UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

DEPARTAMENTO DE FISIOLOGIA E BIOFÍSICA

PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:

FISIOLOGIA E FARMACOLOGIA

ASSIMETRIA E LATERALIZAÇÃO NAS VIAS DESCENDENTES CARDIOVASCULARES ORIUNDAS DO HIPOTÁLAMO DORSOMEDIAL

Carlos Henrique Xavier Custódio

Orientador: Prof. Dr. Marco Antônio Peliky Fontes

Belo Horizonte – Outubro de 2012

UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

DEPARTAMENTO DE FISIOLOGIA E BIOFÍSICA

PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:

FISIOLOGIA E FARMACOLOGIA

ASSIMETRIA E LATERALIZAÇÃO NAS VIAS DESCENDENTES CARDIOVASCULARES ORIUNDAS DO HIPOTÁLAMO DORSOMEDIAL

Tese de doutorado apresentada ao Programa de Pós-Graduação em Fisiologia e Farmacologia do Departamento de Fisiologia e Biofísica. Instituto de Ciências Biológicas (ICB). Universidade Federal de Minas Gerais (UFMG).

Carlos Henrique Xavier Custódio

Orientador: Prof. Dr. Marco Antônio Peliky Fontes

Belo Horizonte – Outubro de 2012

"ASSIMETRIA E LATERALIZAÇÃO NAS VIAS DESCENDENTES CARDIOVASCULARES ORIUNDAS DO HIPOTÁLAMO DORSOMEDIAL"

CARLOS HENRIQUE XAVIER CUSTÓDIO

Tese de Doutorado defendida e aprovada no **dia 31 de outubro de 2012**, pela Banca Examinadora constituída pelos seguintes professores:

Prof. Dr. Eduardo Colombari
Universidade Estadual Paulista/SP

Prof. Dr. Valdir de Andrade Braga
Universidade Federal da Paraíba/PB

Prof. Dr. Jerome Paul Armand L. Baron
Universidade Federal de Minas Gerais

Prof. Dr. Márcio Flávio Dutra Moraes
Universidade Federal de Minas Gerais

Prof. Dr. Marco Antônio Peliky Fontes
Universidade Federal de Minas Gerais
Orientador

Programa de Pós-Graduação em Ciências Biológicas-Fisiologia e Farmacologia
Instituto de Ciências Biológicas – Universidade Federal de Minas Gerais – UFMG

Belo Horizonte, 31 de outubro de 2012.

“Ensinar não é transferir conhecimento, mas criar as possibilidades para a sua própria produção ou a sua construção”

Paulo Freire – Educador

DEDICATÓRIA

Dedico aos meus familiares que me apoiam e me incentivam em todas as decisões. Em especial minha mãe e minha irmã pelo suporte emocional. Vô Nico e vó Maria, obrigado por me proporcionarem o prazer de tê-los por tantos anos. Que muitos outros ainda sejam possíveis.

Dedico especialmente à minha esposa, minha Danny, por fazer parte da minha vida. Sem seu apoio, toda essa caminhada teria muito mais obstáculos.

À família Ianzer por me receber como um dos seus com tanto amor.

AGRADECIMENTOS

À Deus, por ter me proporcionado viver tão intensamente com as pessoas que mais amo e por ter me guiado e protegido nas minhas escolhas.

À população do meu país, que mesmo com tantas mazelas e dificuldades, pôde me proporcionar a oportunidade de investir em minha formação.

Ao Prof. Dr. Marco Antônio Peliky Fontes por tantas oportunidades, pela confiança e amizade, pelas publicações, pela orientação e incentivo diário ao meu crescimento.

Ao Prof. Eugene Nalivaiko pelas oportunidades e ensinamentos.

Aos professores Shaun Morrison e Chris Madden pelas oportunidades e ensinamentos.

Ao Prof. Dr. Deoclécio Chianca-Jr pela amizade, apoio e oportunidades.

Aos professores Dr. Robson Santos, Dra. Andréia Haibara e Maria José Campagnole Santos pelos ensinamentos e pelas colaborações.

Ao Prof. Dr. Gustavo Menezes pela oportunidade de colaboração, pela apoio e disponibilidade.

À Profa. Maria da Glória Rodrigues Machado pelas primeiras oportunidades e pela confiança.

Ao Prof. Rodney Saldanha pelo apoio e incentivo.

À minha Danny, Dra. Danielle Ianzer, pelo carinho, amor, dedicação, aprendizado, pelos projetos e publicações que tivemos.

Aos amigos Fernando Barroso, Flávio Oliveira, Di Borges, Rafael e Simone Rocha, Fernanda Santos, Domenico Tupone, Martina Mancini, Andrea Lima, Taciana Dias, João Furtado, Rafael Barreto, Andreia Fiedler e Thomaz Fiedler, Fernanda Gravina e Ramatis Oliveira.

Aos meus afilhados Bruno e Arthur que sempre me animam com seu amor e alegria.

Aos amigos da Peladinha do Floresta – Vlad, Ze, Joel, Bolota, Warley, Marcelo, Dudu, Fred, Amandio, Vinição, Anderson, Dedé e outros.

À todos os colegas e amigos do Laboratório de hipertensão e do ICB que me apoiaram, divertiram e incentivaram. Agradeço especialmente ao Zezé, Gisele Vaz, Marcelo Coelho, Priscila, Nivia e Daniel, Quênia e Kyan, Rodrigo e Fabiana, Augusto, Flávia, Ana Paula, Patrícia, Edson, Marilene, Renatinha Bessa, Nathália, Janaína, Fernanda, Marcelo, Samuel e a todos que estiveram comigo.

Aos colegas e amigos do Departamento de Ciências Biológicas da UFOP.

Aos novos colegas do Departamento de Ciências Fisiológicas da UFG.

À todos que de alguma forma colaboraram para realização deste trabalho.

RESUMO

Situações de estresse causam respostas cardiovasculares e neuroendócrinas centralmente mediadas. Entretanto, as causas potenciais da variabilidade interindividual nas amplitudes dessas respostas ainda permanecem pobremente compreendidas. Desta forma, torna-se importante a investigação da contribuição das áreas centrais que organizam as respostas cardiovasculares de diferentes magnitudes. Através da desinibição unilateral do hipotálamo dorsomedial (DMH) pela nanoinjeção do antagonista $GABA_A$, bicuculina metiodida (BMI), nós previamente demonstramos que há assimetria no controle cronotrópico cardíaco pelo DMH (com predominância do DMH direito) e lateralização no controle da atividade simpática renal. No presente trabalho, objetivando estender este estudo anterior, avaliamos a existência de lateralização e assimetria anátomo-funcional nas vias descendentes cardiovasculares oriundas do DMH. Três diferentes focos foram dados no presente trabalho: i) Na busca da implicação funcional de assimetria do DMH sobre a função cardíaca, observamos que a ativação do DMH direito pela nanoinjeção de BMI provoca maiores respostas cronotrópicas e inotrópicas comparadas àquelas evocadas pelo DMH esquerdo. Tais respostas contráteis foram independentes das influências cronotrópica e de pós-carga que sabidamente modificam o inotropismo cardíaco. Também foi visto que a ativação do DMH provoca batimentos ectópicos cardíacos, novamente em maior numero após desinibição do DMH direito; ii) Sabendo que após a remoção do tônus Gabaérgico pelo antagonismo farmacológico causado pela BMI há predominância da liberação de aminoácidos excitatórios (EAA), o segundo foco foi avaliar a contribuição dos receptores EAA para as respostas

assimétricas e lateralizadas evocadas pelo DMH unilateral. Também investigamos se estas respostas são mediadas pela substância cinzenta periaquedutal (PAG). Em animais anestesiados, a estimulação do DMH e da PAG provocou respostas lateralizadas para o nervo renal. Entretanto, as respostas cronotrópicas evocadas pelo DMH direito foram mais amplas do que aquelas observadas para o DMH esquerdo ou PAG unilateral. Experimentos anatômicos revelaram uma projeção bidirecional entre o DMH e a PAG. A inibição dos receptores EAA em animais não-anestesiados atenuou as respostas taquicárdicas evocadas por exposição ao estresse agudo. Novamente, os receptores EAA do DMH direito foram determinantes de respostas cronotrópicas mais amplas provocadas pelo estresse; iii) Os neurônios da *Raphe Pallidus* (RP) recebem densas projeções do DMH e abrigam os neurônios pré-motores simpáticos cardíacos. Assim, buscamos entender a contribuição dos neurônios da RP para a assimetria nas respostas cardíacas causadas pela estimulação do DMH unilateral. A estimulação da RP provocou respostas cronotrópicas e inotrópicas cardíacas semelhantes àquelas evocadas pelo DMH direito. A inibição dos neurônios da RP aboliu as diferenças na amplitude das respostas cronotrópicas e inotrópicas causadas pela estimulação do DMH unilateral. Nós concluímos que: i) a assimetria no controle cardíaco pelo DMH depende de receptores EAA e dos neurônios da RP; ii) as respostas lateralizadas evocadas pelo DMH dependem de uma via descendente lateralizada até a PAG. Estudos complementares são necessários para elucidar os mecanismos envolvidos nessas respostas assimétricas e lateralizadas.

ABSTRACT

Stress exposure causes centrally mediated cardiovascular and neuroendocrine responses. However, the mechanisms underlying the interindividual variability in the amplitude of these responses remain to be further investigated. Then, it is worth attempting efforts to investigate the central areas that organize these differential cardiovascular responses. Previous studies using disinhibition of unilateral dorsomedial hypothalamus (DMH) by nanoinjecting the $GABA_A$ antagonist, bicuculline methiodide (BMI), revealed asymmetry in the cardiac control by DMH (with right-sided predominance) and lateralization in the control of the renal sympathetic activity. Aiming to extend these previous data, we next evaluated anatomic and functional asymmetry in the descending cardiovascular pathways from DMH. There were three different focuses: i) Seeking to reveal whether the DMH asymmetry implicates in the cardiac performance, we observed that injection of BMI into right DMH provokes greater cardiac positive chronotropy and inotropy. Such inotropic responses were likely independent on chronotropy and afterload influences, likely known to modify the cardiac contractility. It was also detected cardiac ectopic beats, again greatly numerous following right DMH activation; ii) Assuming that the removal of the Gabaergic tone acts over DMH by injecting BMI allows an excitatory input, our second aim was to assess the contribution of EAA receptors (EAA) to the asymmetry and lateralization evoked from unilateral DMH. We also evaluated whether these responses would be mediated by periaqueductal gray. In anesthetized animals, injection of NMDA into unilateral DMH or periaqueductal gray (PAG) caused lateralized renal sympathetic responses. However, the tachycardia evoked from right DMH was greater that

left DMH or left and right PAG. Anatomic experiments unraveled a bidirectional pathway between DMH and PAG. In non-anesthetized rats, inhibition of EAA receptors attenuated the stress-evoked tachycardia, but EAA receptors in the right DMH seem to govern the full tachycardia caused by stress; iii) Neurons in *Raphe Pallidus* (RP) receive dense inputs from DMH and rule pre-sympathetic cardiac neurons. In this regard, we shall to investigate the contribution of RP neurons to the asymmetry in the cardiac responses evoked from unilateral DMH. Stimulation of RP neurons caused positive chronotropism and inotropism similar to that observed after stimulation of right DMH. Inhibition of RP abolished the differences in the cardiac responses evoked from right and left DMH. We conclude that the asymmetry in the cardiac control by DMH depends on the EAA receptors and on the recruitment of RP neurons. Furthermore, the lateralized responses evoked from unilateral DMH depend on a descending lateralized synapse between from DMH to PAG. Complimentary analyses are required to better elucidate the mechanisms involved in these asymmetric and lateralized responses.

SUMÁRIO

ABREVIATURAS

AP – ântero-posterior

BAT – tecido adiposo marrom

BMI – bicuculina metiodida

DMH – hipotálamo dorsomedial

DV – dorso-ventral

EAA – aminoácido excitatório

FC – freqüência cardíaca, HR

$GABA_A$ – receptor para ácido gama amino butírico (subtipo A)

i.m. – intramuscular

i.p. – intraperitoneal

i.v. – intravenosa

KYN – ácido quinorênico

L- – esquerdo, left

LL – látero-lateral

LVP – pressão no ventrículo esquerdo cardíaco

NMDA – N-metil-D-aspartato

NTS – núcleo do trato solitário

PAG – substância cinzenta periaquedutal

PAM – pressão arterial média, MAP

PAP – pressão arterial pulsátil

PFA – paraformoldeído

PVN – núcleo paraventricular do hipotálamo

R- – direito, right

RP – *Raphe Pallidus*

RSNA – atividade simpática para o nervo renal

RVLM – bulbo ventrolateral rostral

RVMM – bulbo ventromedial rostral

VMH – hipotálamo ventromedial

1 – INTRODUÇÃO

Diferenças nas propriedades do funcionamento dos lados direito e esquerdo do sistema nervoso central são conhecidas como lateralização da função. Este fenômeno tem sido observado em diferentes estruturas cerebrais, incluindo o hipotálamo de diferentes espécies (Toga and Thompson, 2003, Stephan et al., 2007). Estudos têm mostrado que, em algumas condições, a atividade assimétrica de regiões do cérebro podem resultar em lateralização e desequilíbrio do controle autonômico cardíaco, implicando em arritmias cardíacas (Harris et al., 1996, Critchley et al., 2005), e isso inclui condições de estresse mental (Lane et al., 1992a, Lane et al., 1992b).

Foi observado em humanos que há uma divisão de funções entre os hemisférios. O hemisfério esquerdo governa a geração da atividade parassimpática para o coração, enquanto o hemisfério direito controla a performance contrátil cardíaca (Wittling et al., 1998a, Wittling et al., 1998b). Através de exames de imagem foi visto que o estresse mental e a assimetria central podem ser um mecanismo de morte súbita em humanos. Curiosamente, a ativação mesencefálica unilateral foi relacionada à predisposição a arritmias cardíacas (Critchley et al., 2005).

Estudos têm demonstrado que a assimetria de funções centrais pode resultar em desequilíbrio autonômico com implicações para o sistema cardiovascular. Anatomicamente, as projeções de núcleos que participam do controle autonômico para a coluna intermédio lateral são predominantemente lateralizadas (Amendt et al., 1979, Loewy, 1981, Taylor and Weaver, 1992, Jansen et al., 1995, Huang et al., 2002). Adicionalmente, segmentos medulares

torácicos que comportam neurônios pré-ganglionares do nervo simpático renal também apresentam distribuição anatômica e funcional ipsilateral (Amendt et al., 1979, Blessing et al., 1981, Zagon and Smith, 1993). Há também evidências de que a inervação cardíaca apresenta distribuição diferencial (Randall et al., 1972, Kamosinska et al., 1989). Os nervos simpáticos originários de gânglios superiores (cervicais) e localizados do lado direito da cadeia paravertebral inervam predominantemente os átrios cardíacos e exercem maior influência sobre o cronotropismo. Por sua vez, os nervos simpáticos provenientes de gânglios inferiores (torácicos) e localizados do lado esquerdo da cadeia paravertebral adentram predominantemente o sincício ventricular, e consequentemente controlam o inotropismo cardíaco (Yanowitz et al., 1966, Randall et al., 1972, Kamosinska et al., 1989, Furukawa et al., 1990). É possível especular que, durante situações de maior demanda energética como estresse emocional, para alcançar o desempenho cardíaco máximo, deve haver o recrutamento de um maior número de neurônios pré-motores, pré-ganglionares medulares e nervos simpáticos cardíacos.

Situações de estresse psicológico induzem simpatoexcitação (Julius, 1993) com conseqüente alteração na função cardíaca, na resistência vascular e no fluxo sanguíneo (Halliwill et al., 1997, Harris, 2000, Carter et al., 2005, Hayashi et al., 2006) que em conjunto com outras alterações fisiológicas, resultam na conhecida síndrome de emergência ou reação de luta ou fuga (Cannon, 1929) . No entanto, em situações extremas o estresse psicológico pode ter consequências cardiovasculares como: arritmias cardíacas (Lathers and Schraeder, 2006, Oppenheimer, 2006); hipertensão arterial (Leor et al., 1996); infarto do miocárdio (Folkow, 1982, Davies et al., 1999, Gerin et al.,

2005) e até morte súbita (Parati et al., 2001, Gerin et al., 2005). Embora existam dados sobre variabilidade individual nas respostas cardiovasculares a evento aversivos (Smith, 2004, Critchley et al., 2005, Gerin et al., 2005), as causas potenciais e as áreas centrais envolvidas na elaboração dessas respostas diferenciais permanecem pouco compreendidas.

O papel do sistema nervoso central na elaboração de respostas ao estresse foi inicialmente considerado ao observar que um hipotálamo intacto seria essencial para a geração de respostas adequadas de luta ou fuga (Bard, 1928). Posteriormente, utilizando técnicas e metodologia mais precisas e refinadas, foram descritas subregiões diencefálicas que organizam estas repostas (Schmidt and DiMicco, 1984, Smith, 1990, Diamant, 1992, Lovallo and Gerin, 2003), dentre elas, o hipotálamo dorsomedial (DMH) (Fontes et al., 2011). Um crescente número de trabalhos tem avaliado o papel do DMH e de suas vias descendentes na organização de respostas fisiológicas a eventos aversivos, além de sugerir sua participação em processos fisiopatológicos [para revisão, ver (DiMicco et al., 2002, Xavier et al., 2008, Fontes et al., 2011)].

1.1- O HIPOTÁLAMO DORSOMEDIAL

O hipotálamo dorsomedial (DMH) localiza-se adjacentemente ao terceiro ventrículo, e além de conexões intrahipotalâmicas, conecta-se com telencéfalo e com outros núcleos diencéfalicos e bulbares (Paxinos and Watson, 1986, Thompson et al., 1996, DiMicco et al., 2002, Xavier et al., 2008). Anatomicamente, as vias intrahipotalâmicas e descendentes a partir do DMH parecem ser predominantemente ipsilaterais. Assim, uma porção unilateral do DMH parece não se comunicar diretamente com seu correspondente

contralateral. Da mesma forma, as vias descendentes do DMH têm aspecto de espelhos ipsilaterais (ter Horst and Luiten, 1986, Thompson et al., 1996).

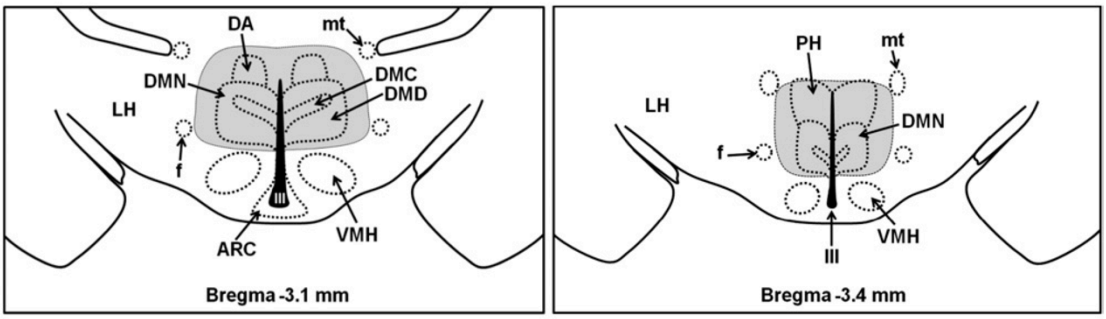


Figura 1. Desenho esquemático demonstrando a região hipotalâmica dorsomedial (DMH – demarcada em cinza). Indicadas pelas setas estão as estruturas e referências anatômicas que compõem e cercam o hipotálamo dorsomedial. DA: aréa hipotalâmica dorsal; mt: trato mamilar; f: fornix; LH: hipotálamo lateral; ARC: núcleo arqueado; III: terceiro ventrículo; VMH: hipotálamo ventromedial; PH: hipotálamo posterior; DMN: núcleo hipotalâmico dorsomedial – porções compacta (DMC) e difusa (DMD). Extraído do artigo de revisão - *The dorsomedial hypothalamus and the central pathways involved in the cardiovascular response to emotional stress. Fontes MA, Xavier CH, Menezes RCA and DiMicco J. – Neuroscience 184 (2011) 64-74.*

Existem numerosas evidências da participação do DMH nas respostas ao estresse. Algumas manobras farmacológicas têm sido utilizadas no estudo do papel do DMH na organização de respostas cardiovasculares, comportamentais e neuroendócrinas ao estresse. Dados da literatura evidenciam que os neurônios do DMH estão sob constante inibição pela ação de projeções gabaérgicas (DiMicco and Abshire, 1987). Nesse sentido, a injeção do antagonista dos receptores GABA subtipo A, bicuculina metiodida

(BMI), tem sido uma das intervenções mais usadas para provocar desinibição / ativação dos neurônios do DMH [para revisão, ver anexo 2 (Fontes et al., 2011)]. O bloqueio dos receptores $GABA_A$ no DMH produz respostas simpatoexcitatórias tipicamente observadas em estresse emocional experimental, como aumentos de freqüência cardíaca (FC), de atividade simpática para o nervo renal (RSNA) e pressão arterial (PA) (DiMicco et al., 1996, Fontes et al., 2001, Cao et al., 2004, Horiuchi et al., 2004, Xavier et al., 2009). Tem sido proposto que a exposição ao estresse emocional reduz a ação deste tônus inibitório que age sobre neurônios do DMH, permitindo assim sua ativação. Assim, no intuito de confirmar o papel da inibição GABA no controle da atividade de neurônios do DMH e suas respostas, a injeção do agonista de receptores $GABA_A$, muscimol, tem sido a estratégia comumente empregada. A injeção de muscimol no DMH reduz a taquicardia e a hipertensão em ratos submetidos ao estresse (Lisa et al., 1989a, Lisa et al., 1989b, Stotz-Potter et al., 1996a, Stotz-Potter et al., 1996b, Xavier et al., 2009).

A despeito das substanciais evidências da existência de projeções inibitórias sobre os neurônios do DMH, é importante salientar que essas células não possuem atividade tônica e para serem estimuladas, necessitam de um "*input*" excitatório. Na busca do melhor entendimento sobre os mecanismos neuroquímicos envolvidos no controle da atividade de neurônios do DMH, foi relatada a participação dos receptores de aminoácido excitatório (EAA) na estimulação desses neurônios, que ocorre concomitantemente à remoção do tônus GABA. Estudos descrevem que a remoção do tônus GABA permite a ação de uma sinapse excitatória sobre o DMH, permitindo que estes neurônios sejam estimulados / excitados. Injeções de agonistas de EAA provocaram

respostas cardiovasculares, neuroendócrinas e comportamentais semelhantes àquelas obtidas após o bloqueio dos receptores $GABA_A$ pela BMI (Soltis and DiMicco, 1991a, b, 1992, De Novellis et al., 1995, Soltis et al., 1998). Por outro lado, a injeção de drogas como o ácido quinurênico (Kyn), que bloqueia os receptores EAA, atenua as respostas provocadas por diferentes estímulos aversivos, inclusive estresse emocional (Soltis and DiMicco, 1991b, 1992, Queiroz et al., 2011).

A ação de dois tipos de sinapses diferentes (inibitória – GABA e excitatória – EAA) no controle do DMH sugere que este núcleo recebe projeções de diferentes áreas, que de maneira coordenada ativa os neurônios provocando assim as respostas acima descritas. Nesse sentido, as projeções oriundas da área preóptica medial (mPOA) foram descritas como uma importante fonte gabaérgica que controle o DMH (Hunt et al., 2010). De maneira diferente, um estudo prévio relatou que a amigdala (Amg), uma estrutura central límbica, é uma das responsáveis pela excitação dos neurônios do DMH (Soltis et al., 1998). Mais recentemente foi descrito que neurônios da substância cinzenta periaquedutal (PAG) também fornecem os "*inputs*" excitatórios necessários para o recrutamento dos neurônios do DMH (de Menezes et al., 2009, Horiuchi et al., 2009). Embora estudos anatômicos fomentem hipóteses sobre a participação de outras estruturas no controle do DMH, como as regiões pré-frontal medial (mPFC) (Hurley et al., 1991, Vertes, 2004) e insular (IC) (Cechetto and Chen, 1990) do córtex, estudos fisiológicos / funcionais complementares são necessários para avaliar se essas áreas exercem algum controle sobre o DMH.

1.2- VIAS DESCENDENTES LATERALIZADAS ORIUNDAS DO DMH

Há evidências anatômicas de que um lado do DMH parece não se comunicar diretamente com seu correspondente hipotalâmico contralateral. Adicionalmente, as vias anatômicas descendentes oriundas do DMH parecem ser lateralizadas, como espelhos ipsilaterais (ter Horst and Luiten, 1986, Thompson et al., 1996). Estes dados despertaram nosso interesse, levando à formulação de hipóteses acerca das possíveis implicações funcionais destas particularidades anatômicas do DMH. Os estudos funcionais anteriores utilizaram abordagens unilaterais randômicas e/ou bilaterais, e assim, não forneciam dados consistentes sobre a possível existência de lateralização e assimetria nas vias oriundas do DMH. Ainda não existiam estudos comparando as respostas evocadas pelos lados direito e esquerdo deste núcleo. Então, realizamos uma avaliação criteriosa, comparando a amplitude de respostas cardiovasculares evocadas pelos lados direito e esquerdo do DMH. Nossos dados recentes (anexo 1) demonstram assimetria e lateralização nas respostas funcionais causadas pela ativação unilateral do DMH. Através da injeção de agonistas e antagonistas dos receptores $GABA_A$, concluímos que o DMH unilateral conduz o controle autonômico no hemicorpo ipsilateral. Além disso, a participação do DMH direito parece ser crítica para atingir a taquicardia máxima durante o estresse emocional (Xavier et al., 2009). A partir destes dados, os questionamentos levantados foram: i) quais seriam os mecanismos de controle hemodinâmico e cardíaco existentes durante os efeitos causados pela ativação do DMH (bloqueio $GABA_A$); ii) os receptores de EAA contribuem para as respostas assimétricas e lateralizadas previamente descritas? Estas perguntas

embasaram, respectivamente, o primeiro e parte do segundo objetivos específicos da presente tese (ver seção 2 – objetivos).

Apesar de todas as evidências do envolvimento do DMH na integração das respostas simpatoexcitatórias ao estresse, este núcleo não envia projeções diretas para a coluna intermediolateral (ter Horst and Luiten, 1986, Thompson et al., 1996), e por isto necessita conectar-se a outros núcleos para produzir seus efeitos. Estudos demonstram densas projeções do DMH para núcleos como a substância cinzenta periaquedutal (PAG), o bulbo ventrolateral rostral (RVLM) e a *Raphe Pallidus* (RP), localizada no bulbo ventromedial rostral (RVMM) (ter Horst and Luiten, 1986, Hosoya et al., 1987, Thompson et al., 1996, Farkas et al., 1998). Algumas das regiões envolvidas na circuitaria que organiza as respostas cardiovasculares ao estresse emocional e as projeções oriundas do DMH estão resumidas na Figura 2.

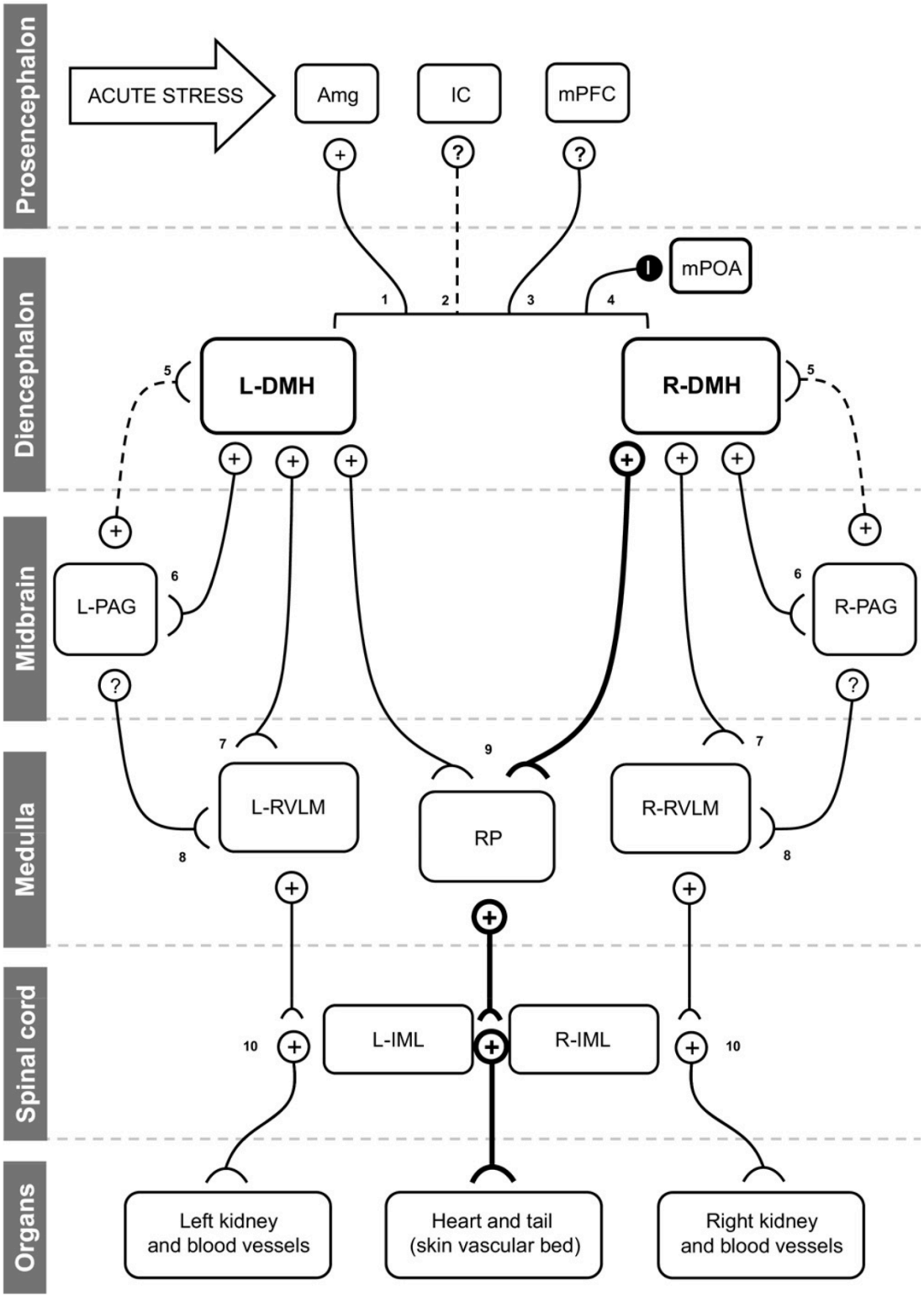


Figura 2. Desenho esquemático demonstrando as vias centrais que participam da organização de respostas cardiovasculares ao estresse. O hipotálamo dorsomedial é evidenciado como um núcleo chave na integração de regiões topograficamente superiores (prosencéfalo) e inferiores (mesencéfalo e bulbo). Em consonância ao demonstrado no esquema acima, há evidências de lateralização nas projeções do DMH para núcleos bilaterais como a PAG e

RVLM. Todavia, o DMH se conecta com a RP, um núcleo central localizado exatamente na região medial do tronco encefálico. Através de uma sinapse excitatória com a RP, o DMH produz respostas cardíacas e termogênicas. Sinais positivos (+), negativos (-) e interrogação (?) indicam sinapses excitatórias, inibitórias e desconhecidas, respectivamente. Marcações L- e R- indicam localização à esquerda e à direita de núcleos bilaterais, respectivamente. Amg: amígdala; IC: córtex insular; mPFC: córtex pré-frontal medial; mPOA: área pré-optica medial; DMH: hipotálamo dorsomedial; PAG: substância cinzenta periaquedutal; RVLM: bulbo ventrolateral rostral; RP: raphe pallidus; IML: coluna intermédio-lateral espinhal. Extraído do artigo de revisão - *The dorsomedial hypothalamus and the central pathways involved in the cardiovascular response to emotional stress. Fontes MA, Xavier CH, Menezes RCA and DiMicco J. – Neuroscience 184 (2011) 64-74.*

A PAG é considerada uma região chave na organização de respostas comportamentais e autonômicas a eventos aversivos (Lovick, 1993, Farkas et al., 1998, Vianna and Brandao, 2003, de Menezes et al., 2008), anatomicamente organizada em um sistema colunar lateralizado (Farkas et al., 1998). Foi visto que as alterações de PA e principalmente de FC evocadas por estresse e pela ativação do DMH foram revertidas e/ou atenuadas pela inibição da PAG (da Silva et al., 2003, da Silva et al., 2006, de Menezes et al., 2006, de Menezes et al., 2008, de Menezes et al., 2009). Além disso, somente a inibição da PAG ipsilateral atenuou as respostas pressora e cronotrópica evocadas pela estimulação unilateral do DMH (da Silva et al., 2003). Isto sugere a existência de uma via funcionalmente lateralizada do DMH até a PAG. De maneira inversa, dois estudos recentes observaram que é necessária a atividade de neurônios no DMH, para que se alcancem as respostas evocadas pela estimulação da PAG (de Menezes et al., 2009, Horiuchi et al., 2009). Entretanto, ainda não foi avaliado se a estimulação unilateral da PAG causa respostas semelhantes àquelas observadas para o DMH. Assim, a pergunta

que remete à parte restante do segundo objetivo específico da presente tese é: existe assimetria e lateralização nas respostas evocadas pela PAG mediadas pelo DMH?

Estudos que avaliaram a participação de áreas bulbares nas respostas evocadas pelo DMH demonstraram que as elevações de PA e RSNA produzidas pela ativação do DMH foram atenuadas mediante inibição dos neurônios do bulbo ventrolateral rostral (RVLM) (Fontes et al., 2001, Cao et al., 2004). Vale ressaltar que a inibição ipsilateral da RVLM reduz de maneira significativa os aumentos de RSNA evocadas pela ativação do DMH ipsilateral. Entretanto, a taquicardia parece não ser mediada pela RVLM (Fontes et al., 2001).

Como citado anteriormente, a ausência de influência dos neurônios pré-motores simpáticos da RVLM sobre a resposta taquicárdica causada pela ativação do DMH (Fontes et al., 2001) revelou que: i) a RVLM governa predominantemente os efeitos vasomotores e simpatoexcitatórios renais causados pela ativação do DMH; ii) a reatividade cardíaca / taquicardia evocada pelo DMH é controlada por um outro grupo de neurônios pré-motores simpáticos, possivelmente com maior influência cardíaca. Estudos anatômicos mostraram que a região do bulbo ventromedial rostral (RVMM), que compreende a *Raphe Pallidus* (RP), *Raphe Magnus* (RM) e neurônios parapiramidais (PPy), contêm neurônios pré-motores simpáticos cardíacos e para o tecido adiposo marrom interescapular (Loewy, 1981, Ter Horst et al., 1996, Nakamura et al., 2004). Funcionalmente, foi avaliada a participação da RVMM nas repostas cronotrópicas e inotrópicas cardíacas. A inibição da RP suprimiu a taquicardia gerada pela ativação do DMH (Samuels et al., 2002,

Zaretsky et al., 2003, Cao et al., 2004, Samuels et al., 2004). Além disso, foi observado que a estimulação da RP produziu amplas respostas cronotrópicas e inotrópicas positivas (Salo et al., 2009) e que a amplitude dessas respostas taquicárdicas foi similar àquelas observadas para o DMH (Samuels et al., 2002, Cao and Morrison, 2003). No entanto, ainda não se sabe se há assimetria e lateralização nas respostas controladas pela RP, um núcleo central e único. Sabendo que a RP é o núcleo que controla as respostas cronotrópicas evocadas pelo DMH, as perguntas a seguir relacionam-se ao terceiro e último objetivo específico da presente tese: i) os neurônios da RP contribuem para a assimetria que determina diferenças nas respostas cardiovasculares evocadas pelo DMH unilateral (Xavier et al., 2009)]?; ii) os neurônios da RP controlam as respostas assimétricas de contratilidade cardíaca evocadas pelo DMH (obtidas a partir do cumprimento do primeiro objetivo específico)?

Considerando as diferenças na amplitude das respostas evocadas pelos lados direito e esquerdo do DMH (Xavier et al., 2009), as densas projeções do DMH para PAG e RP (ter Horst and Luiten, 1986, Thompson et al., 1996) e seu papel na organização da resposta cardiovascular ao estresse (Fontes et al., 2011), pode-se especular que o recrutamento diferencial desses núcleos causaria diferentes respostas funcionais. Uma vez que esse recrutamento diferencial e assimetria de funções cerebrais pode resultar em alterações autonômicas, torna-se importante avaliar o envolvimento do eixo DMH – PAG – RP na elaboração destas respostas diferenciais.

2 – OBJETIVO GERAL

Comparar as respostas cardiovasculares provocadas pela ativação / estimulação unilateral do hipotálamo dorsomedial (lado direito *vs.* lado esquerdo) e a participação dos centros integradores inferiores, substância cinzenta periaquedutal (PAG) e *Raphe Pallidus* (RP).

2.1- OBJETIVOS ESPECÍFICOS

Sempre objetivando avaliar a possível assimetria funcional envolvendo o DMH, os objetivos específicos desta tese são:

2.1.1 - Comparar as respostas eletrocardiográficas, cronotrópicas e inotrópicas cardíacas provocadas pela desinibição dos lados direito e esquerdo do hipotálamo dorsomedial;

2.1.2 - Avaliar a contribuição de receptores de aminoácidos excitatórios nos lados direito e esquerdo do hipotálamo dorsomedial e colunas lateral e dorsolateral da substância cinzenta periaquedutal para as respostas simpatoexcitatória, pressórica e cronotrópica cardíaca;

2.1.3 - Avaliar o envolvimento da *Raphe Pallidus* nas respostas assimétricas cronotrópicas e inotrópicas cardíacas provocadas pela estimulação dos lados direito e esquerdo do hipotálamo dorsomedial.

3 – MATERIAL

3.1- DROGAS

As drogas utilizadas foram: i) antagonista $GABA_A$: bicuculina metiodida (BMI 40pmol/100nL); ii) o agonista de receptores $GABA_A$: muscimol (12mM/60nL); iii) o agonista de receptores de aminoácidos excitatórios (EAA) N-metil D-aspartato (NMDA) (0,2mM/60nL e 100pmol/100nL); iv) o antagonista de EAA: ácido quinurênico (Kyn) 1nmol/100nL; adquiridas da Sigma e diluídas em salina estéril (NaCl – 0,9%); v) o bloqueador da corrente marcapasso cardíaca (corrente ativada por hiperporlarização - If ou corrente funny): zatebradina (ou UL-FS 49) (1mg/Kg); vi) o antagonista de receptores beta adrenérgicos cardíacos: atenolol (2mg/Kg); vii) os anestésicos: isofluorano; tribromoetanol (250mg/Kg – i.p.); uretana (1,2 a 1,4g/Kg i.p.); cloralose (120mg/kg i.p.); lidocaína (2%) + epinefrina (1:50.000) s.c.; viii) o bloqueador neuromuscular tubocurarina (0,6mg); ix) o agonista adrenérgico fenilefrina (10 µg/kg i.v.).

Para experimentos em animais não-anestesiados, após os procedimentos cirúrgicos, os animais receberam antibiótico (pentabiótico 0,2mL i.m.) analgésico e anti-inflamatório Banamine® (1,1-2,2mg/Kg i.m.).

3.2- ANIMAIS

Foram utilizados ratos Wistar, com peso entre 250 e 350g, fornecidos pelo Centro de Bioterismo das respectivas instituições nas quais este trabalho foi desenvolvido: i) Instituto de Ciências Biológicas (ICB) da Universidade Federal de Minas Gerais (MG - Brasil); ii) School of Biomedical Sciences and Pharmacy da University of Newcastle (NSW - Australia); iii) Department of

Neurological Surgery da Oregon Health and Sciences University (OR – Estados Unidos). Os procedimentos estiveram de acordo com as regras estabelecidas pelo CETEA, pelo U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals e como recomendado pelas diretrizes de cada instituição envolvida. Todos os esforços foram feitos para minimizar o número de animais necessários para a conclusão dos experimentos.

4 – MÉTODOS

4.1- PROCEDIMENTOS CIRÚRGICOS

4.1.1- TRAQUEOSTOMIA

Na superfície ventral do pescoço, próximo ao manúbrio esternal, foi realizada uma pequena incisão na pele do animal. Foram divulsionadas e rebatidas as estruturas musculares, e após a localização da traquéia foi feito um pequeno orifício entre dois anéis cartilaginosos para a inserção de uma cânula de diâmetro compatível (JELCO Plus - n° 14G 7068 – Johnson & Johnson MEDICAL). Dessa forma, possibilitou-se aspiração endotraqueal (se necessário) e a manutenção da perviedade das vias aéreas. À porção externa da cânula traqueal, foi posicionado um tubo em “Y” conectado a um transdutor de pressão respiratória que permitiu o cálculo da frequência respiratória.

4.1.2- CANULAÇÃO DE ARTÉRIA E VEIA FEMORAIS

Os ratos foram posicionados em decúbito dorsal e receberam uma incisão na região inguinal unilateral para dissecação do feixe vásculo-nervoso femoral. Cânulas de polietileno PE-50 (15cm) soldados a PE-10 (2cm para veia e 4cm para artéria) foram implantadas e fixadas por amarraduras. A cânula venosa foi utilizada para injeção de drogas e a extremidade livre da cânula inserida na artéria foi acoplada a um transdutor de pressão (strain-Gauge – DT-XX Viggo-spectramed) ligado a um amplificador e a um sistema de conversão analógico-digital para aquisição dos valores de PA e FC.

4.1.3- CRANIOTOMIA E LOCALIZAÇÃO DOS NÚCLEOS CENTRAIS

As coordenadas utilizadas foram baseadas no Atlas de Paxinos e Watson (Paxinos and Watson, 1986). Os animais foram posicionados em um aparelho estereotáxico (STOELTING, IL, USA) com a cabeça posicionada 3,3mm acima da linha interaural. As coordenadas de localização de núcleos a partir do bregma foram: i) DMH - ântero-posterior (AP): -3,1mm, dorso-ventral (DV): -8,5mm e látero-lateral (LL): ±0,6mm; ii) l/dlPAG – AP: -6,8mm, DV: -4,8mm e LL: ±0,6mm. As coordenadas para localização da RP/RVMM a partir do lambda foram: -3,0mm do lambda e movidas caudalmente (-0,5mm) de forma gradual até que se identificasse o local de injeção que produzisse maior resposta taquicárdica e termogênica; DV: -10,6mm e LL: 0mm. Após a realização de uma craniotomia local, uma micropipeta de vidro de ponta ultrafina foi posicionada para nanoinjeção de drogas.

Nos protocolos que utilizam animais acordados, cânulas-guia medindo 16mm foram posicionadas bilateralmente próximas ao DMH e l/dlPAG através do uso de resina acrílica que fixou tais cânulas a parafusos previamente colocados no crânio dos animais marginalmente à craniotomia de acesso ao DMH.

4.1.4- LOCALIZAÇÃO E ISOLAMENTO DO NERVO RENAL

Os animais tiveram o flanco lateral esquerdo tricotomizado. Uma área delimitada entre a última costela e a pata traseira foi demarcada com caneta dermatográfica e exposta pela retirada da pele. Em seguida, foi divulsionada a musculatura paravertebral da região retroperitoneal até a localização do rim. Foram fixadas a um campo operatório as extremidades da abertura cirúrgica

para visualização e acesso a aorta abdominal, nervo e artéria renais. Com o auxílio de uma lupa (aumento de 20x) (Opto SM 2002), o nervo renal foi cuidadosamente localizado e dissecado. O nervo renal foi posicionado sobre um par de eletrodos de prata acoplados a um micromanipulador. Os *bursts* de atividade simpática do nervo renal captados pelo eletrodo foram filtrados (100 a 1000Hz), amplificados e enviados simultaneamente a um amplificador de som e a um osciloscópio (Tektronix 546B) para visualização e identificação da freqüência de descarga que caracterizam a atividade simpática. Além disso, a atividade simpática captada foi conduzida a uma placa analógico-digital e a um sistema de aquisição de dados (Power Lab 4/20 – ADInstruments). Através de um software (Chart 7.2 for Windows), o sinal captado foi registrado como sinal bruto, medido por unidades arbitrárias e simultaneamente convertido em sinal integrado para serem avaliados a variação da RSNA (%).

Dentro do campo cirúrgico foi adicionado, durante todo o experimento, óleo mineral (Nujol – Schering-Plough) previamente aquecido (± 37,5° C) para evitar ressecamento dos tecidos expostos e isolar a captação do sinal do nervo renal de ruídos externos. Toda essa preparação permaneceu em observação por cerca de 30 minutos ou até estabilização dessas medidas para que se iniciassem os procedimentos experimentais.

4.1.5- POSICIONAMENTO DE ELETRODOS PARA ELETROCARDIOGRAFIA

Após tricotomia na região anterior do pescoço, incisão na porção anterior do pescoço e exposição do apêndice xifóide, eletrodos para registro eletrocardiográfico foram posicionados no porção superior do mediastino e posteriormente ao xifóide e em seguida fixados à musculatura próxima com

suturas cirúrgicas. Todo este procedimento ocorreu como previamente descrito (Sgoifo et al., 1996).

4.1.6- CATETERIZAÇÃO DO VENTRÍCULO ESQUERDO CARDÍACO

Sob efeito de anestesia, a artéria carótida direita do animal foi localizada e dissecada. Um cateter Millar (SPR-249, Millar Instruments) foi inserido na artéria e dirigido ao interior da ventrículo esquerdo cardíaco. O registro de pressão foi acompanhado e o posicionamento do catéter no interior da câmara cardíaca foi confirmado quando o valor da pressão diastólica atingia valores próximos a zero. Este catéter permitiu aferir pressão ventricular esquerda (LVP) e sua derivativa (LVdp/dt).

A confirmação do posicionamento do cateter no interior do ventrículo esquerdo cardíaco se deu como exemplificado abaixo (Figura 3). A seta indica o momento em que o cateter adentra a câmara cardíaca, a partir do qual o sinal de pressão se diferencia. Valores aproximados de pressão arterial sistêmica sistólica e diastólica variam de 120 a 80mmHg, respectivamente. Nota-se que, no registro de pressão ventricular, o valor de pressão sistólica é similar ao achado da pressão arterial sistêmica. Entretanto, a pressão diastólica é próxima a 0mmHg.

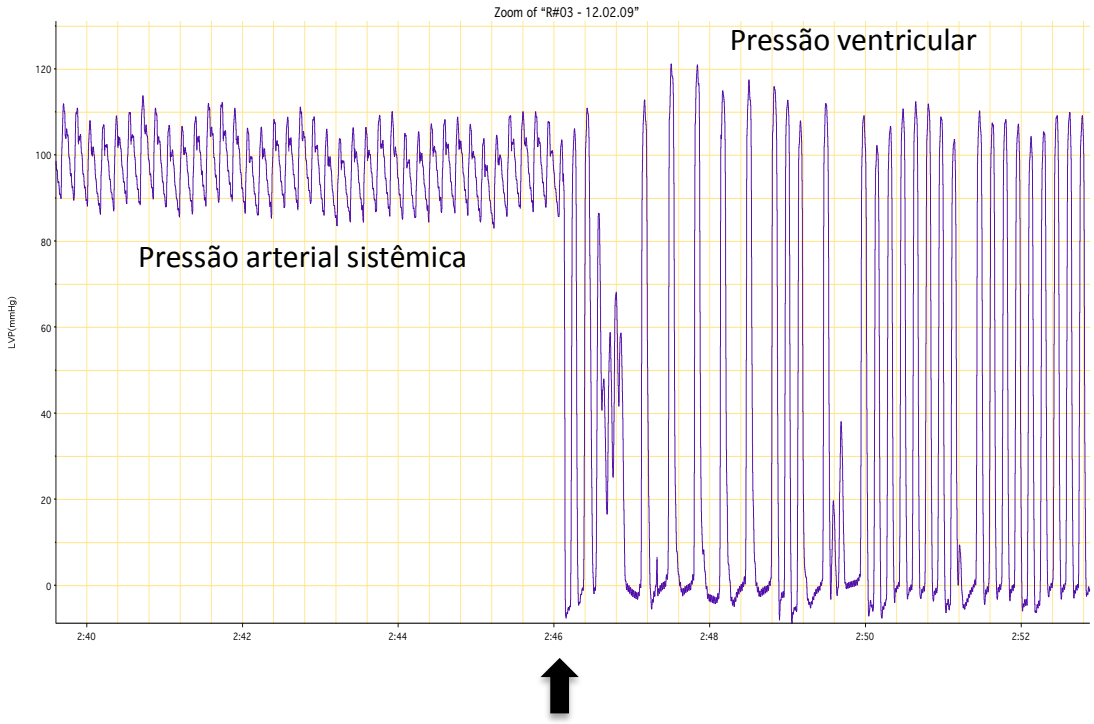


Figura 3. Sinal de pressão sanguínea evidenciando as diferenças entre os registros de pressão arterial periférica e pressão ventricular. A seta indica o momento em que o cateter adentra o ventrículo esquerdo cardíaco.

4.1.7- POSICIONAMENTO DE SENSORES DE TEMPERATURA NO TECIDO ADIPOSO MARROM INTERESCAPULAR E RETAL

Após tricotomia na região interescapular, foi identificado o tecido adiposo marrom (BAT). Uma cânula de 0,5cm foi inserida no BAT de maneira cuidadosa, para que se preservasse a rica vascularização deste tecido, composta por vasos que confluem ventralmente e são responsáveis por conduzir o sangue que capta e dissipa o calor gerado. No interior desta cânula foi posicionada a ponta de um probe de temperatura. Além disso, outros dois sensores de temperatura foram posicionados no cólon do animal, a 1,5cm do esfíncter anal e na cauda dos animais, para medida de temperatura corporal e caudal, respectivamente. O sistema de aquisição de dados térmicos utilizado

foi o TC 1000 Thermocouple Meter (Sable Systems), que fornece precisão de 0,2° C.

4.2- PROCEDIMENTOS EXPERIMENTAIS

Devido ao número de protocolos e grupos experimentais, esta sub-seção foi dividida de acordo com cada objetivo específico, visando facilitar o entendimento do leitor.

4.2.1 – OBJETIVO ESPECÍFICO 1: COMPARAR AS RESPOSTAS ELETROCARDIOGRÁFICAS, CRONOTRÓPICAS E INOTRÓPICAS CARDÍACAS PROVOCADAS PELA DESINIBIÇÃO DOS LADOS DIREITO E ESQUERDO DO HIPOTÁLAMO DORSOMEDIAL

Todos os procedimentos experimentais realizados para atingir o objetivo específico 1 estiveram de acordo com o recomendado pela instituição envolvida: University of Newcastle (Callagham/NSW - Australia). Esta série experimental foi realizada no Neurocardiology Laboratory, School of Biomedical Sciences and Pharmacy, University of Newcastle, como parte do doutorado sanduíche (CNPq – 143005/2008-1) de janeiro a abril de 2009. Orientador: Prof. Eugene Nalivaiko, MD, PhD.

4.2.1.1- ANESTESIA

Os animais foram anestesiados com isofluorano. Após terem sua artéria e veia femorais canuladas (para medida de PAM e FC), receberam lentamente a injeção endovenosa da solução uretana 500mg + cloralose 40mg/mL (aprox.

3mL/Kg) e concomitantemente a inalação de isofluorano foi gradativamente reduzida. Suplementações de uretana foram dadas por via intravenosa (i.v.) quando necessário.

4.2.1.2- DESENHO EXPERIMENTAL - *Grupo 1*

Após anestesia, os animais (n=7) foram submetidos aos procedimentos cirúrgicos de traqueostomia (item 4.1.1), canulação de artéria e veia femorais (item 4.1.2), cateterização do ventrículo esquerdo cardíaco (item 4.1.6), posicionamento de eletrodos para registro de ECG (item 4.1.5) e craniotomia para acesso ao DMH (item 4.1.3). As coordenadas utilizadas (AP: -3,1; LL: ±0,6; DV: -8,5mm) seguiram os dados de mapeamento do DMH realizados por Tanaka and McAllen, que observaram que a região superior e anterior do DMH produz respostas cardíacas mais proeminentes (Tanaka and McAllen, 2008). Durante os procedimentos cirúrgicos e experimentais, a temperatura corporal foi mantida em torno de 37° C por uma placa aquecedora e uma lâmpada incandescente.

Para obter os registro controle das respostas cardiovasculares, injeção de bicuculina metiodida (BMI - 40pmol/100nL) foi feita no DMH unilateral. O lado escolhido para primeira injeção central foi feito aleatoriamente. Após 40min ou até as variáveis retornarem a valores próximos ao basal, uma segunda injeção de BMI foi feita no DMH contralateral.

No intuito de reduzir o efeito da frequência cardíaca sobre a contratilidade (DiFrancesco, 1994) permitindo assim avaliar o efeito direto do DMH sobre a contratilidade cardíaca, foi injetado endovenosamente zatebradina (1mg/Kg), um bloqueador da corrente de marcapasso cardíaco (I*f*).

Após um período de aproximadamente 15 minutos para a estabilização do efeito da zatebradina, as injeções de BMI no DMH unilateral foram então repetidas como descrito anteriormente.

Para determinar se as respostas cronotrópicas e inotrópicas observadas envolviam ativação de receptores β-adrenérgicos cardíacos foi feita a injeção i.v. do bloqueador adrenérgico atenolol (2mg/Kg). Após um período de estabilização de aproximadamente 15 minutos, as injeções de BMI no DMH unilateral foram então repetidas, respeitando os intervalos previamente descritos. Ao final, com o intuito de conhecer a contribuição da pós-carga para o aumento na contratilidade, injeções i.v. do agonista adrenérgico fenilefrina (10μg/Kg) foram realizadas e seus efeitos sobre a relação PAM / LVdp/dt foram comparados com aqueles causados pela ativação do DMH unilateral. Ao final dos experimentos, os animais receberam nanoinjeção do corante alcian blue e foram perfundidos com paraformaldeído (PFA) à 4%. Os cérebros foram retirados, mantidos em PFA 4% por 24 horas, e em seguida transferidos para solução de sacarose 30% por 48 horas para posterior corte (40μm) em criostato. Para análise histológica, os cortes foram montados em lâminas previamente gelatinizadas e corados com vermelho neutro.

A sequência experimental do grupo 1 está esquematizada na Figura 4.

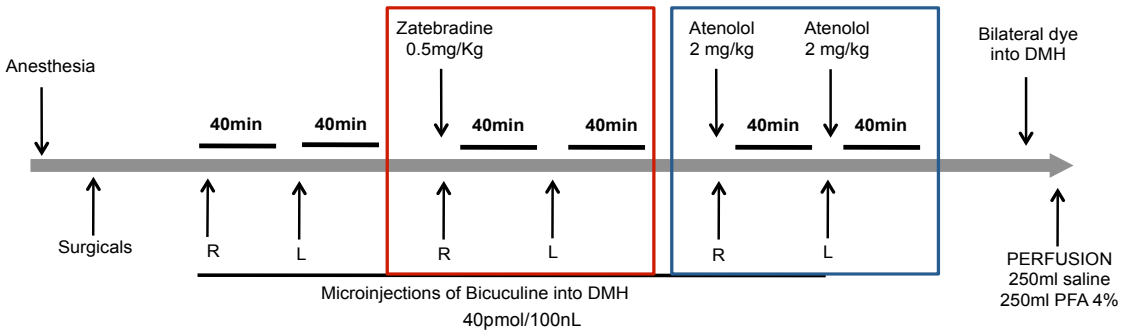


Figura 4. Sequência experimental do grupo 1 – objetivo específico 1.

4.2.1.3- DESENHO EXPERIMENTAL - *Grupo 2*

Outro grupo de animais (n=4), foi submetido a experimentos de "pacing cardíaco". Através de estímulos elétricos diretamente no coração a frequência cardíaca era controlada, predominando somente os efeitos da ativação unilateral do DMH sobre a contratilidade cardíaca. Após anestesia (como descrito no item 4.2.1.1), os animais foram submetidos aos procedimentos cirúrgicos de canulação de artéria e veia femorais (item 4.1.2), craniotomia (item 4.1.3), traqueostomia (item 4.1.1) e cateterização do ventrículo esquerdo cardíaco (item 4.1.6).

Os animais receberam injeções de zatebradina (1mg/Kg), um inibidor do marcapasso cardíaco, com o objetivo de reduzir a frequência cardíaca a valores próximos a 270bpm. Em seguida, os animais foram conectados à ventilação artificial e sofreram toracotomia para o posicionamento de eletrodos no átrio direito. A estimulação elétrica cardíaca foi ajustada para que a FC fosse mantida em aproximadamente 360bpm (pulsos retangulares de 1ms; voltagem entre 1 e 2V; frequência de 8,5Hz), como descrito anteriormente (Salo et al., 2009). As nanoinjeções unilaterais de BMI no DMH foram realizadas enquanto o "pacing" foi mantido durante 15 minutos.

A sequência experimental e de injeções centrais estão apresentadas na Figura 15 (painel A).

4.2.2- OBJETIVO ESPECÍFICO 2: AVALIAR A CONTRIBUIÇÃO DE RECEPTORES DE AMINOÁCIDOS EXCITATÓRIOS NOS LADOS DIREITO E ESQUERDO DO HIPOTÁLAMO DORSOMEDIAL E COLUNAS LATERAL E DORSOLATERAL DA SUBSTÂNCIA CINZENTA PERIAQUEDUTAL PARA AS RESPOSTAS SIMPATOEXCITATÓRIA, PRESSÓRICA E CRONOTRÓPICA CARDÍACA.

Todos os procedimentos experimentais realizados para alcançar o objetivo específico 2 estiveram de acordo com o recomendado pelo comitê de ética da Universidade Federal de Minas Gerais (UFMG), Brasil (protocolo CETEA n°136/2006). Esta série experimental foi realizada no Departamento de Fisiologia e Biofísica da Instituto de Ciências Biológicas da UFMG. Orientador: Prof. Dr. Marco Antônio Peliky Fontes.

Nestes experimentos que avaliaram a contribuição dos receptores de aminoácido excitatório do DMH e da l/dl PAG para a assimetria e lateralização nas respostas cardiovasculares, duas diferentes abordagens foram feitas: i) estimulação unilateral dos receptores de EAA do DMH e l/dl PAG em animais anestesiados e ii) inibição unilateral dos receptores de EAA do DMH e l/dl PAG em animais acordados expostos a estresse agudo.

4.2.2.1 – ANESTESIA - EXPERIMENTOS EM ANIMAIS ANESTESIADOS

Os animais receberam injeção intraperitoneal de uretana (1,4g/Kg). A eficácia da anestesia foi verificada pela ausência de reflexo de retirada ao

estímulo nociceptivo em um dos membros inferiores do animal. Adicionalmente, os animais receberam injeções subcutâneas de anestésico local com vasoconstritor (0,2mL de lidocaína 2% + epinefrina 1:50.000) na região em que posteriormente seria realizada craniotomia. Durante todos os procedimentos experimentais a temperatura dos animais foi mantida em torno de 37,5° C por uma placa aquecedora e uma lâmpada incandescente.

4.2.2.2- DESENHO EXPERIMENTAL – *Grupos 1, 2 e 3*

Para os grupos experimentais foram utilizados: i) animais que receberam injeção de NMDA no DMH direito e esquerdo (grupo 1); ii) animais que receberam injeção de NMDA nos lados direito e esquerdo da l/dl PAG (grupo 2); iii) animais que receberam injeção de NMDA nos lados direto e esquerdo da PAG após injeção de antagonista de receptores de EAA no DMH (grupo 3).

Os procedimentos experimentais foram semelhantes para os grupos 1 e 2 que receberam injeção de NMDA nos lados direito e esquerdo do DMH (n=5) ou da PAG (n=5), respectivamente. Subsequente à anestesia (item 4.2.2.1), foram realizados os procedimentos cirúrgicos de traqueostomia (item 4.1.1), canulação de artéria e veia femorais (item 4.1.2), localização do nervo renal (item 4.1.4) e craniotomia (item 4.1.3) para acesso de micropipetas de vidro ao DMH ou PAG. Depois de um período de estabilização mínimo de 20 minutos, injeções de NMDA (100pmol/100nl) foram realizadas unilateralmente. Subsequente a um período de 20 minutos ou até os parâmetros cardiovasculares retornarem ao basal, NMDA foi injetado no lado contralateral à primeira injeção. Após um período mínimo de 20 minutos, os animais receberam overdose de anestésico (i.v.) para extração do ruído da RSNA. A

amplitude das respostas à nanoinjeção de NMDA unilateral foram comparadas com aquelas vistas contralateralmente.

No terceiro grupo experimental (n=5), o antagonista de receptores de EAA, ácido quinurênico (Kyn) foi injetado no DMH esquerdo para avaliar se uma sinapse ascendente oriunda da PAG se conecta ipsilateralmente ao DMH. Subsequente à anestesia (item 4.2.2.1), foram realizados os procedimentos cirúrgicos de traqueostomia (item 4.1.1), canulação de artéria e veia femorais (item 4.1.2), localização do nervo renal (item 4.1.4) e craniotomia (item 4.1.3) para acesso de micropipetas de vidro ao DMH e à PAG. Após um período de estabilização mínimo de 20 minutos, injeções de Kyn (1nmol/100nl) foram realizadas no DMH esquerdo. Um período de 20 minutos ou até a estabilização dos parâmetros cardiovasculares, foi aguardado para que injeções de NMDA (100pmol/100nl) foram realizadas na PAG unilateralmente. Após os mesmos 20 minutos, NMDA foi injetado no lado contralateral. Após um período mínimo de 20 minutos, os animais receberam overdose de anestésico (i.v.) para extração do ruído da RSNA. A amplitude das respostas à nanoinjeção de NMDA unilateral foi comparada com aquela vista contralateralmente. Além disso, a amplitude das respostas evocadas pela estimulação unilateral e contralateral da PAG foram comparadas entre os grupos de animais que receberam Kyn no DMH esquerdo e o grupo que recebeu somente estimulação unilateral da PAG.

Após eutanásia, os animais dos três grupos experimentais receberam nanoinjeção do corante alcian blue, foram perfundidos com PFA 4%. Os cérebros foram retirados, mantidos em PFA 4% por 24 horas e em seguida transferidos para solução de sacarose 30% por 48 horas para posterior corte

(40μm) em criostato. Para análise histológica, os cortes foram montados em lâminas previamente gelatinizadas e corados com vermelho neutro.

4.2.2.3 – ANESTESIA - EXPERIMENTOS EM ANIMAIS NÃO ANESTESIADOS

Os animais receberam injeção intraperitoneal de tribromoetanol (250mg/Kg) para procedimento cirúrgico de craniotomia (item 4.1.3), posicionamento das cânulas-guia bilaterais no DMH ou na l/dl PAG e fixação com acrílico odontológico. No intuito de aliviar a dor e evitar processos infecciosos advindos do procedimento cirúrgico, os animais receberam injeções intramusculares de antibióticos (Pentabiotico – 0,2mL) e analgésico / antiinflamatório (Banamine Pet® – 1,1mg/Kg) após a cirurgia.

Após 5 dias, os animais foram novamente anestesiados com tribromoetanol (250mg/Kg, i.p.) para realização do procedimento cirúrgico de canulação de artéria e veia femorais (item 4.1.2).

4.2.2.4- DESENHO EXPERIMENTAL – *Grupos 4 e 5*

Após 24 horas de recuperação do procedimento cirúrgico de canulação, iniciou-se os registros. Para as nanoinjeções no sistema nervoso central nestes grupos de animais não-anestesiados, utilizaram-se agulhas odontológicas gengivais (30G curta – Becton Dickson Ind.). Em seguida a cânula foi conectada a uma das extremidades de um polietileno PE 10, preenchido com a droga a ser injetada. À outra extremidade do PE 10 conectou-se uma seringa Hamilton (5μL – Reno, Nevada, USA) preenchida com água deionizada e posicionada a um suporte para administração exata de um volume fixo de 100nL. As injeções foram realizadas unilateralmente.

Os animais foram expostos ao estresse por jato de ar em 3 dias consecutivos, como descrito a seguir. No primeiro dia, depois de um período de estabilização mínimo de 30 minutos, os animais receberam nanoinjeção de ácido quinurênico (Kyn - 1nmol/100nl) no DMH (Grupo 4) ou l/dl PAG (Grupo 5) unilateral e em seguida foram colocados em um contensor de acrílico. Após 10 minutos, os animais foram submetidos ao estresse por jato de ar (10L/min) durante 10 minutos. Em seguida, o contensor foi aberto e foi aguardado um período mínimo (20min) de observação/estabilização dos parâmetros cardiovasculares. No segundo dia experimental, os animais receberam nanoinjeção de Kyn no lado contralateral àquele do primeiro dia experimental DMH (Grupo 4) ou l/dl PAG (Grupo 5) e então repetiram-se os procedimentos experimentais de exposição ao estresse por jato de ar acima descritos. No terceiro e último dia experimental, todos os animais receberam injeção bilateral de veículo (NaCl 0,9% - 100nL) e foram também submetidos ao mesmo estresse por jato de ar. Ao final de todos os experimentos os animais foram anestesiados (tribromoetanol 250mg/Kg), receberam nanoinjeção do corante alcian blue. Em seguida, os animais foram perfundidos (PFA 4%) e tiveram seus cérebros retirados, mantidos em PFA 4% por 24 horas, e transferidos para solução de sacarose 30% por 48 horas para posterior corte (40µm) em criostato. Para análise histológica, os cortes foram montados em lâminas previamente gelatinizadas e corados com vermelho neutro.

A sequência experimental realizada nos grupos de animais não-anestesiados está representada Figura 5.

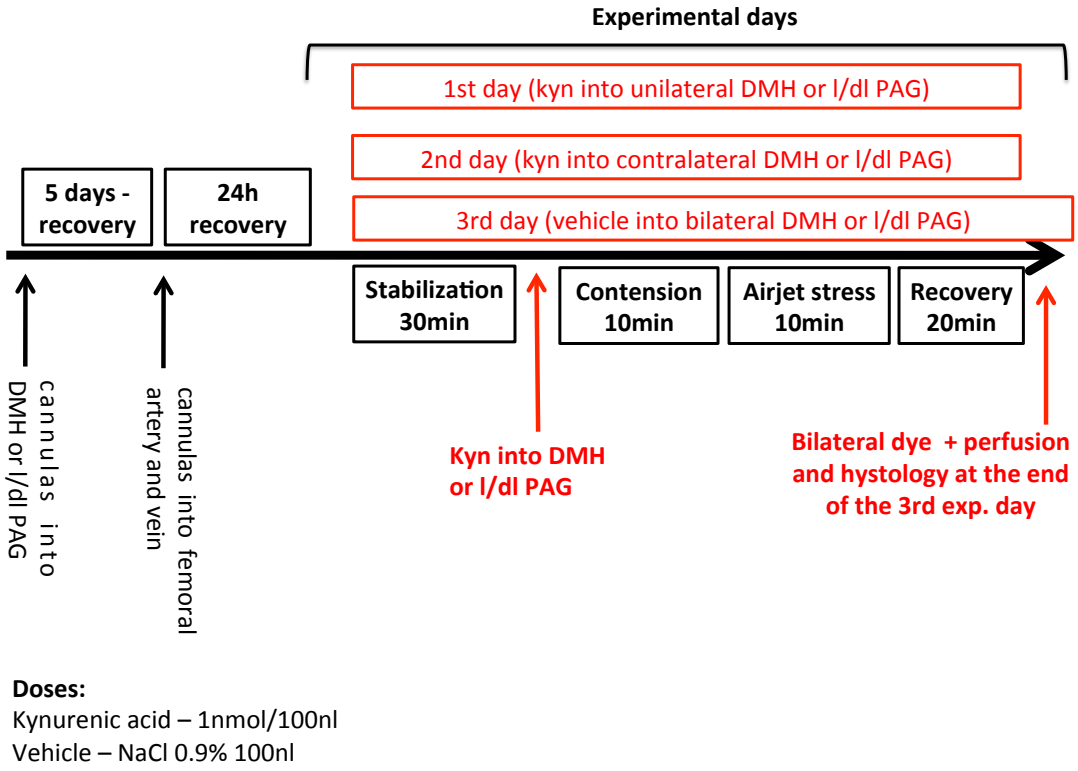


Figura 5. Sequência experimental realizada nos grupos de animais não-anestesiados (Grupos 4 e 5 – objetivo específico 2).

4.2.2.5- DESENHO EXPERIMENTAL – *Grupo 6*

Para verificar as projeções anatômicas entre o DMH e PAG, os animais foram anestesiados com tribromoetanol conforme descrito no item 4.2.2.3 e submetidos a pequenas craniotomias (item 4.1.3) para posicionamento de micropipetas de vidro no DMH direito e na l/dl PAG esquerda em aparelho estereotáxico. As coordenadas utilizadas foram: DMH (3,2 mm posterior, +0,6 mm lateral, 8,5 mm ventral); l/dl PAG (6,8mm posterior, -0,6mm lateral, 4,8mm ventral). Os procedimentos foram realizados de acordo com o descrito previamente por Apps e Ruigork (Apps and Ruigrok, 2007). Em um animal foi injetado 100nL de microesferas verdes e vermelhas [Retrobeads^{TM} (Luma Fluor)] não diluídas no DMH direito e na l/dl PAG esquerda, respectivamente.

Tais esferas são traçadores neuronais retrógrados monossinápticos, ou seja, viajam do terminal sináptico onde foram injetadas até o corpo celular dos neurônios que se projetam diretamente ao sítio de injeção. Ao contrário da projeção anatômica PAG-DMH, a projeção DMH-PAG é extensivamente descrita. Assim, para assegurar se havia mesmo uma projeção anatômica ascendente que parte da PAG até o DMH, em outro animal, microesferas verdes foram injetadas somente no DMH direito. Um período de 7 dias, suficiente para visualizar as Retrobeads nos corpos celulares de neurônios, foi aguardado até que se realizassem os procedimentos de perfusão e histologia descritos adiante. Os animais foram novamente anestesiados (item 4.2.2.3) e submetidos a perfusão transcardíaca com 250mL de salina (NaCl 0,9%) heparinizada (5000U.I.) seguida de 250mL de PFA 4%. Os cérebros foram removidos, mantidos em PFA 4% por 24 horas e então transferidos para uma solução de sacarose 30%, onde permaneceram durante as 48 horas seguintes. Antes de serem cortados (40µm) em criostato, durante o congelamento os cérebros receberam uma marca (orifício – *"fiducial mark"*) produzida pela inserção da ponta de uma agulha em uma região distante do DMH e da PAG, no intuito de identificar um dos lados dos hemisférios. Os cortes foram montados em lâminas previamente silanizadas e foi aguardado um período de 24 horas para secagem. As lâminas foram então cobertas pelo meio de montagem "anti-sombra" ProLong Gold® (Invitrogen). Análises em microscópio de fluorescência foram feitas utilizando excitação de 460 e 530nm / emissão de 505 e 590nm para as esferas verde e vermelha (fluoresceína e rodamina), respectivamente.

A Figura 6 esquematiza a sequência experimental descrita acima. Estão demonstradas, com suas respectivas cores, os sítios (pequenos círculos preenchidos em verde ou vermelho) onde as Retrobeads foram injetadas e os locais (quadrados com borda verde ou vermelha) onde foi analisada a possível marcação retrógrada.

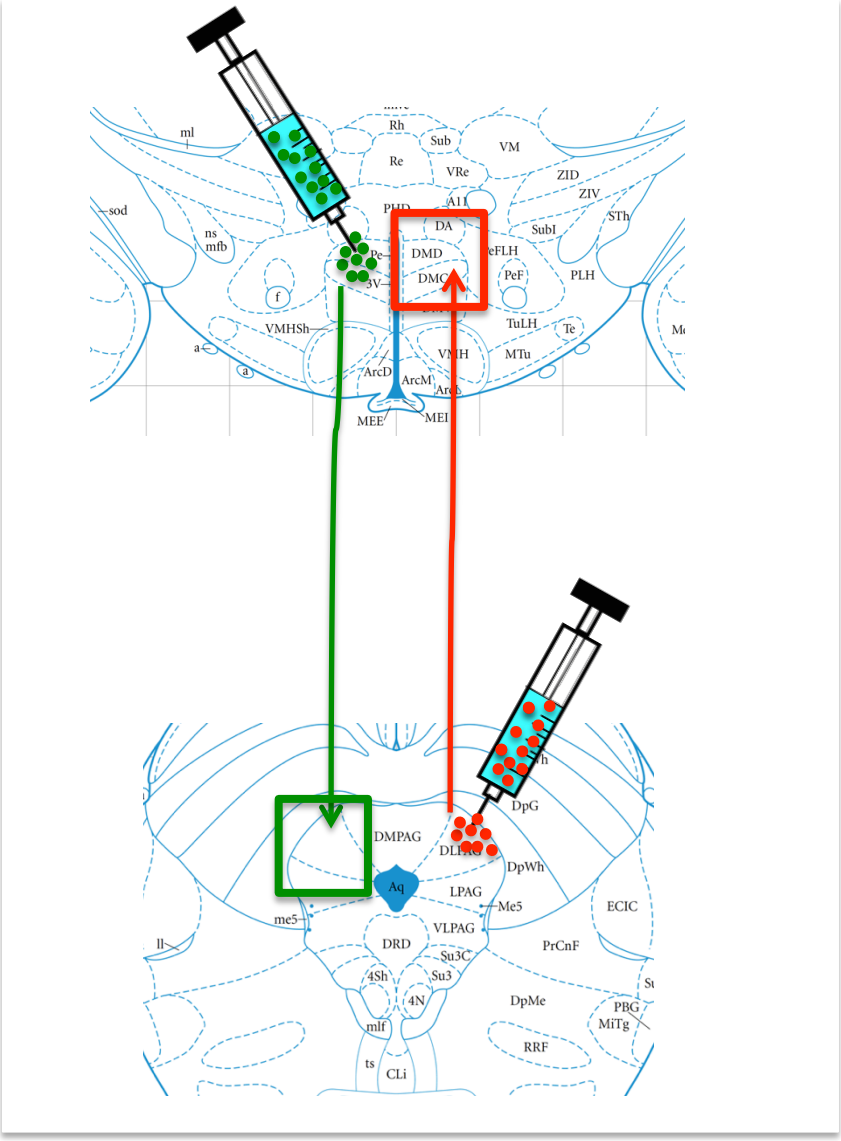


Figura 6. Esquema demonstrando os sítios de injeção e locais onde foram analisadas as marcações retrógradas (quadrados). Adaptado do Atlas (Paxinos and Watson, 2005).

4.2.3- OBJETIVO ESPECÍFICO 3: AVALIAR O ENVOLVIMENTO DA *RAPHE PALLIDUS* NAS RESPOSTAS CRONOTRÓPICAS E INOTRÓPICAS CARDÍACAS PROVOCADAS PELA ESTIMULAÇÃO DOS LADOS DIREITO E ESQUERDO DO HIPOTÁLAMO DORSOMEDIAL

Todos os procedimentos experimentais realizados estiveram de acordo com o recomendado pela instituição envolvida: Oregon Health and Sciences University - OHSU (Portland/OR - USA). Esta série experimental foi realizada no Department of Neurological Surgery da Oregon Health and Sciences University. Doutorado sanduíche (CNPq – 201351/2010-2) de janeiro a julho de 2011. Orientador: Prof. Shaun F. Morrison, PhD.

4.2.3.1- ANESTESIA

Os animais foram anestesiados com isofluorano. Após terem sua artéria e veia femorais canuladas (para medida de PAM e FC), receberam lentamente a injeção endovenosa da solução uretana 500mg + cloralose 40mg / mL (aproximadamente 3mL/Kg), na medida em que a inalação de isofluorano foi gradativamente reduzida. Suplementações de uretana foram dadas por via intravenosa (i.v.) quando necessárias.

4.2.3.2- DESENHO EXPERIMENTAL – *Grupo 1*

Após anestesia, os animais (n=8) foram submetidos aos procedimentos cirúrgicos de traqueostomia (item 4.1.1), canulação de artéria e veia femorais (item 4.1.2), cateterização do ventrículo esquerdo cardíaco (item 4.1.6), posicionamento de eletrodos para registro de ECG (item 4.1.5), posicionamento

de sensores de temperatura corporal (TC), caudal e no tecido adiposo marrom (BAT) (item 4.1.7) e craniotomia para acesso ao DMH e a RP (item 4.1.3).

Os animais receberam injeção endovenosa do bloqueador muscular tubocurarina (inicialmente 0,6mg; suplementações de 0,2mg/h). Os parâmetros ventilatórios foram de acordo com o descrito anteriormente (Cao and Morrison, 2003): 100% de O_2 a um volume/minuto de 140 a 180mL; frequência respiratória foi ajustada para que o CO_2 expirado fosse mantido entre 3,5% e 4%). O analisador de gases utilizado foi o CapStar 100 – Carbon Dioxide Analyzer.

Foram feitas nanoinjeções de NMDA (0,2mM – 60nL) na RP iniciadas a -3,0mm do lambda e movidas caudalmente (-0,5mm) de forma gradual até que se identificasse o local de injeção que produzisse maior resposta taquicárdica e termogênica. É importante salientar que a dose de NMDA escolhida não causa neurotoxicidade após repetidas injeções, como demonstrado anteriormente (Nakamura and Morrison, 2008). Entre cada nanoinjeção foi aguardado um período mínimo de 15min ou até que as varáveis retornassem a níveis basais.

NMDA (0,2mM – 60nL) foi nanoinjetado no DMH unilateral para que se obtivesse um padrão de respostas cardiovasculares. O lado para primeira estimulação do DMH foi escolhido aleatoriamente e a segunda injeção foi realizada no lado contralateral à primeira. Em seguida, o agonista $GABA_A$ muscimol (12mM – 60nL) foi injetado no local mais responsivo da RP, seguido por repetidas estimulações do DMH unilateral com NMDA (0,2mM – 60nL). Para determinar se as respostas cronotrópicas e inotrópicas observadas envolviam ativação de receptores β-adrenérgicos cardíacos, o bloqueador

adrenérgico atenolol (2mg/Kg) foi administrado endovensamente. Após um período de aproximadamente 15 minutos para a estabilização do efeito do atenolol, as injeções de BMI no DMH unilateral foram então repetidas, respeitando os intervalos previamente descritos. Ao final dos experimentos, os animais receberam nanoinjeção microesferas fluorescentes de Poliestireno (Fluo-Spheres, models F8797, F8801, or F8803; Molecular Probes, Eugene OR) de diferentes cores na RP e no DMH. Os animais foram perfundidos utilizando 200mL de PFA 4%. Os cérebros foram retirados, mantidos em PFA 4% por 24 horas, e em seguida transferidos para solução de sacarose 30% por 48 horas para posterior corte (40µm) em criostato. Para análise histológica, os cortes foram montados em lâminas previamente gelatinizadas e analisados em microscópio de fluorescência.

A figura 7 apresenta a sequência experimental deste protocolo.

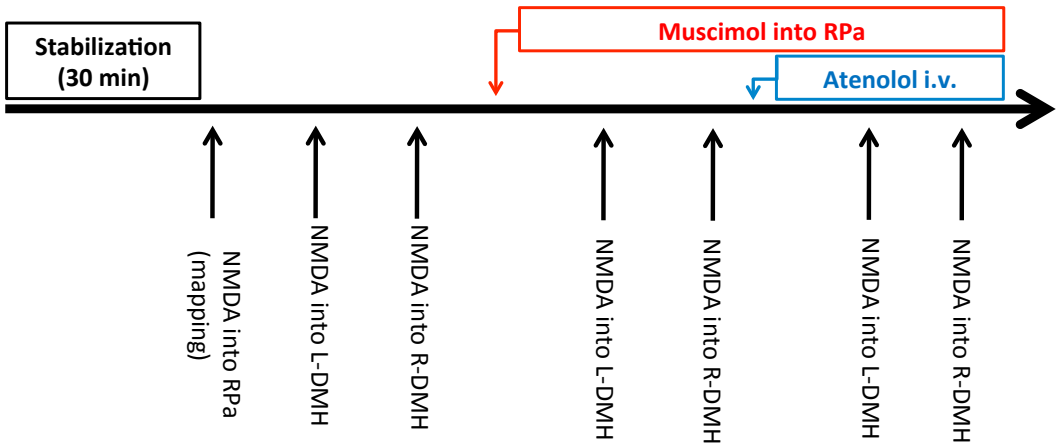


Figura 7. Sequência experimental usada para avaliar a influência da RP nas assimetria no controle cardíaco pelo DMH unilateral.

4.3- ANÁLISE DOS DADOS

Esta sub-seção também foi dividida de acordo com cada objetivo específico, seguindo os procedimentos experimentais descritos na sub-seção anterior. Em geral, os dados estão apresentados como valores absolutos basais e máximos, e como a variação da resposta (Δ), obtida pela subtração entre os valores basais de um animal e a resposta máxima vista no mesmo animal.

4.3.1 – OBJETIVO ESPECÍFICO 1: COMPARAR AS RESPOSTAS ELETROCARDIOGRÁFICAS, CRONOTRÓPICAS E INOTRÓPICAS CARDÍACAS PROVOCADAS PELA DESINIBIÇÃO DOS LADOS DIREITO E ESQUERDO DO HIPOTÁLAMO DORSOMEDIAL

Os valores basais foram obtidos a partir de médias extraídas dos 5 minutos anteriores às injeções de BMI. Em seguida os valores médios máximos de resposta em cada parâmetro foram extraídos do período compreendido entre o momento da injeção no DMH e o 15° minuto pós-injeção. Após detectado um período onde as respostas cardiorrespiratórias evocadas pelo DMH atingiam e mantinham valores máximos, foi selecionado um período de 1 minuto, considerado como uma média do pico de resposta. O número de batimentos ectópicos e suspiros foram contados por 10 minutos imediatamente após injeção no DMH. Para avaliar os efeitos da pós-carga sobre a contratilidade cardíaca, nós estabelecemos um índice, produto da divisão entre a variação (max-min) nas respostas de LVdP/dtpeak e MAP [AP dependence

contractility index = (max-min LVPdp/dtpeak) / (max-min MAP)] – ver figura 13 da seção de resultados.

4.3.2 – OBJETIVO ESPECÍFICO 2: AVALIAR A CONTRIBUIÇÃO DE RECEPTORES DE AMINOÁCIDOS EXCITATÓRIOS NO HIPOTÁLAMO DORSOMEDIAL E COLUNAS LATERAL E DORSOLATERAL DA SUBSTÂNCIA CINZENTA PERIAQUEDUTAL PARA AS RESPOSTAS SIMPATOEXCITATÓRIA, PRESSÓRICA E CRONOTRÓPICA CARDÍACA.

Nos experimentos com animais anestesiados (grupos 1 e 2), os valores basais foram obtidos através de médias extraídas dos 5 minutos anteriores ao início dos procedimentos experimentais. Após injeções centrais de NMDA, o período compreendido entre o momento da injeção e o 5° minuto pós-injeção foi considerado para amostragem das respostas máximas.

No grupo de animais não-anestesiados, os valores de FC e PAM basais (anteriores à entrada do animal no contensor), após a entrada no contensor, durante o estresse por jato de ar, subsequente ao fim do jato de ar e ao fim do experimento foram avaliados considerando os valores máximos das respostas cronotrópicas de cada período. Os valores basais foram obtidos através de médias extraídas dos 5 minutos anteriores ao início dos procedimentos experimentais. Períodos de 30 segundos foram amostrados a cada 2 minutos.

4.3.3 – OBJETIVO ESPECÍFICO 3: AVALIAR O ENVOLVIMENTO DA RAPHE PALLIDUS NAS RESPOSTAS ASSIMÉTRICAS CRONOTRÓPICAS E INOTRÓPICAS CARDÍACAS PROVOCADAS PELA ESTIMULAÇÃO DOS LADOS DIREITO E ESQUERDO DO HIPOTÁLAMO DORSOMEDIAL.

Os valores basais foram obtidos através de médias extraídas dos 5 minutos anteriores ao início dos procedimentos experimentais. Para avaliar o curso temporal das respostas, períodos de 10 segundos foram amostrados a cada 30 segundos durante todo o experimento. O período compreendido entre o momento da injeção e o 5° minuto pós-injeção foi considerado para amostragem das respostas máximas causadas pelas injeções de NMDA. Após injeção central de muscimol na RP e endovenosa de atenolol, o período compreendido entre o momento da injeção e o 10° minuto pós-injeção foi considerado para amostragem das respostas máximas.

4.4 – ANÁLISE ESTATÍSTICA

Os testes estatísticos utilizados foram, *Student t test,* e as análises de variância *One-way* e *Two-way* ANOVA (fatores droga e tempo) seguidas de pós-testes de Newman-Keuls e Bonferroni, respectivamente. Os testes aplicados em cada grupo de resultados estão descritos nas respectivas legendas. O nível de significância considerado foi $P<0,05$ e todos os valores estão apresentados como média ± erro padrão da média (EPM).

5 – RESULTADOS

5.1- RESPOSTAS CRONOTRÓPICAS E INOTRÓPICAS CARDÍACAS PROVOCADAS PELA ATIVAÇÃO DOS LADOS DIREITO E ESQUERDO DO HIPOTÁLAMO DORSOMEDIAL (ver anexo 3)

A Figura 8 exemplifica um corte de cérebro ao nível do DMH onde as nanoinjeções foram feitas.

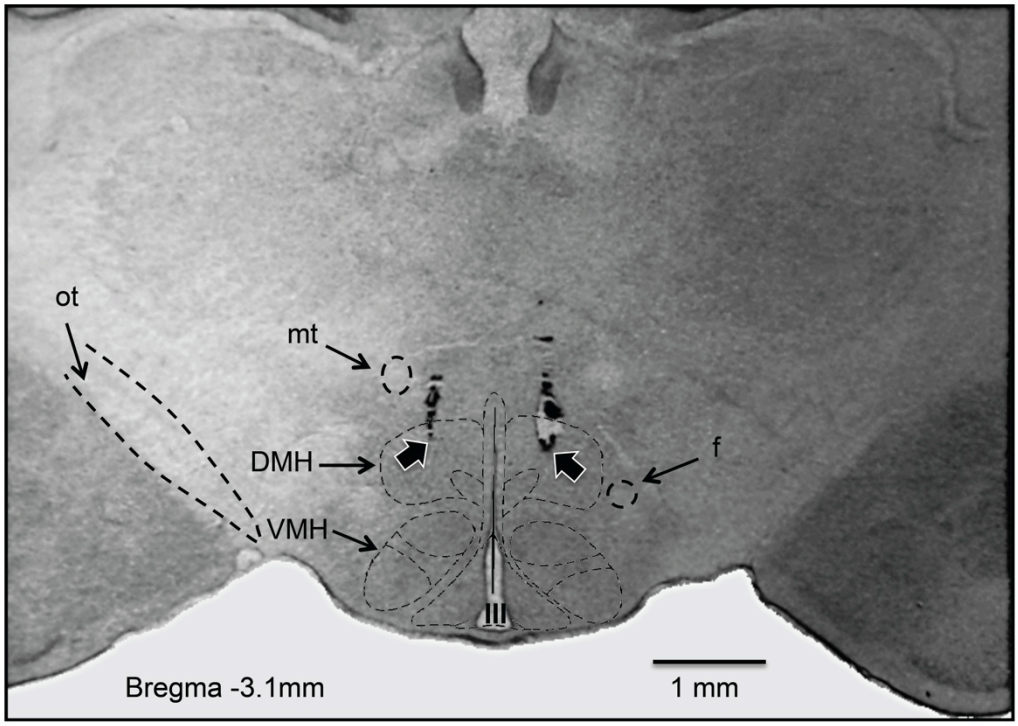


Figura 8. Representação esquemática de corte histológico ao nível do DMH, onde as injeções de BMI foram realizadas. Setas negras indicam sítios de injeção, com deposição do corante alcian blue. DMH: hipotálamo dorsomedial; VMH: hipotálamo ventromedial; mt: trato mamilar; f: fornix; ot: trato óptico.

Nanoinjeções unilaterais de BMI no DMH causaram aumentos transientes na FC, PAM, LVP, LVdp/dt e na frequência respiratória. Foi observado também que durante a taquipnéia, houveram eventos respiratórios, interpretados como suspiros ou “ciclos respiratórios” aumentados (ver exemplo da Figura 9 e discussão). As respostas evocadas pelo DMH foram, de maneira geral, evidentes a partir do primeiro minuto após injeção de BMI no DMH, alcançando valores máximos entre 5 e 7 minutos e então retornando gradualmente ao nível basal dentro de 25 a 30 minutos. Respostas cronotrópicas (FC) e inotrópicas positivas (LVPpeak e LVdp/dt peak) provocadas pela estimulação do DMH direito foram significativamente maiores comparadas àquelas causadas pela injeção de BMI no DMH esquerdo (Figura 9, gráficos de barras). A ativação do DMH unilateral não foi capaz de alterar os valores de pressão diastólica final no ventrículo esquerdo. De maneira diferente ao observado para os outros parâmetros cardiovasculares, a magnitude das respostas pressora e taquipnéica não diferiu entre os lados do DMH. Além disso, não houve diferença entre o número de suspiros avaliados durante 10 minutos após injeção de BMI no lados direito e esquerdo do DMH.

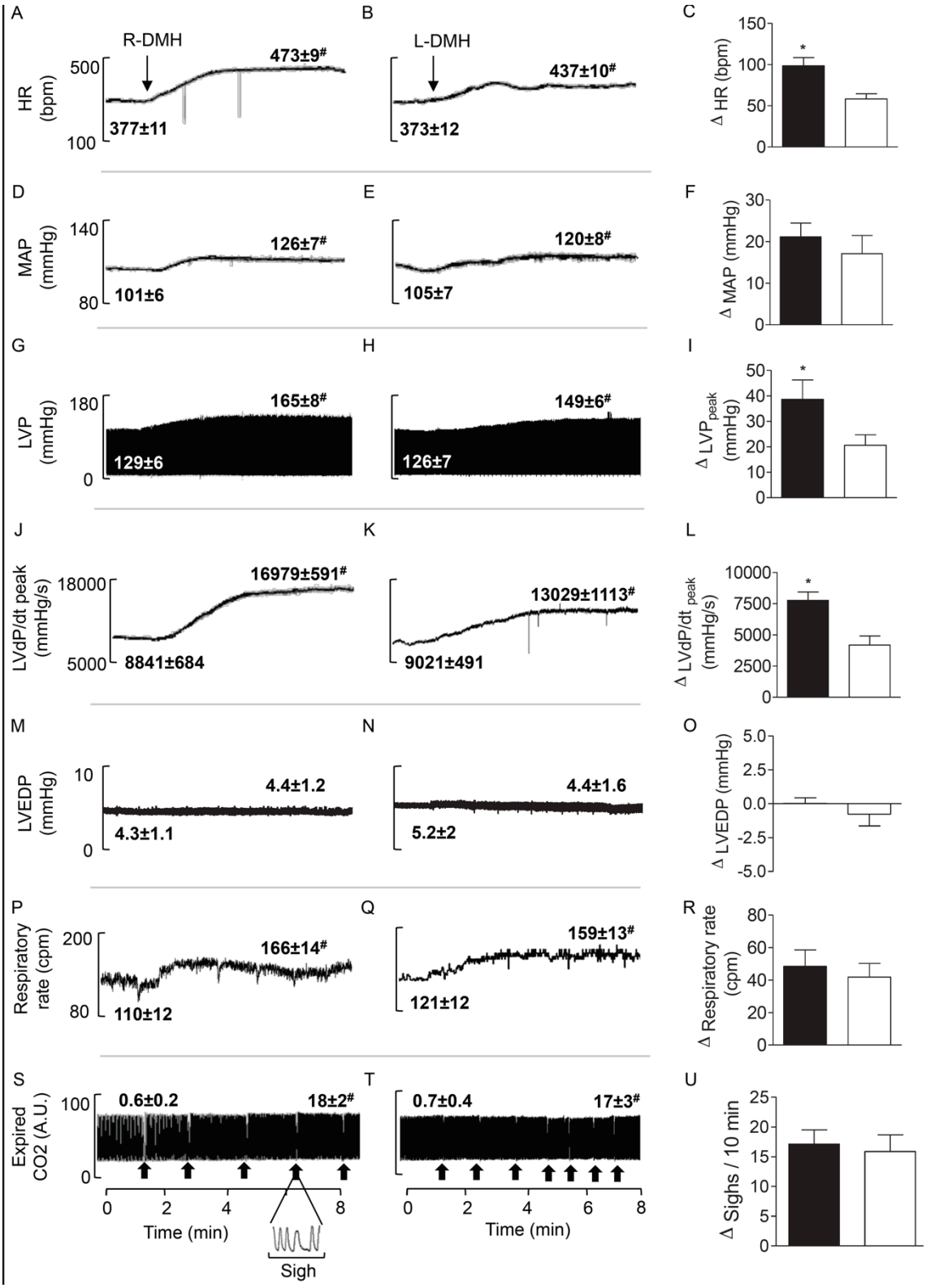


Figura 9. Valores basais e respostas cardiorrespiratórias máximas evocadas pela nanoinjeção de BMI no lados direito e esquerdo do DMH (L- e R-DMH, respectivamente). HR: frequência cardíaca (A,B,C); MAP: pressão arterial média (D,E,F); LVP: pressão no ventrículo esquerdo (G,H); LVP peak: valores de pico de pressão ventricular esquerda (I); LVdP/dt peak: valores de pico da derivativa da pressão ventricular esquerda (J,K,L); LVEDP:

valores de pressão ventricular ao fim da diástole (M,N,O); Respiratory rate: frequência respiratória (P,Q,R); Expired CO_2: dióxido de carbono expirado (S,T); Sighs: suspiros ou ciclos respiratórios aumentados (U). Time (min): tempo em minutos. # *P*<0,05 *vs*. Basal; * *P*<0,05 (R- *vs.* L-DMH). ANOVA two-way e Student t test.

A Figura 10 apresenta o número de batimentos ectópicos ventriculares e supraventriculares medidos durante 10 minutos após a administração de BMI no DMH.

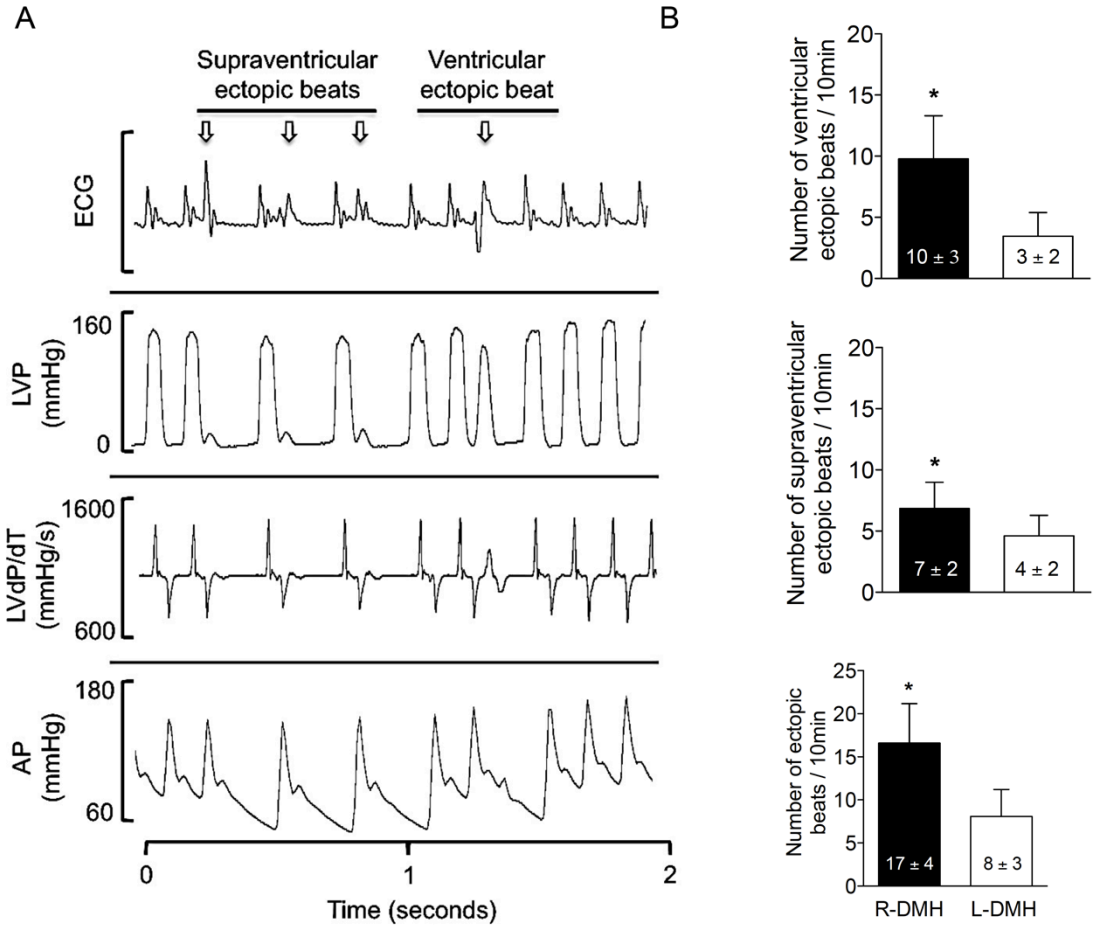


Figura 10. Número de batimentos ectópicos ventriculares (VEB – ventricular ectopic beats) e supraventriculares (SVEB – supraventricular ectopic beats) contados em um período de 10 minutos subsequente a nanoinjeção de BMI nos lados direito e esquerdo do DMH. As setas indicam os sinais típicos eletrocardiográficos dos VEBs e dos SVEBs e suas consequências nos sinais de pressão arterial periférica (AP), pressão ventricular esquerda (LVP) e sua derivativa (LVdP/dt). **P*<0,05 (R- vs. L-DMH). ANOVA two-way.

De acordo com os dados de taquicardia obtidos, a estimulação do DMH causou redução dos intervalos RR do ECG, com uma diferença mais pronunciada após nanoinjeção no DMH direito (DMH-D: -28±9 *vs.* DMH-E: -15±4 msec; *P*<0,05). Interessantemente, a desinibição do DMH causou batimentos ectópicos ventriculares (Figura 10), com incidência significativamente maior após desinibição do DMH direito (D= 16±5 *vs*. E= 8±3 batimentos ectópicos/10min, *P*<0,05). Tais batimentos ectópicos foram ausentes no período basal pré-injeção. Os batimentos ectópicos foram diferenciados entre batimentos ectópicos ventriculares (VEB) e supraventriculares (SVEB). A ativação do DMH direito provocou um número maior de ambos, VEBs e SVEBs (Figura 10).

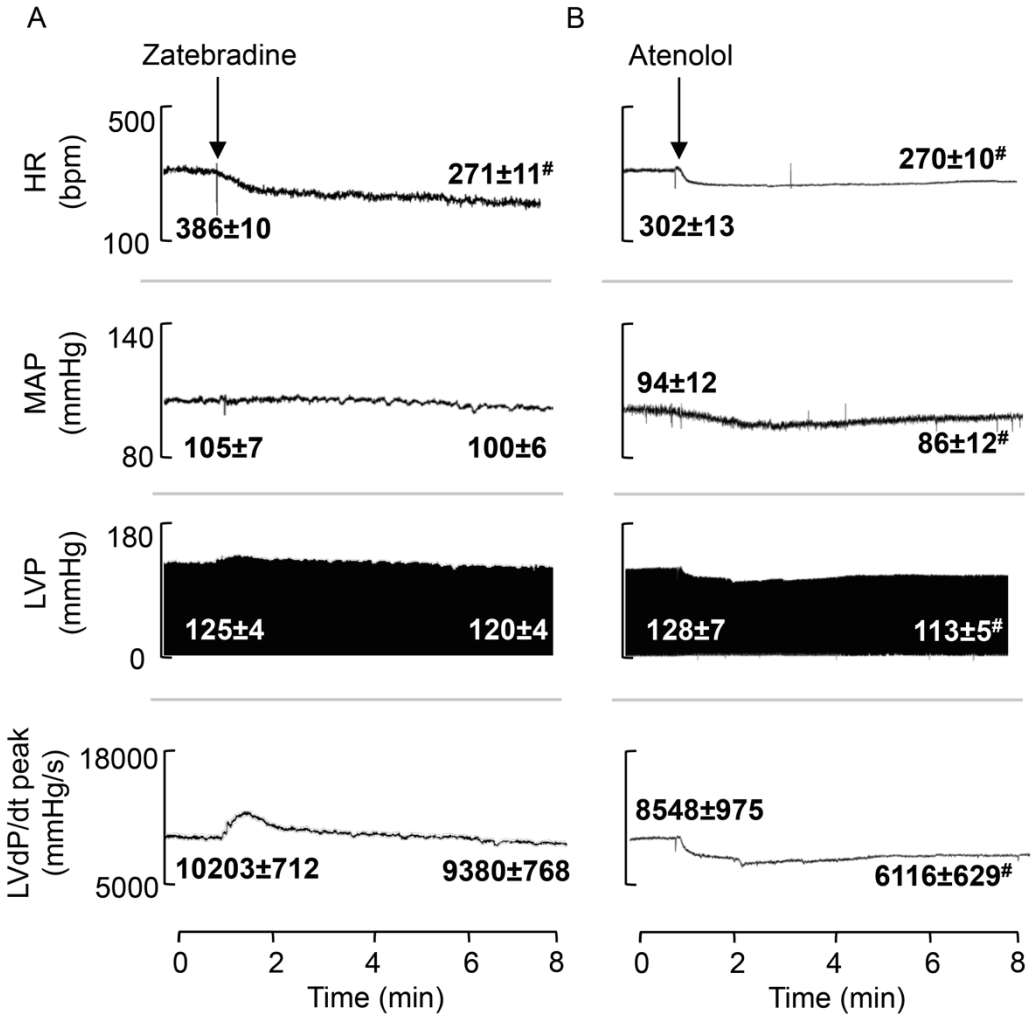


Figura 11. Respostas cardiovasculares provocadas pela injeção endovenosa de zatebradina (coluna A) e atenolol (coluna B). HR: frequência cardíaca; MAP: pressão arterial média; LVP: pressão ventricular esquerda; LVdP/dt peak: valores de pico da derivada da pressão ventricular esquerda; Time: tempo em minutos. # *P*<0,05 *vs.* Basal. Student t test.

O bloqueio farmacológico do marcapasso cardíaco, pela administração endovenosa de zatebradina, reduziu a FC (∆FC = -104±4bpm, *P*<0,05 *vs.* basal) sem alterar os outros parâmetros medidos. Entretanto, durante a fase inicial da queda de FC foi observado um aumento transiente na medida de contratilidade cardíaca ($\Delta LVdP/dt_{peak}$ = 1293±496mmHg/s *vs.* basal; *P*<0,05). Este efeito durou menos de 3 minutos e após o 5° minuto, os parâmetros se mostraram novamente estáveis (Figura 11, painel A).

A Figura 12 apresenta as respostas cardiovasculares causadas pela ativação dos lados direito e esquerdo do DMH durante bloqueio farmacológico do marcapasso cardíaco com zatebradina.

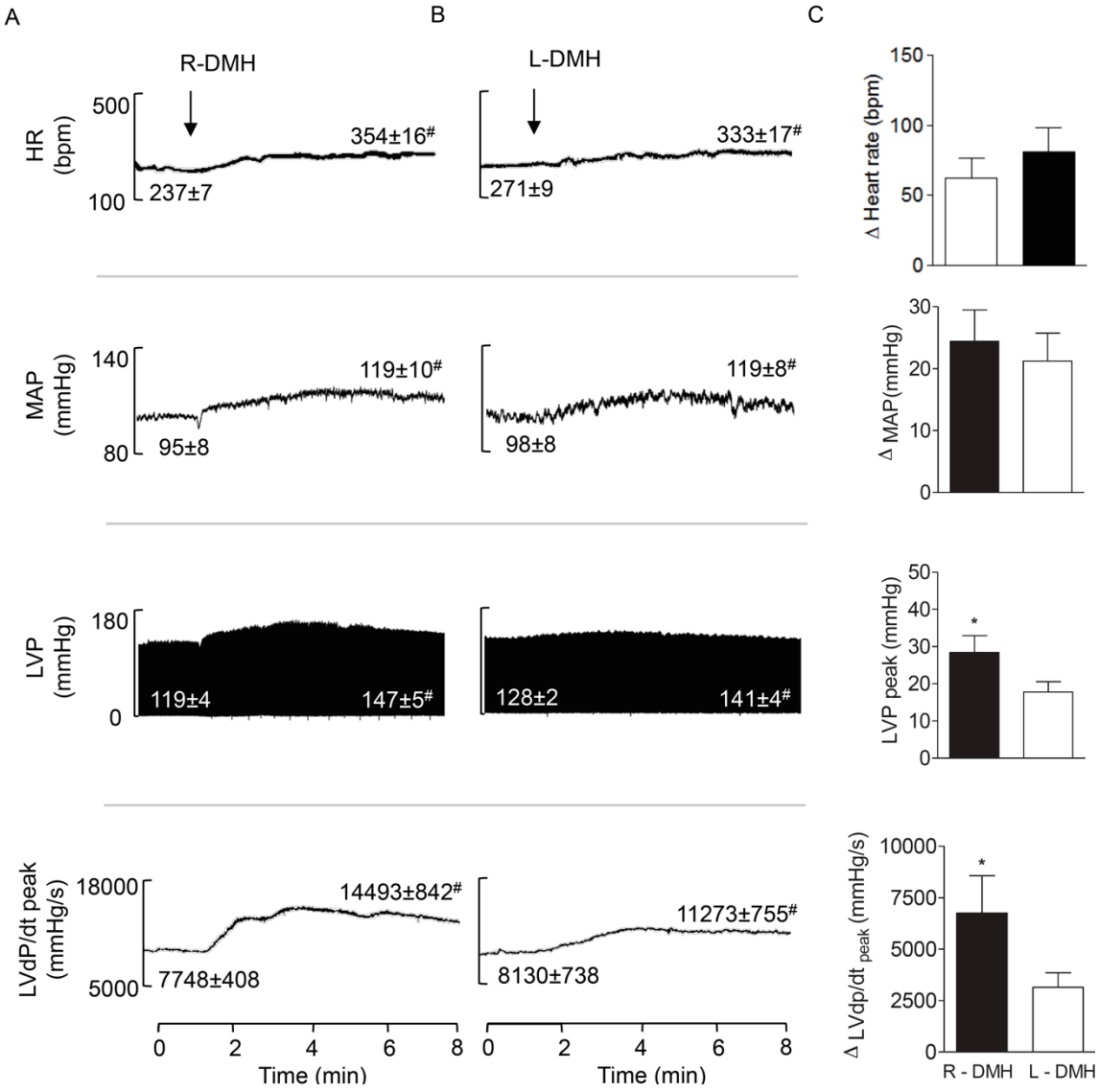


Figura 12. Valores absolutos basais e respostas cardiovasculares máximas provocadas pela nanoinjeção de BMI no DMH direito (coluna A; R-DMH) e esquerdo (coluna B; L-DMH) durante efeito de zatebradina. A coluna (C) apresenta as mudanças máximas evocadas pelo DMH. HR: frequência cardíaca; MAP: pressão arterial média; LVP: pressão ventricular esquerda; LVdP/dt peak: valores de pico da derivada da pressão ventricular esquerda; Time: tempo em minutos. # P<0,05 *vs.* Basal; * P<0,05 (R- *vs.* L-DMH). ANOVA two-way e Student t test.

A ativação do DMH unilateral durante os efeitos da zatebradina causou aumentos de FC e contratilidade cardíaca, novamente com predominância do lado direto do DMH (Figura 12). A amplitude das alterações de PAM, LVP e $LVdP/dt_{peak}$ não diferiram daquelas obtidas antes do efeito da zatebradina [(∆PAM E = 17±4 *vs*. 21±5 e D= 21±3 *vs*. 24±5mmHg); (∆LVP E = 21±4 *vs*. 18±3 e D = 39±8 *vs*. 29±5mmHg); ($\Delta LVdP/dt_{peak}$ E = 4188±730 *vs*. 3143±706 e D = 7769±672 *vs*. 6745±1816mmHg/s); antes *vs*. depois da zatebradina, respectivamente]. A magnitude das respostas taquipnéicas não diferiu da condição pré-zatebradina e foi similar para ambos os lados da injeção no DMH (∆RR E= 47±8 *vs*. 43±9 e D= 54±9 *vs*. 47±8cpm; antes *vs*. após zatebradina, respectivamente). Também não houve diferença na incidência de suspiros ("sighs") provocado pela estimulação dos lados direito e esquerdo do DMH (∆ = 16±2 *vs*. 17±2 sighs/10min, E *vs*. D respectivamente).

Por outro lado, quando comparada a amplitude das respostas controle, a FC máxima alcançada após ativação unilateral do DMH foi menor durante o efeito da zatebradina (E= 437±9 *vs*. 333±16 e D= 472±8 *vs*. 355±17bpm; *P*<0,05 antes *vs*. após zatebradina, respectivamente). O bloqueio do marcapasso cardíaco também reduziu a incidência de batimentos ectópicos supraventriculares desencadeados pela ativação do DMH direito (D=7±2 *vs.* 2±1 SVEB/10min; *P*<0,05); em contraste, a incidência de batimento ectópicos ventriculares não foi afetada (E= 3±2 *vs*. 2±1 e D= 9±4 *vs*. 6±2 VEB/10min; antes *vs*. após zatebradina, respectivamente). Nenhuma diferença foi observada na amplitude das alterações de frequência cardíaca causada pela ativação dos lados direito e esquerdo do DMH comparando antes e após o

efeito da zatebradina (ΔFC E = 58±6 *vs*. 62±14 e D= 99±10 *vs*. 90±18bpm; antes *vs*. após zatebradina, respectivamente).

A Figura 13 apresenta as respostas cardiovasculares causadas pela nanoinjeção de BMI dos lados direito e esquerdo do DMH durante efeito do atenolol.

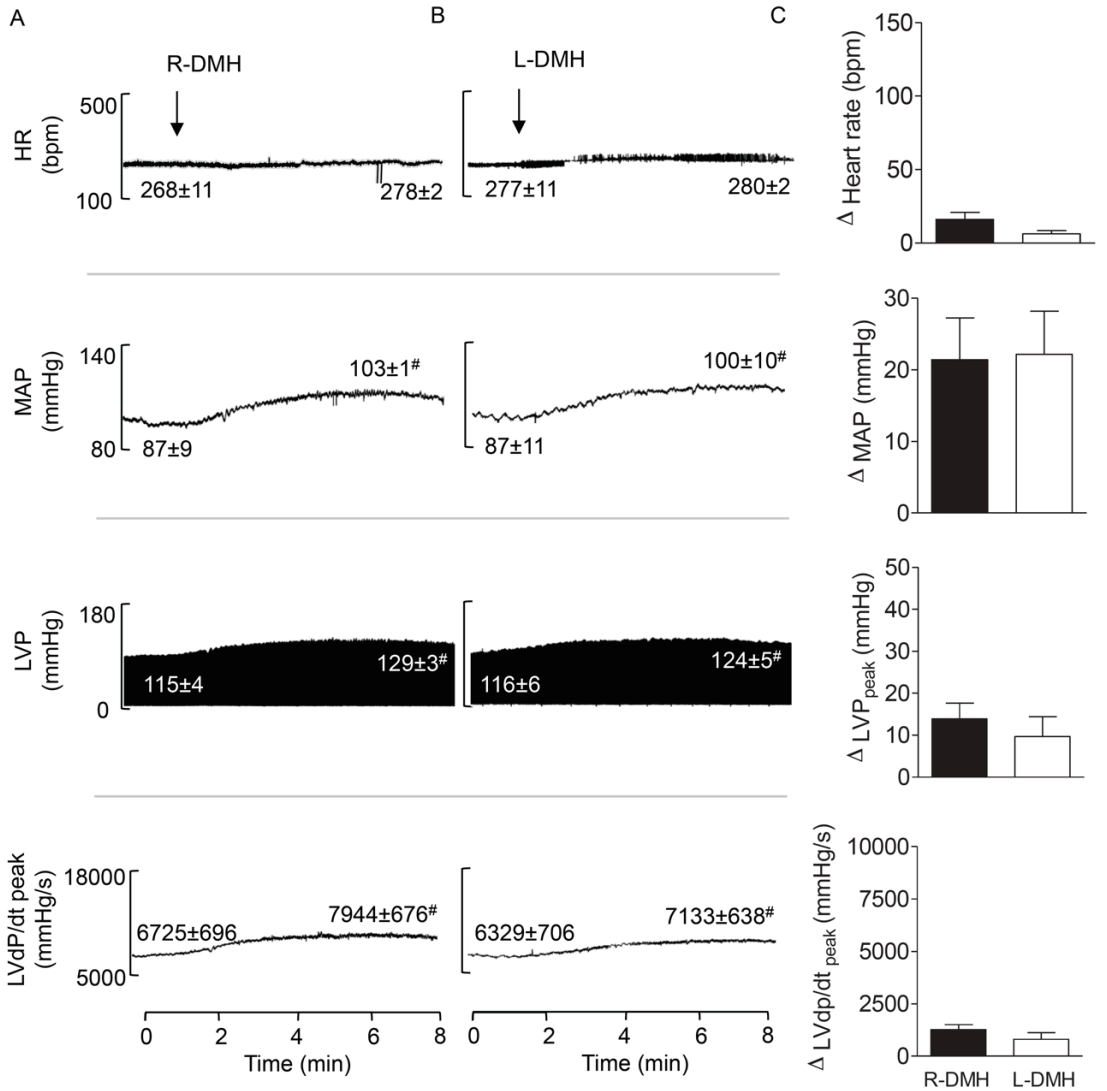


Figura 13. Valores absolutos basais e respostas cardiovasculares máximas provocadas pela nanoinjeção de BMI no DMH direito (coluna A; R-DMH) e esquerdo (coluna B; L-DMH) durante efeito do atenolol. A coluna (C) apresenta as mudanças máximas causadas evocadas pelo DMH. HR: frequência cardíaca; MAP: pressão arterial média; LVP: pressão ventricular esquerda; LVdP/dt peak: valores de pico da derivada da pressão ventricular esquerda; Time: tempo em minutos. # *P*<0,05 vs. Basal. ANOVA two-way e Student t test.

O bloqueio dos receptores beta-adrenérgicos cardíacos com atenolol causou queda na LVP e LVdP/dt (Figura 11B). O atenolol bloqueou completamente as respostas cronotrópicas cardíacas causadas pela nanoinjeção de BMI em ambos os lados do DMH. Porém, a ativação do DMH ainda causou um significativo efeito pressor associado com um efeito menor, mas também significativo, sobre a contratilidade cardíaca. Novamente, nenhuma assimetria foi observada na amplitude destas respostas pressoras (Figura 13). O atenolol aboliu completamente os batimentos ectópicos supraventriculares causados pela estimulação dos lados direito e esquerdo do DMH. Entretanto, um número pequeno de batimentos ectópicos ventriculares ainda persistiu em 3 dos 7 animais (L-DMH = 3±1 e R- DMH = 6±4 VEB/10min).

Para determinar se os aumentos de contratilidade ventricular foram secundários aos aumentos na pós-carga, ou seja, devido a aumentos na resistência vascular periférica, foram comparadas as variações de contratilidade (ΔLVdP/dt peak) e pressão arterial média (ΔMAP) causadas pela ativação dos lados direito e esquerdo do DMH com aquelas provocadas pela administração endovenosa de fenilefrina (Figura 14).

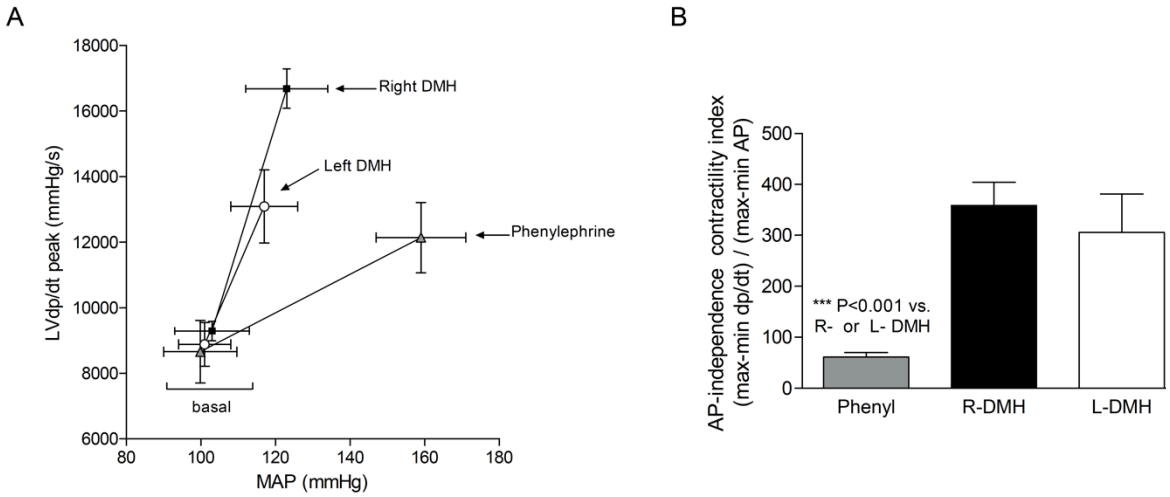


Figura 14. <u>Painel A</u> - Variações na pressão arterial média (MAP) e na medida de contratilidade (valor de pico da derivada da pressão ventricular esquerda - LVdp/dt peak) causadas pelas ativação dos lados direito e esquerdo do DMH (R- e L-DMH) e injeção endovenosa do agonista adrenérgico fenilefrina. <u>Painel B</u> – Índice que mede a influência da pós-carga sobre a contratilidade calculado em cada uma das situações experimentais citadas anteriormente (para detalhes, ver métodos). One-way ANOVA.

Como demonstrado pela Figura 14, a ativação de ambos DMH direito e esquerdo provocaram aumentos pronunciados de contratilidade como aumento discreto de PAM. Em contraste, a injeção de fenilefrina causou aumento substancial de PAM concomitante a aumentos moderados de contratilidade (LVdp/dt peak). A partir desta análise é evidente que a resposta contrátil provocada pelo DMH poderia ser pobremente associada à discreta resposta pressora que ocorre durante esta intervenção (±15mmHg). A Figura 14 também mostra que, para um pequeno e similar efeito pressor, a ativação do DMH provocou maiores respostas inotrópicas.

As respostas cardíacas observadas nos animais submetidos ao "pacing" cardíaco estão demonstradas na Figura 15.

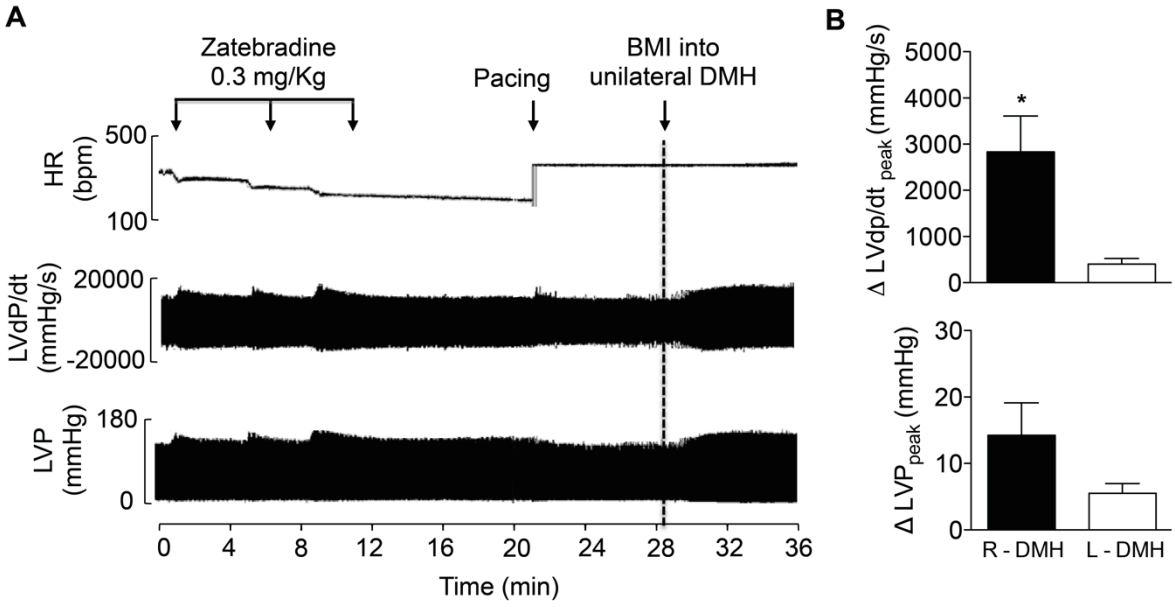


Figura 15. Respostas cardíacas à injeção endovenosa de zatebradina, ao pacing e à ativação unilateral do DMH (painel A). Variações máximas nos valores de pico pressão ventricular esquerda (LVP peak) e sua derivada (LVdp/dt peak) (painel B). Time: tempo em minutos. **P*<0,05 R- *vs.* L-DMH. ANOVA two-way e Student t test.

Ainda sem iniciar o "pacing", o bloqueio farmacológico da corrente marcapasso cardíaco provocou mudanças similares àquelas descritas anteriormente (Figura 11A). A desinibição unilateral do DMH durante "pacing" cardíaco evocou aumentos nos valores de pico da pressão ventricular esquerda e sua derivada (LVPpeak e LVdP/dt peak, respectivamente) (Figura 15, painel A). De maneira similar ao observado em experimentos anteriores,

efeitos inotrópicos evocados pelo DMH direito foram significativamente maiores do que o DMH esquerdo (Figura 15, painel B).

5.2- CONTRIBUIÇÃO DE RECEPTORES DE AMINOÁCIDOS EXCITATÓRIOS NO HIPOTÁLAMO DORSOMEDIAL E COLUNAS LATERAL E DORSOLATERAL DA SUBSTÂNCIA CINZENTA PERIAQUEDUTAL PARA AS RESPOSTAS SIMPATOEXCITATÓRIA, PRESSÓRICA E CRONOTRÓPICA CARDÍACA (ver anexo 4)

5.2.1 EXPERIMENTOS EM ANIMAIS ANESTESIADOS

As Figuras 16 e 17 apresentam as respostas cardiovasculares causadas pela nanoinjeção unilateral de NMDA no DMH (Figura 16) e PAG (Figura 17).

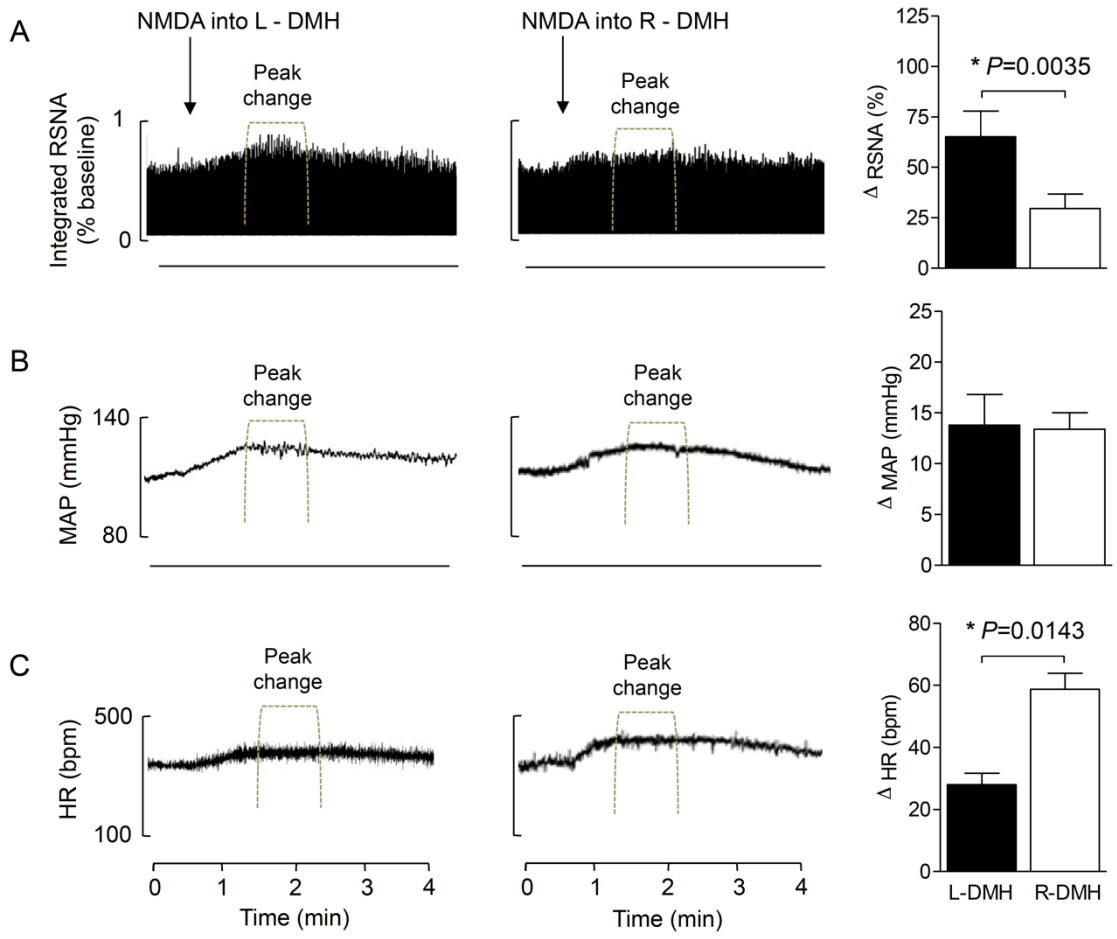


Figura 16. Registros representativos e mudanças máximas na atividade simpática para o nervo renal esquerdo (RSNA – linha A), pressão arterial media (MAP – linha B) e frequência cardíaca (HR – linha C) causadas pela nanoinjeção de NMDA (100pmol/100nL) nos lados direito (barras brancas) e esquerdo (barras negras) do DMH. Na figura estão indicados os períodos de aproximadamente um minuto dentro dos quais as respostas máximas foram marcadas **P*<0,05 R- *vs.* L-DMH – valores exatos de *P* estão indicados nos gráficos (paired Student *t* test).

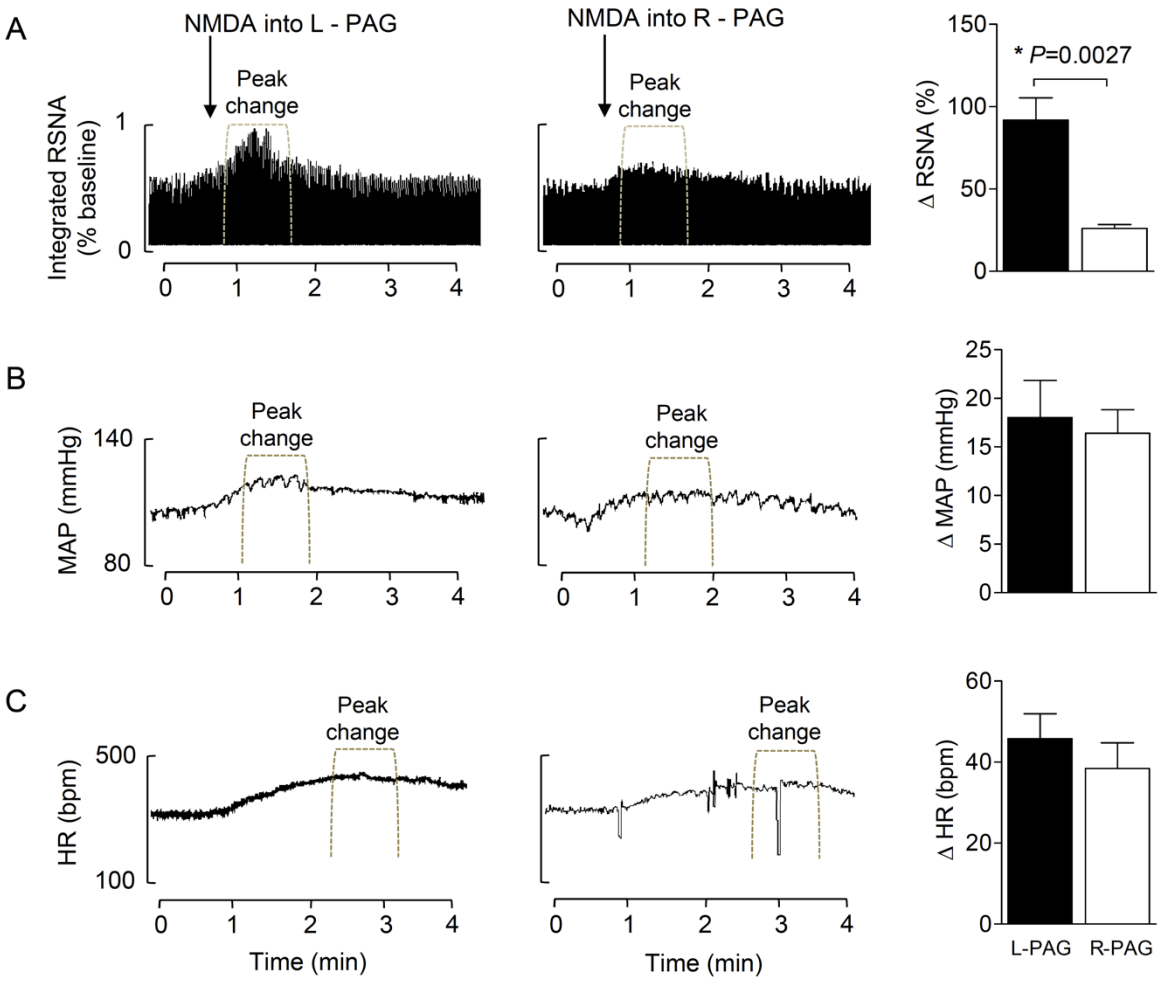


Figura 17. Registros representativos e mudanças máximas na atividade simpática para o nervo renal esquerdo (RSNA – linha A), pressão arterial media (MAP – linha B) e frequência cardíaca (HR – linha C) causadas pela nanoinjeção de NMDA (100pmol/100nL) nos lados direito (barras brancas) e esquerdo (barras negras) da PAG. Na figura estão indicados os períodos de aproximadamente um minuto dentro dos quais as respostas máximas foram marcadas **P*<0,05 R- *vs.* L-DMH – valor exato de *P* está indicado no gráfico (paired Student *t* test).

Estas respostas foram evidentes à partir do primeiro minutos após injeção e duraram por aproximadamente 5 minutos, com os parâmetros cardiovasculares retornando a valores basais em torno do décimo minuto após injeção. Foram observados aumentos equipotentes de pressão arterial após injeção unilateral de NMDA no DMH ou PAG (∆MAP DMH-D = 13±2 *vs.* E = 14±3; PAG-D = 16±3 *vs.* E = 18±4mmHg; *P*<0,05 *vs.* basal), mas nenhuma diferença foi detectada na comparação dos lados direito e esquerdo dentro do mesmo grupo experimental.

A estimulação do DMH e PAG com NMDA também causou aumentos na RSNA para o nervo renal esquerdo (P<0,05 *vs.* basal), mas as nanoinjeções no lado ipsilateral ao nervo registrado, ou seja, esquerdo, causaram maiores respostas simpatoexcitatórios para este leito (∆RSNA DMH-D = 30±7 *vs.* E = 66±13; PAG-D = 26±3 *vs.* E = 92±14%; *P*<0,05). Foi também observado aumento de FC após estimulação unilateral do DMH ou PAG, mas a nanoinjeções no lado direito do DMH causaram maiores repostas cronotrópicas cardíacas comparadas ao DMH esquerdo (∆HR DMH-D = 59±5 *vs.* E = 28±4bpm; *P*<0,05) (Figura 16). A taquicardia observada após estimulação unilateral da PAG foi similar aos lados direito e esquerdo da PAG (∆HR PAG-D = 39±6 *vs.* E = 45±6 bpm) (Figura 17).

De maneira intrigante, uma diferença de padrão no curso temporal das respostas causadas pela estimulação unilateral da PAG foi detectada nos experimentos (ver “peak change” na Figura 17). Em geral, as alterações máximas na PAM e RSNA foram próximas, em torno de 30-60 segundos após estimulação de ambos os lados da PAG (Figura 17, painéis A e B). Entretanto, as respostas máximas na FC se mostraram discretamente atrasadas (90-120s

após injeção) (Figura 17C). Nenhuma diferença no curso temporal das respostas evocadas pela estimulação unilateral do DMH foram vistas (Figura 16).

A Figura 18 apresenta a variação nos parâmetros cardiovasculares e a amplitude das respostas causadas pela injeção unilateral de NMDA na PAG após bloqueio dos receptores EAA no DMH esquerdo.

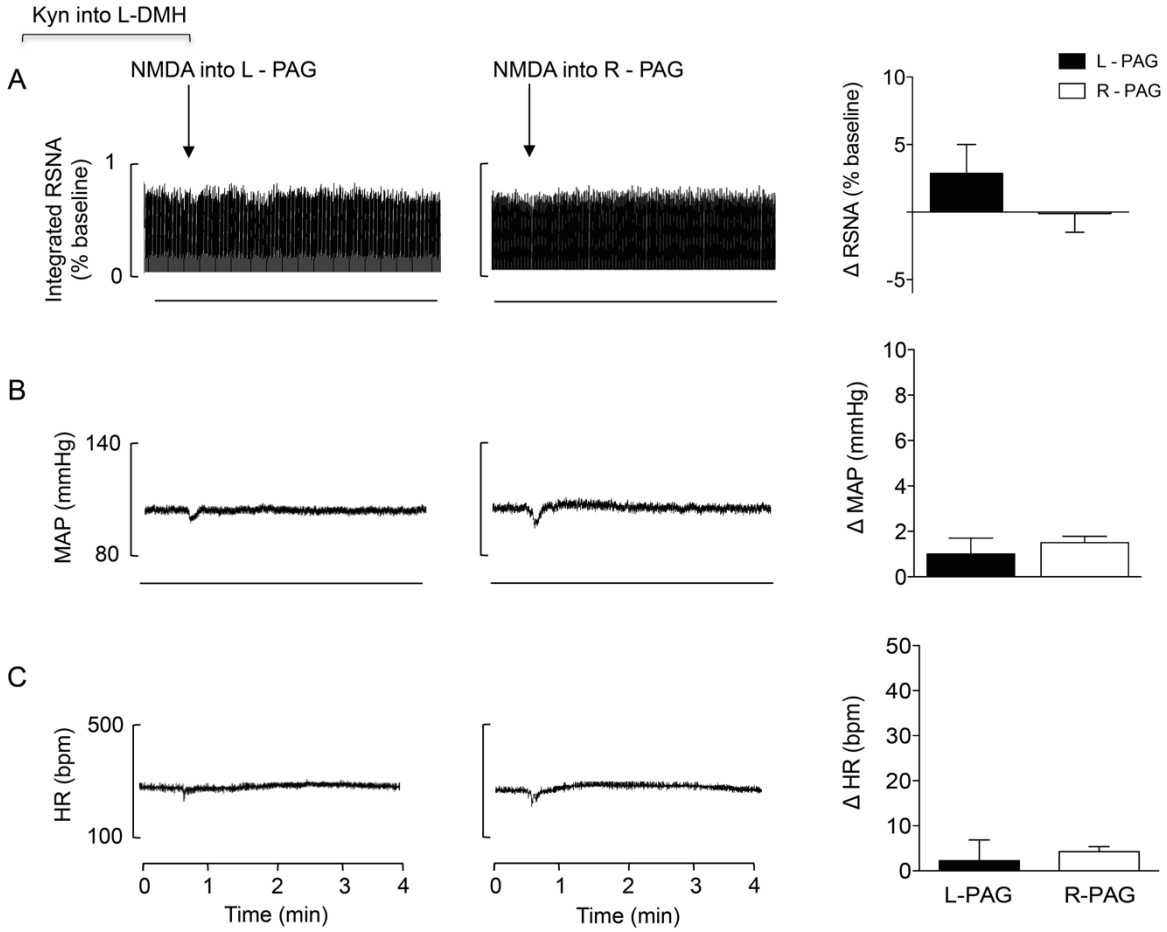


Figura 18. Registros representativos e mudanças máximas na atividade simpática para o nervo renal esquerdo (RSNA – linha A), pressão arterial media (MAP – linha B) e frequência cardíaca (HR – linha C) causadas pela nanoinjeção de NMDA (100pmol/100nL) nos lados direito (barras brancas) e esquerdo (barras negras) da PAG após injeção de Kyn (1nmol/100nL) no DMH esquerdo (Kyn into L-DMH).

O bloqueio farmacológico dos receptores de EAA no DMH esquerdo pela injeção de ácido quinurênico causou discreta redução nos parâmetros cardiovasculares. Após este bloqueio, injeções de NMDA na PAG ipsilateral ou contralateral não causaram respostas com amplitude similar àquelas observadas sem o bloqueio dos EAA no DMH. As respostas de pressão arterial (∆MAP: D = 1±2 *vs*. E = 2±1mmHg), frequência cardíaca (∆HR: D = 3±5 *vs*. E = 4±1bpm) e atividade simpática para o nervo renal esquerdo (∆RSNA: D = -1±2 *vs*. E = 3±5%) foram drasticamente atenuadas. Além disso, a lateralização no controle da atividade simpática para o nervo renal esquerdo foi abolida. As diferenças no tempo de curso das respostas previamente observadas após injeção unilateral de NMDA na PAG antes do bloqueio de EAA no DMH também foram ausentes.

5.2.2 - EXPERIMENTOS EM ANIMAIS NÃO-ANESTESIADOS

A Figura 19 apresenta os parâmetros cardiovasculares durante todo o experimento e as variações máximas na FC e PAM de animais submetidos ao estresse agudo.

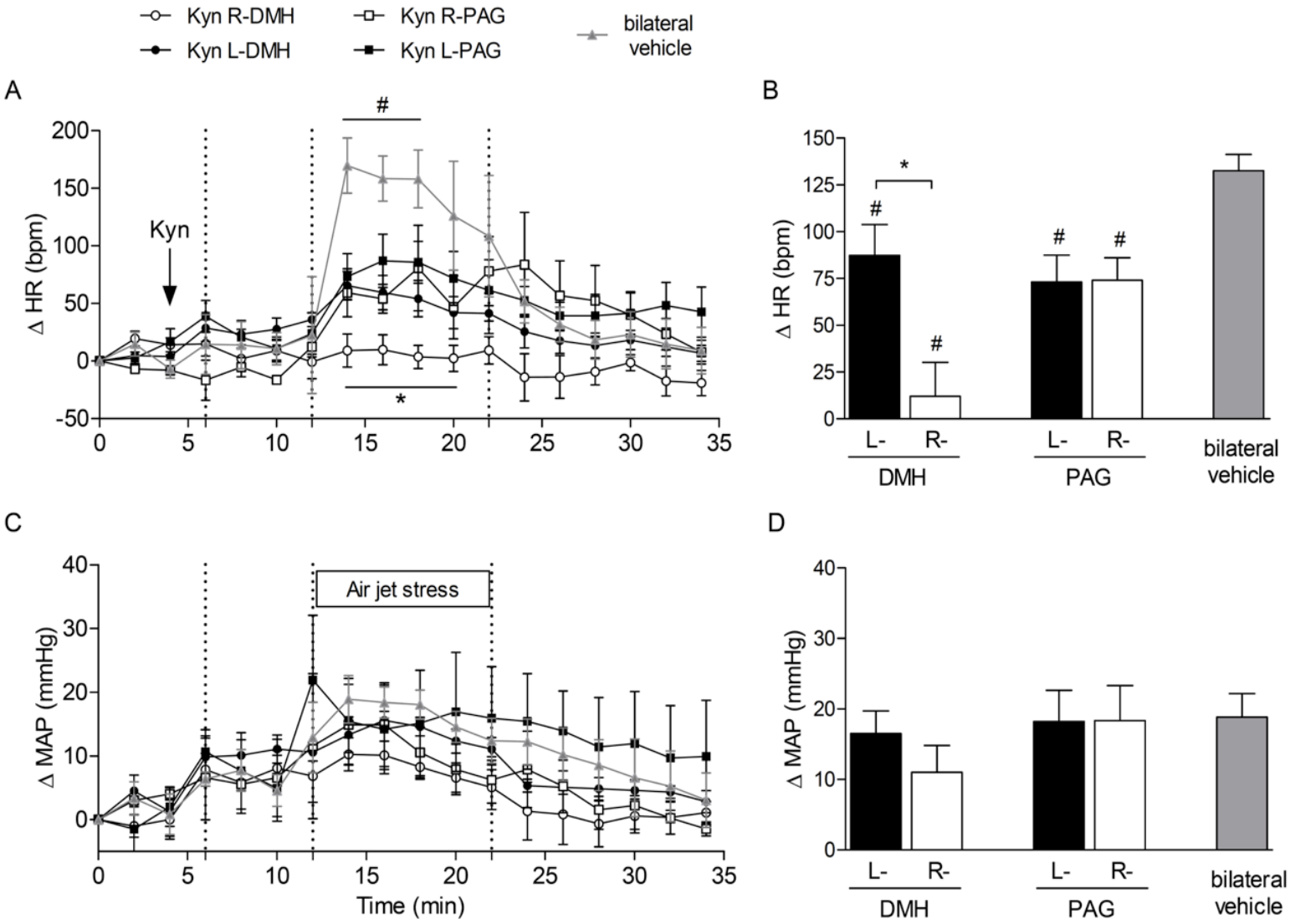

Kyn R-DMH
Kyn L-DMH
Kyn R-PAG
Kyn L-PAG
bilateral vehicle
A
B
C
D
Kyn
Δ HR (bpm)
Air jet stress
Δ MAP (mmHg)
Time (min)
L-
R-
DMH
PAG
bilateral vehicle

Figura 19. Resultados obtidos em animais não-anestesiados submetidos ao estresse por jato de ar. Painéis A e C: curso temporal das alterações na frequência cardíaca (HR) e pressão arterial (MAP) antes e após a injeção de ácido quinurênico (1nmol/100 nl) nos lados direito (R- símbolos vazios) e esquerdo (L- símbolos preenchidos) do DMH (círculos) e l/dl PAG (quadrados); ou injeção bilateral de veículo (triângulos cinzas *P<0.05 R- *vs.* L-DMH; $^{\#}P$<0.05 veículo *vs.* R- ou L-DMH e R- ou L-PAG (Two-way ANOVA seguido de Bonferroni post hoc test). Painéis B e D: mudanças máximas na HR e MAP durante os experimentos de estresse por jato de ar em animais que receberam injeção prévia de ácido quinurênico (1 nmol/100 nl) nos lados direito (barras brancas) e esquerdo (barras negras) do DMH ou l/dl PAG; ou injeção bilateral de veículo (barras cinzas). *P<0.05 R *vs.* L; $^{\#}P$<0.05 *vs.* Veículo bilateral (One-way ANOVA Newman-Keuls post hoc test).

Comparado à amplitude das respostas controle (nanoinjeção bilateral de veículo), a administração de Kyn no lados direito e esquerdo do DMH atenuaram a taquicardia evocada pelo estresse. Entretanto, a taquicardia causada pelo estresse foi praticamente abolida quando os receptores de aminoácidos excitatórios foram inibidos no DMH direito (∆FC DMH-D = 12±18 *vs*. E = 87±17; PAG-D = 73±15 *vs*. E = 74±12; veículo: 132±9bpm; P<0,05) (Figura 22, painéis A e B). O bloqueio destes receptores nos lados direito e esquerdo do DMH ou PAG não alterou a amplitude das respostas pressoras causadas pelo estresse por jato de ar (∆PAM DMH-D = 12±4 *vs*. E = 17±3; PAG-D = 18±4 *vs*. E = 18±5; veículo: 19±3 mmHg) (Figura 19 C e D).

5.2.3 – VIAS ANATÔMICAS ENTRE O DMH E A PAG

Fotomicrografias demonstrando os sítios de injeção das Retrobeads e a região do DMH e PAG, alvos das injeções centrais realizadas neste estudo, estão demonstradas na Figura 20, painéis A e C.

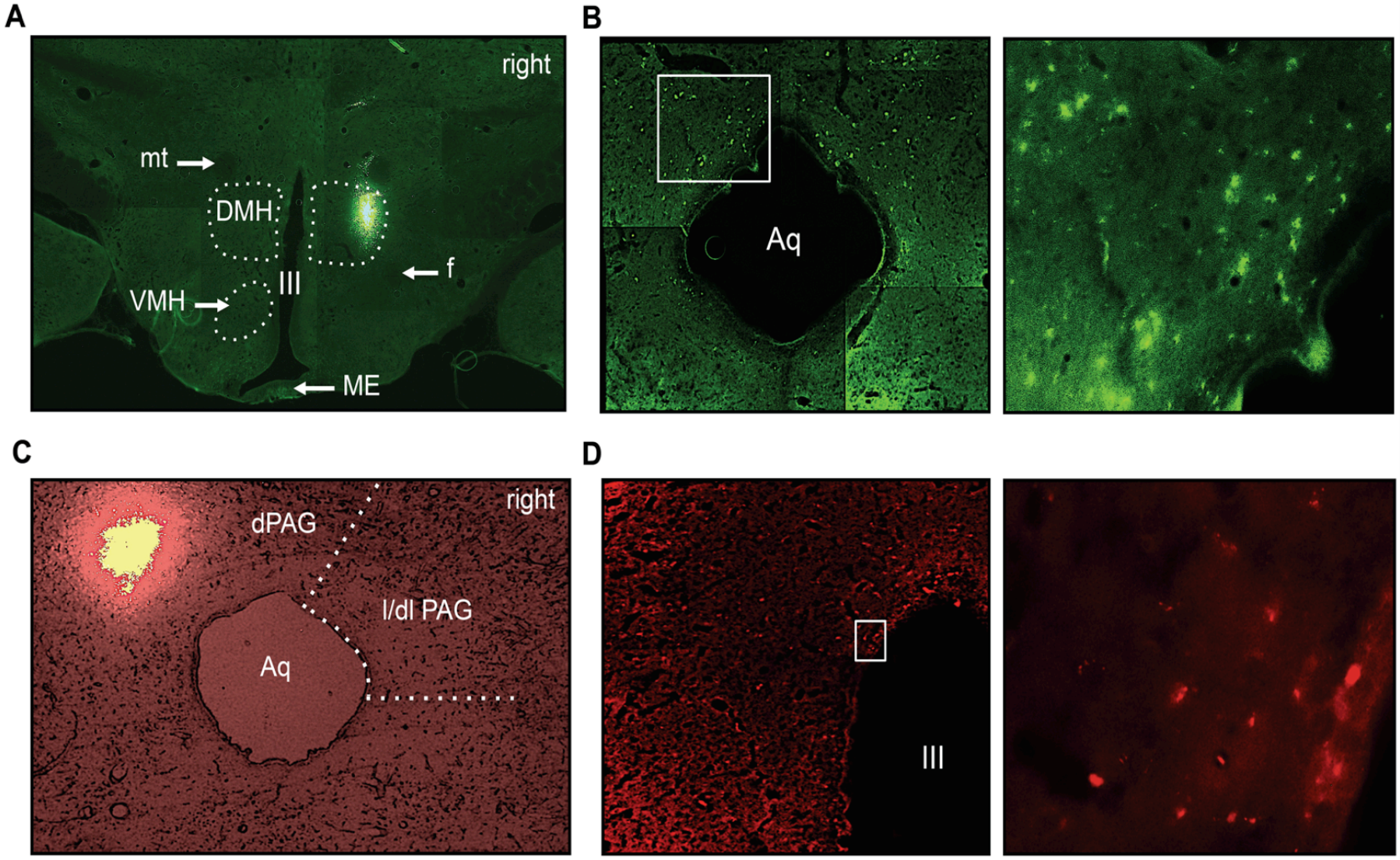

A
right
mt
DMH
III
f
VMH
ME
B
Aq
C
right
dPAG
l/dl PAG
Aq
D
III

Figura 20. <u>Painéis A e C:</u> Fotomicrografias exemplificam os sítios de injeção pretendidos em todos os experimentos desta série (aumento 5x). As injeções acima evidenciadas são de animais que receberam Retrobeads verdes e vermelhas no DMH (A) e PAG (C), respectivamente. <u>Painéis B e D:</u> Maior aumento (20x) exemplificando neurônios retrogradamente marcados com Retrobeads verdes e vermelhas na PAG (B) e DMH (D), respectivamente. Referências anatômicas: DMH – hipotálamo dorsomedial; ME – eminência mediana; mt – trato mamilotalâmico; f- forniz; VMH – hipotálamo ventromedial; III – terceiro ventrículo; l/dl PAG – colunas lateral e dorsolateral da substância cinzenta periaquedutal; Aq – aqueduto.

Como proposto, injeções centrais de microesferas verdes e vermelhas alcançaram o DMH e as colunas lateral/dorsolateral da PAG, respectivamente. No DMH esquerdo, contralateral ao lado onde as esferas verdes foram injetadas, foi possível observar em vermelho neurônios que enviam projeções diretas à PAG. De maneira semelhante, na PAG direita foi possível observar neurônios marcados em verde, que enviam projeções diretas ao DMH. No animal que recebeu apenas injeção de esferas verdes no DMH direito, foi possível observar marcação bilateral, onde poucas células marcadas foram identificadas na região ventral da PAG. Entretanto, a marcação na coluna dorsolateral da PAG ipsilateral pareceu estar ligeiramente mais densa. Conjuntamente estes dados reforçam a existência de uma via bilateral e anatomicamente lateralizada entre o DMH e PAG (Figura 20).

A Figura 21 demonstra corpos celulares neurônios da PAG marcados por esferas fluorescentes oriundas do DMH.

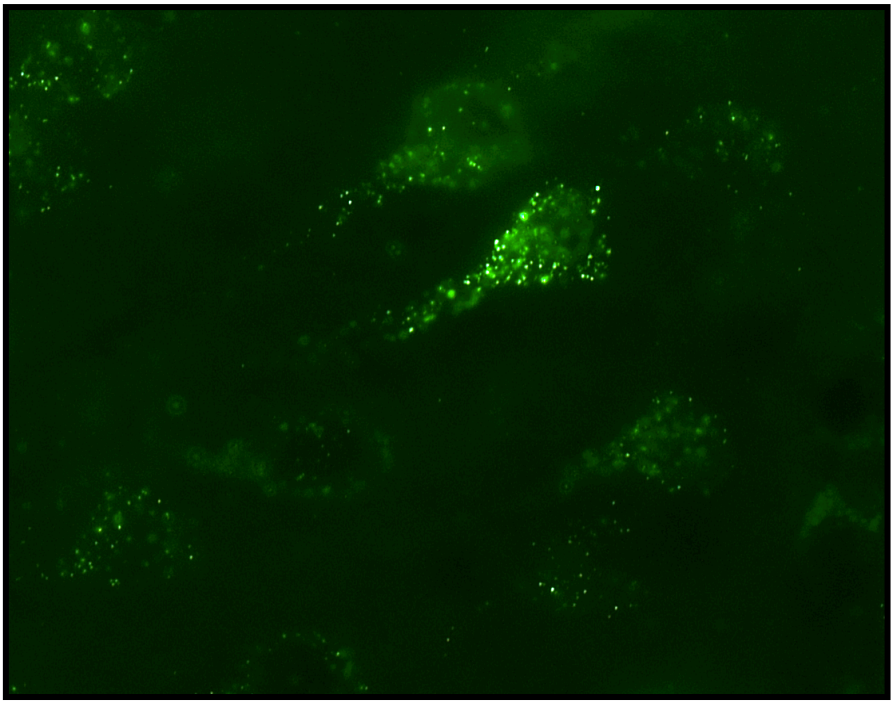


Figura 21. Fotomicrografia em maior aumento (100x) de lâmina de cérebro demonstrando neurônios marcados com Retrobeads verdes injetadas no DMH.

5.3- ENVOLVIMENTO DA *RAPHE PALLIDUS* NAS RESPOSTAS ASSIMÉTRICAS CRONOTRÓPICAS E INOTRÓPICAS CARDÍACAS PROVOCADAS PELA ESTIMULAÇÃO UNILATERAL DO HIPOTÁLAMO DORSOMEDIAL.

Os sítios de injeção nas áreas alvo, hipotálamo dorsomedial e *raphe pallidus* estão demonstrados na Figura 22.

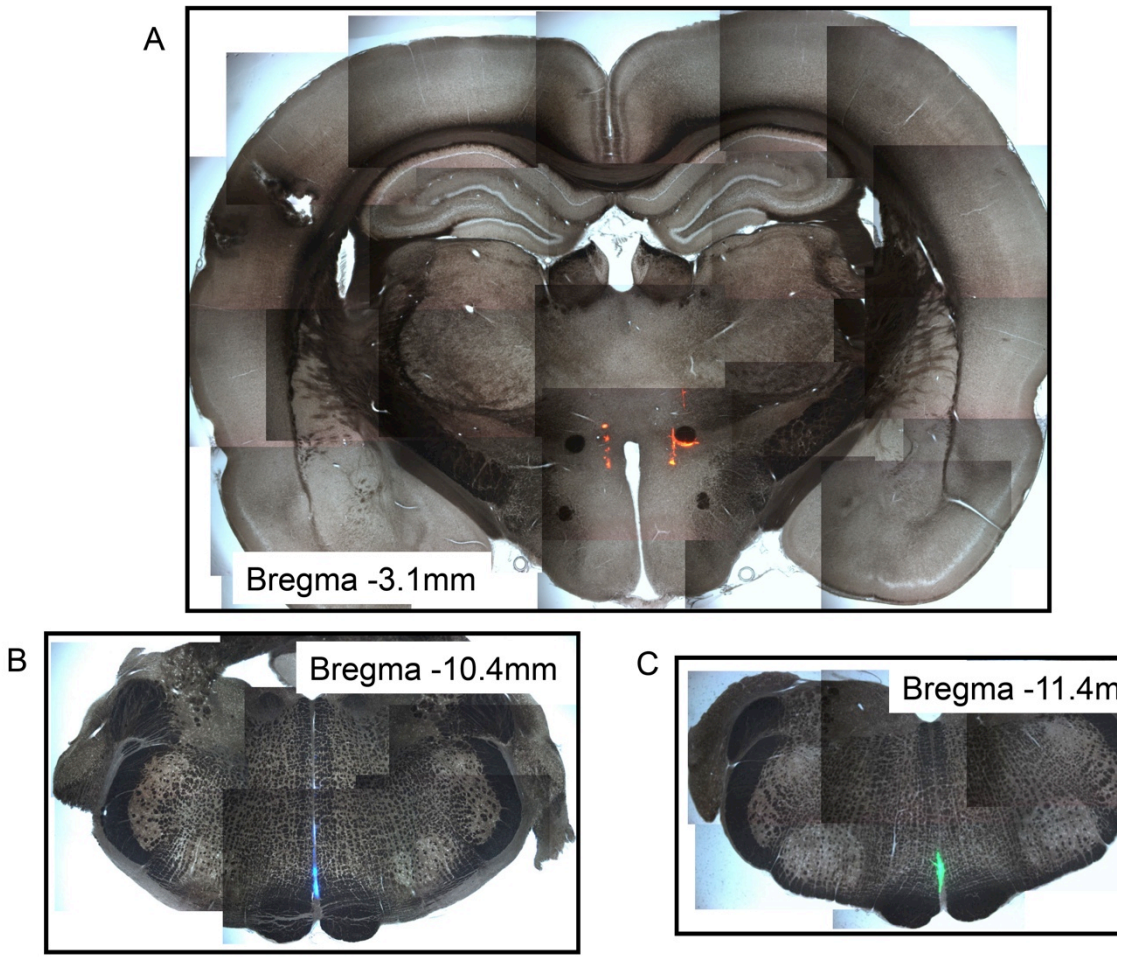


Figura 22: Fotomicrografias demonstrando os sítios de injeção e os níveis dos cortes histológicos do hipotálamo dorsomedial (A – microesferas vermelhas) e raphe pallidus (B e C, rostro-caudais. Fotografias de lâminas em campo claro e filtro para fluorescência específica de cada cor das microesferas. Fatias frescas de cérebro "molhado" posicionadas sobre lâminas de vidros, sem cobertura com lamínula. Referências anatômicas: Atlas (Paxinos and Watson, 1986).

Os painéis B e C da Figura 22 exemplificam sítios de injeção na RP em diferentes níveis rostro-caudais. O painel B exemplifica um sítio de injeção mais rostral.

A Figura 23 é um exemplo de experimento que demonstra as diferenças nas amplitudes das respostas cronotrópicas e inotrópicas cardíacas evocadas pela estimulação da RP nos diferentes níveis rostro-caudais.

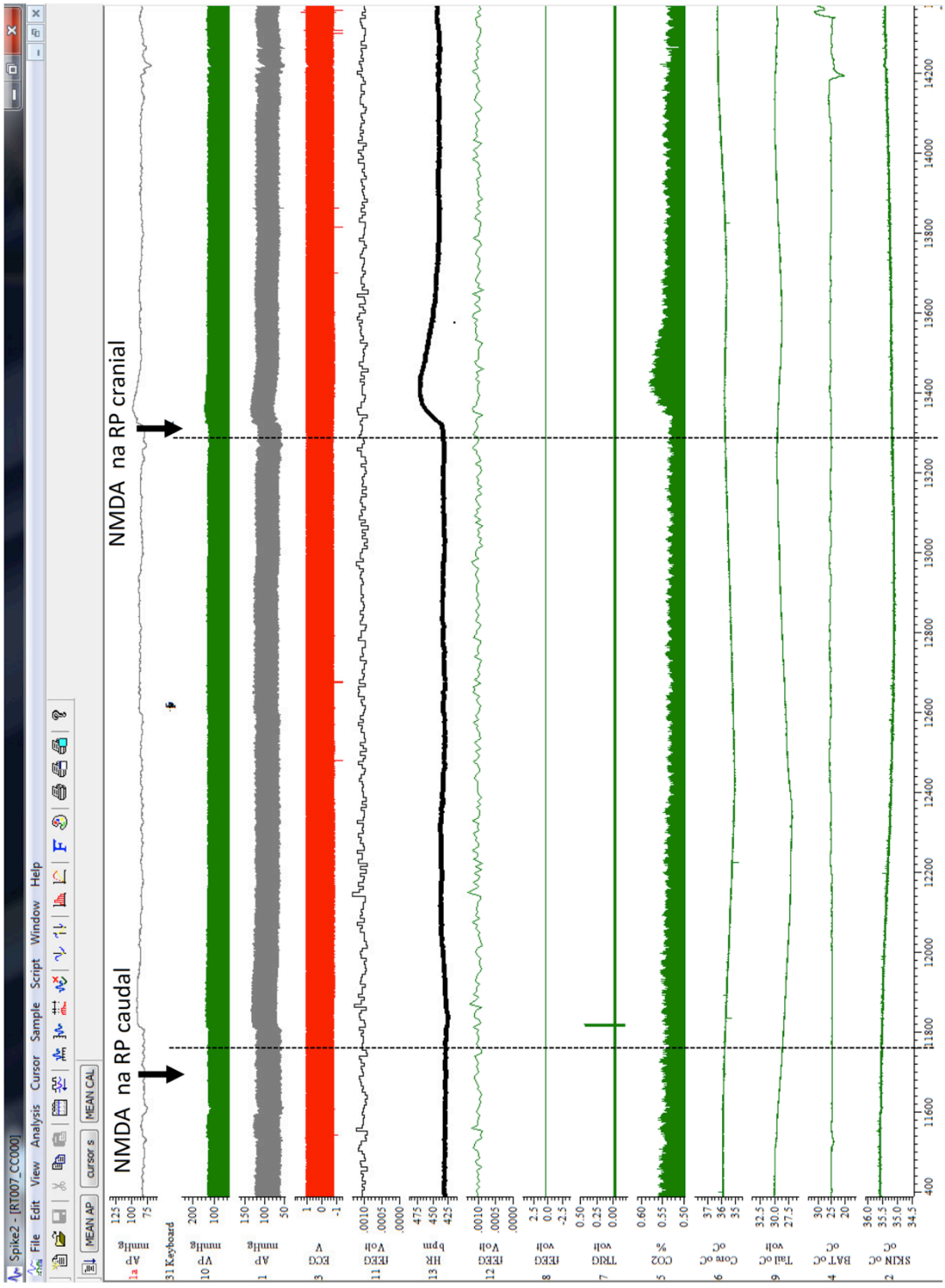


Figura 23. Exemplos de registros demonstrando a amplitude nas respostas cardiovasculares evocaas pela injeção de NMDA na RP em diferentes níveis.

A Figura 24 demonstra os dados coletados em menor escala temporal, detalhando os parâmetros cardiovasculares.

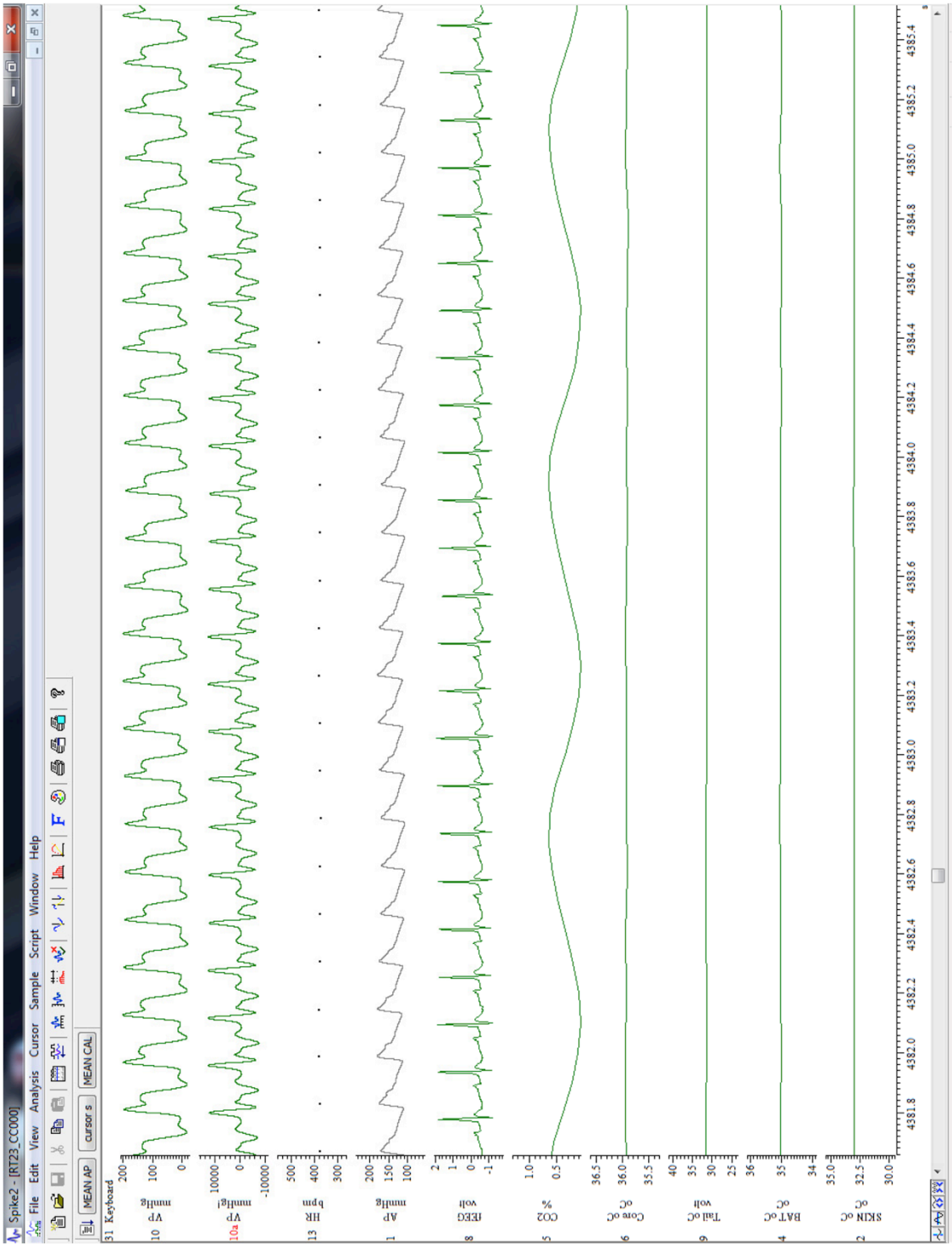


Figura 24. Registro em menor escala temporal evidenciando detalhes de cada parâmetro medido da Figura 23.

A Figura 25 apresenta as respostas cardiovasculares e térmicas evocadas pela nanoinjeção de NMDA na RP e nos lados direito e esquerdo do DMH.

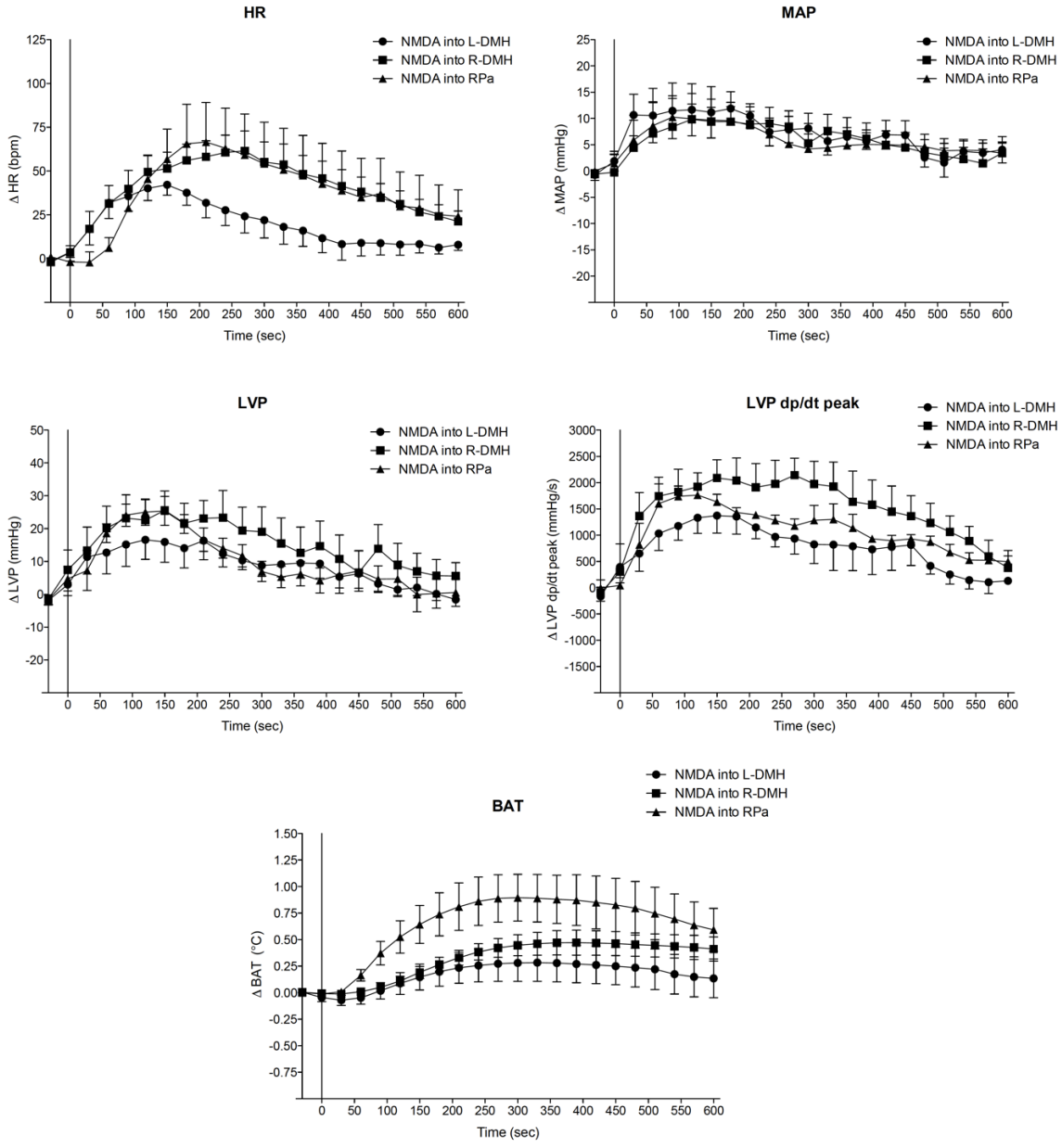


Figura 25. Curso temporal das variações na frequência cardíaca (HR), pressão arterial média (MAP), pressão ventricular esquerda máxima (LVPpeak) e sua derivada (LVdp/dt peak), e temperatura no tecido adiposo marrom (BAT) evocadas pela nanoinjeção de NMDA na raphe pallidus (RP) e hipotálamo dorsomedial direito (R-DMH) e esquerdo (L-DMH).

A injeção de NMDA em ambos RP, e DMH, lados direito e esquerdo (R- e L-DMH) causou respostas pressora (∆PAM D-DMH = 10±3; E-DMH = 12±5; RP = 11±3mmHg; *P*<0,05 *vs.* basal), taquicárdica (∆FC D-DMH = 62±11; E-DMH = 40±6; RP = 67±16bpm; *P*<0,05 vs. basal), aumento da pressão ventricular cardíaca (∆LVP D-DMH = 26±4; E-DMH = 16±6; RP = 26±4mmHg; *P*<0,05 *vs*. basal), contrátil (∆LVdp/dt peak D-DMH = 2089±346; E-DMH = 1355±333; RP = 1964±111mmHg/sec; *P*<0,05 *vs.* basal) e termogênica. A nanoinjeção de NMDA na RP provocou maior aumento na BAT comparado aos lados direito e esquerdo do DMH (∆BAT D-DMH = 0,5±0,1; E-DMH = 0,4±0,2 *vs*. RP = 0,9±0,2 °C; *P*<0,05 vs. Basal; *P*<0,05 RP *vs.* D- e E-DMH). Não houve diferença na amplitude das respostas pressoras causadas pela estimulação da RP e DMH direito e esquerdo. A magnitude dos aumentos de FC, LVP e LVdp/dt evocadas pelo DMH direito e RP foi similar, e de maneira geral, maior que aquela evocada pelo DMH esquerdo (Figura 25).

As Figuras 26 e 27 apresentam, respectivamente, as respostas cardiovasculares e térmicas causadas pela inibição de neurônios da RP e pela subsequente injeção de NMDA nos lados direito e esquerdo do DMH.

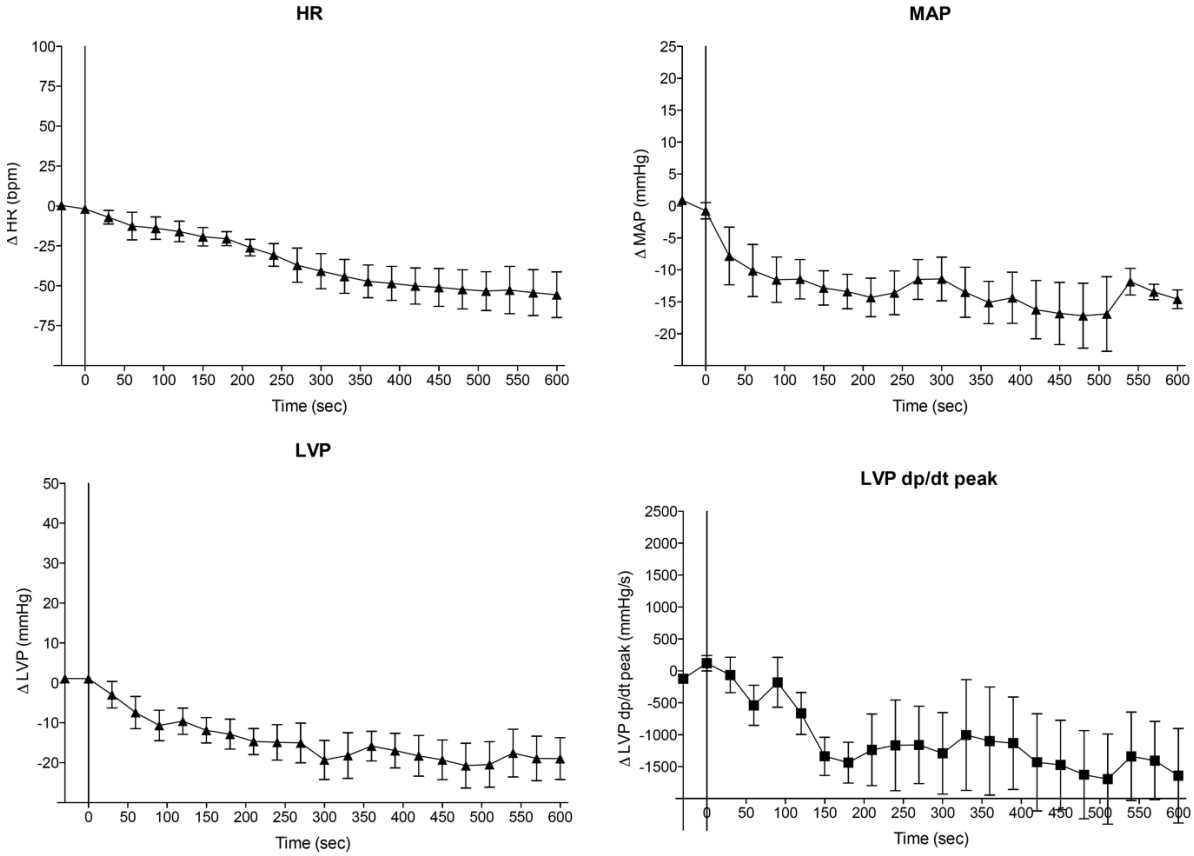


Figura 26. Curso temporal das variações na frequência cardíaca (HR), pressão arterial média (MAP), pressão ventricular esquerda máxima (LVPpeak) e sua derivada (LVdp/dt peak) evocadas pela injeção de muscimol na raphe pallidus (RP).

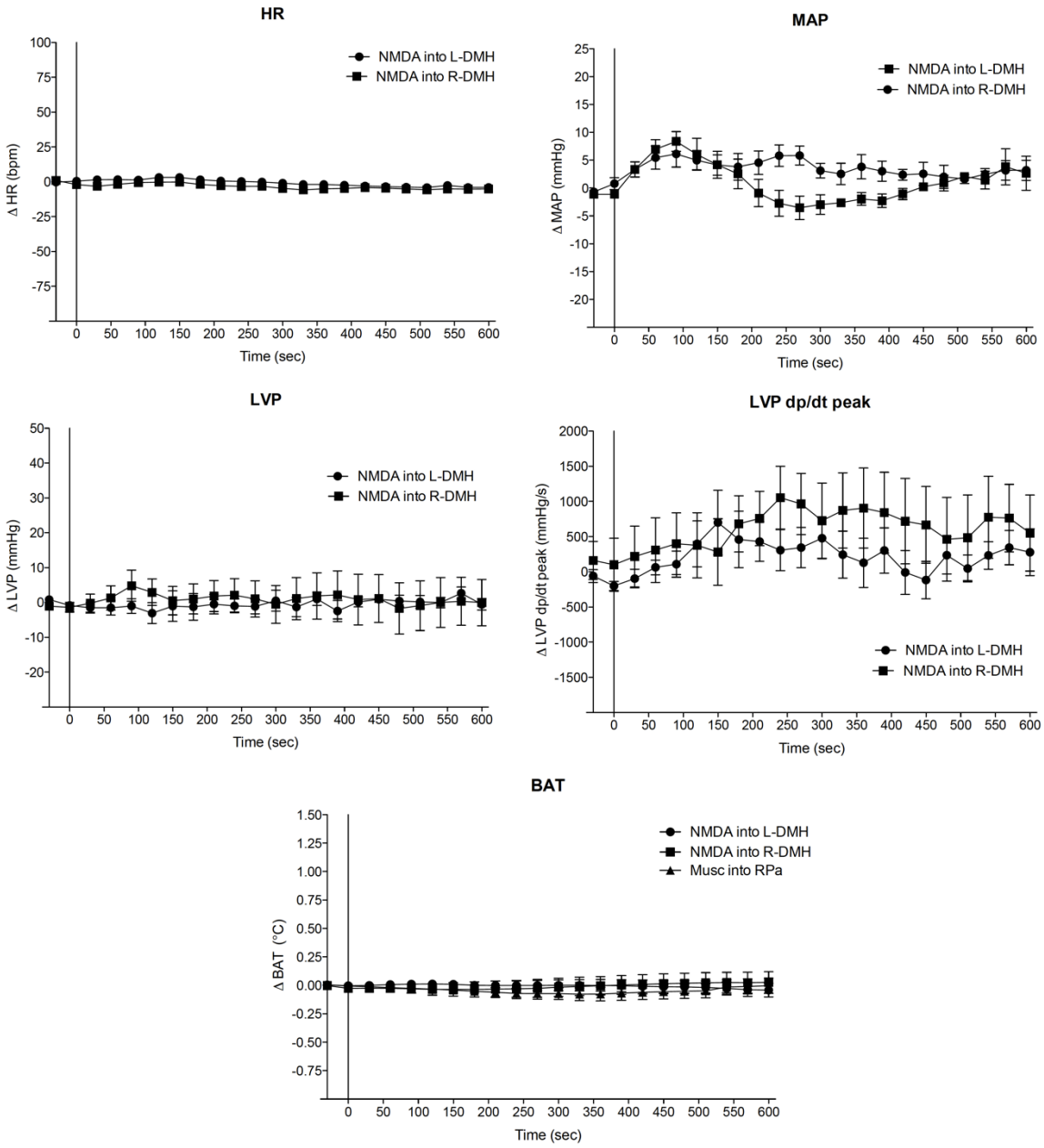


Figura 27. Curso temporal das variações na frequência cardíaca (HR), pressão arterial média (MAP), pressão ventricular esquerda máxima (LVPpeak) e sua derivada (LVdp/dt peak), e temperatura no tecido adiposo marrom (BAT) evocadas pela nanoinjeção de NMDA no hipotálamo dorsomedial direito (R-DMH) e esquerdo (L-DMH) após inibição de neurônios da raphe pallidus (RP) pela nanoinjeção de muscimol.

A inibição dos neurônios da RP causou respostas cronotrópica e inotrópica negativas (Figura 26). Na ausência de atividade neuronal destes neurônios do bulbo ventromedial rostral, a nanoinjeção de NMDA no DMH unilateral não foi capaz de causar respostas cronotrópicas cardíacas e termogênicas (Figura 27). Assim, a inibição da RP aboliu as diferenças na amplitude das respostas cronotrópicas (∆HR: R-DMH = 2±1 *vs*. L-DMH = 1±3bpm) e inotrópicas (∆LVdp/dt peak R-DMH = 757±435 *vs.* L-DMH = 699±460mmHg/sec) cardíacas causadas pela estimulação dos lados direito e esquerdo do DMH. Contudo, foram observadas respostas pressoras menores (∆MAP: R-DMH = 8±2; L-DMH = 7±1mmHg; *P*<0,05 *vs.* basal) acompanhadas por um discreto e equipotente inotropismo positivo (∆LVdp/dt peak: R-DMH = 757±435; L-DMH = 699±460mmHg/sec; *P*<0,05 *vs.* basal), não dependentes do lado do DMH que recebeu injeção de NMDA.

Com o intuito de avaliar se este inotropismo residual era devido a simpatoexcitação miocárdica ou se era somente consequência do aumento de pós-carga, foi realizada a injeção endovenosa do antagonista beta-adrenérgico, atenolol. A Figura 28 mostra os efeitos cardiovasculares causados pelo injeção endovenosa de atenolol. A Figura 29 apresenta as mudanças nos parâmetros cardiovasculares causadas pela injeção de NMDA nos lados direito e esquerdo do DMH após administração endovenosa de atenolol.

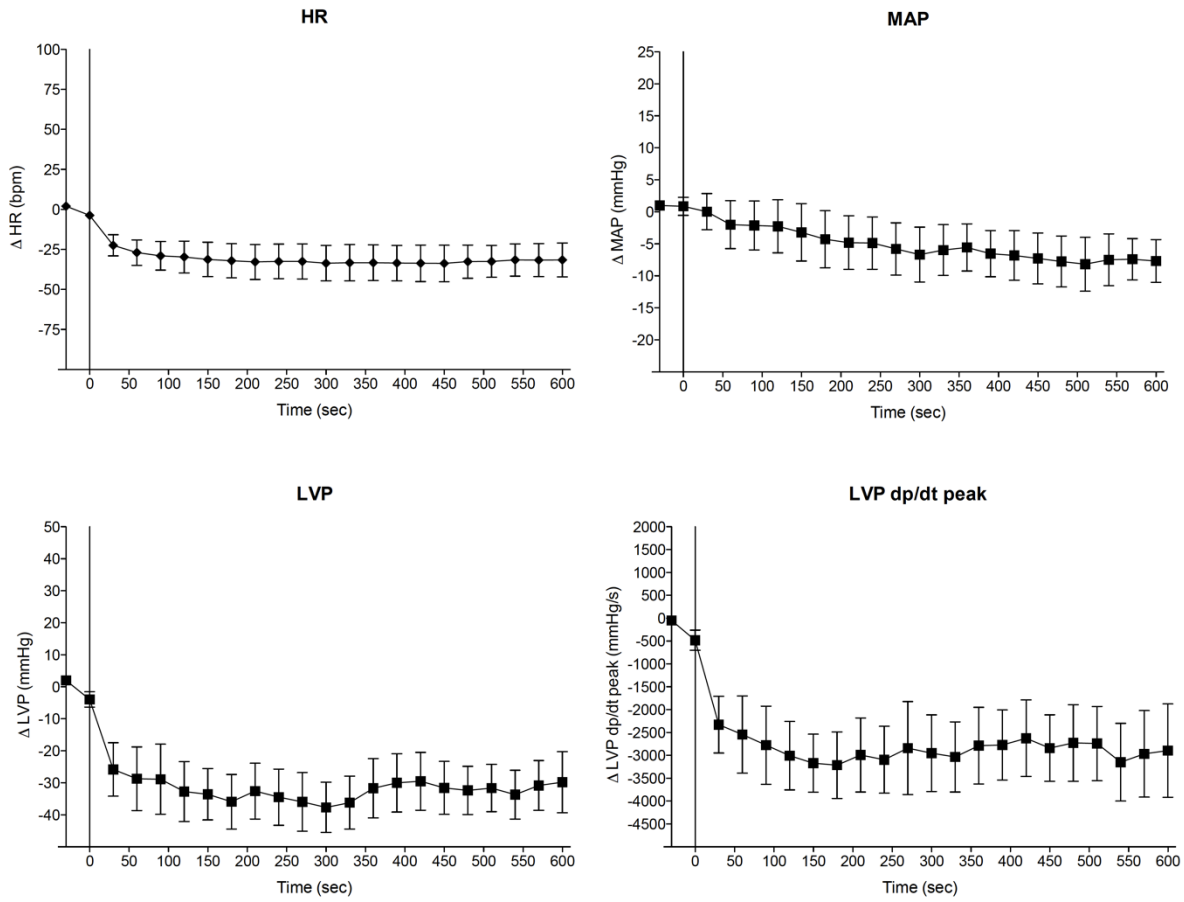


Figura 28. Curso temporal das variações na frequência cardíaca (HR), pressão arterial média (MAP), pressão ventricular esquerda máxima (LVPpeak) e sua derivada (LVdp/dt peak) evocadas pela injeção endovenosa de atenolol.

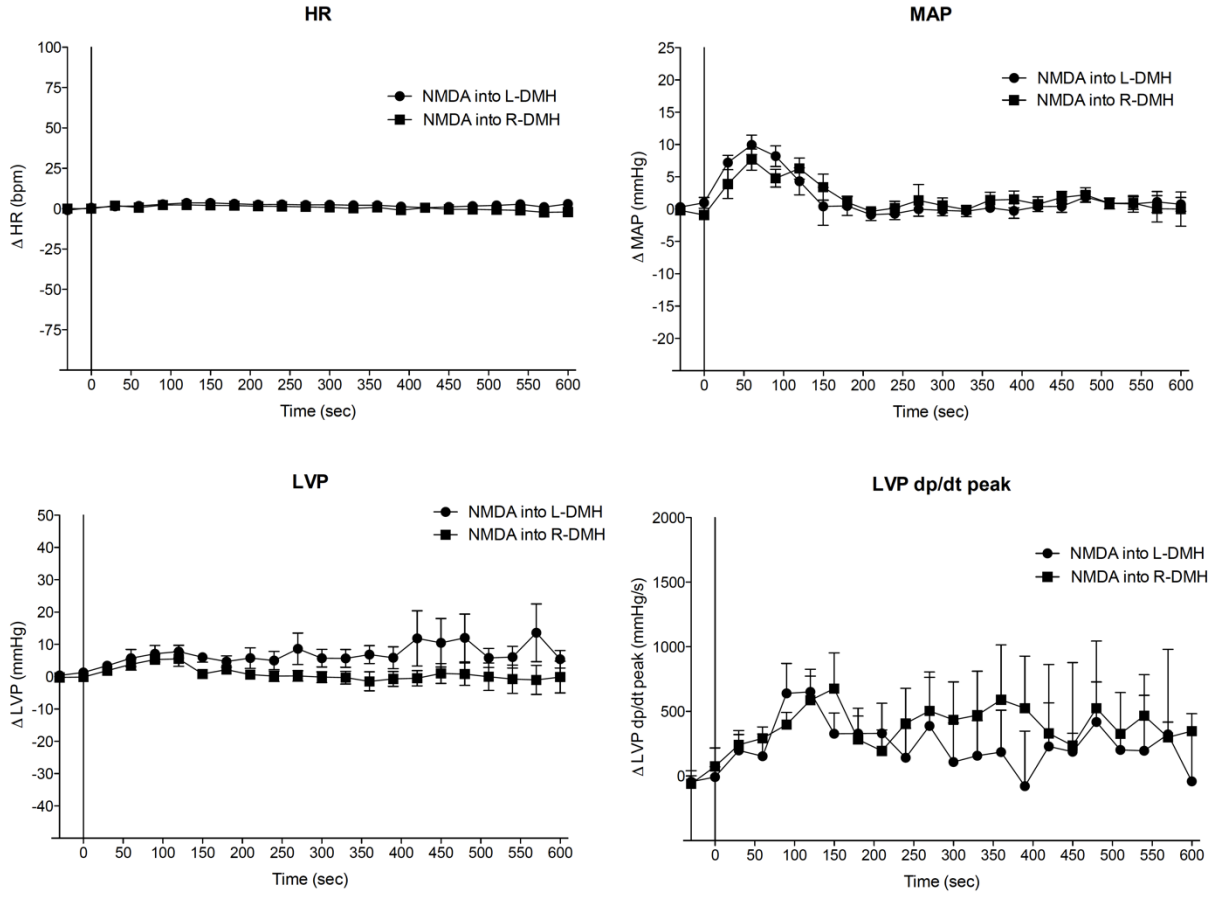


Figura 29. Curso temporal das variações na frequência cardíaca (HR), pressão arterial média (MAP), pressão ventricular esquerda máxima (LVPpeak) e sua derivada (LVdp/dt peak), evocadas pela nanoinjeção de NMDA no hipotálamo dorsomedial direito (R-DMH) e esquerdo (L-DMH) após administração endovenosa de atenolol.

O bloqueio dos receptores adrenérgicos cardíacos causou queda acentuada nos parâmetros cardiovasculares (FC, LVP e LVdp/dt) (Figura 28). Interessantemente, na ausência de atividade de neurônios da RP, o antagonismo beta-adrenérgico cardíaco não alterou a amplitude das respostas pressoras residuais (∆PAM DMH-D = 7±2; DMH-E = 8±2 mmHg; *P*<0,05 *vs.* basal) e das discretas respostas inotrópicas cardíacas (∆LVdp/dt peak DMH-D = 676±276; DMH-E = 640±23mmHg/sec; *P*<0,05 *vs.* basal) provocadas pela estimulação do DMH unilateral (Figura 29).

6 – DISCUSSÃO

Os principais achados deste estudo são: i) existe assimetria no controle cardíaco pelo DMH; ii) a ativação do DMH provoca resposta inotrópica positiva independente das respostas cronotrópicas e de pós-carga que ocorrem simultaneamente; iii) o recrutamento do DMH direito determina respostas cardíacas mais amplas; iv) existe lateralização no controle de atividade simpática renal pelo DMH e PAG; v) os receptores de aminoácido excitatório contribuem para assimetria no DMH e para lateralização em ambos DMH e PAG; vi) não há assimetria nas respostas termogênicas evocadas pelo DMH unilateral; vii) o recrutamento de neurônios da RP são determinantes da assimetria no controle cardíaco, mas não do controle vasomotor pelo DMH.

Estudos em humanos e modelos experimentais suportam nossos dados, demonstrando estreita correlação entre assimetria no funcionamento de áreas do sistema nervoso central e respostas cardiovasculares diferenciadas, como arritmias cardíacas (Wittling, 1997, Wittling et al., 1998a, Wittling et al., 1998b, Critchley et al., 2005, Cechetto and Shoemaker, 2009). Adicionalmente, estudos anatômicos relatam que as vias centrais relacionadas ao controle cardiovascular apresentam projeções predominantemente ipsilaterais (Huang et al., 2002), o que corrobora com os achados funcionais de lateralização no controle do leito simpático renal.

Recentemente, foi demonstrado que a ativação unilateral do DMH provoca aumento na expressão da proteína de ativação neuronal recente, Fos, predominantemente ipsilateral em relés sinápticos bilaterais relacionados ao controle cardiovascular (Zaretskaia et al., 2008). Regiões que recebem projeções do DMH, como os núcleos pré-motores simpáticos PVH e RVLM

apresentaram expressão de Fos ipsilateralmente ao lado do DMH que recebeu injeção de BMI. Especificamente com relação às projeções oriundas do DMH e PAG (ter Horst and Luiten, 1986, Thompson et al., 1996, Farkas et al., 1998), nós acreditamos que o recrutamento de eferências predominantemente lateralizados estaria controlando o lado ipsilateral de leitos simpáticos bilaterais, como o leito renal.

Embora existam dados anatômicos e funcionais que suportem nossos achados sobre lateralização no controle de leitos vasculares, nossos resultados funcionais recentes são sistemáticos e pioneiros, fornecendo dados consistentes sobre o envolvimento de possível lateralização e assimetria na variabilidade das respostas cardiovasculares evocadas por núcleos diencefálicos, mesencefálicos e bulbares, como DMH, PAG e RP, respectivamente. Inicialmente, demonstramos que a desinibição unilateral do DMH, pela nanoinjeção do antagonista $GABA_A$, provoca respostas cardiovasculares lateralizadas e assimétricas. A injeção de BMI no DMH unilteral provoca respostas simpatoexcitatórias lateralizadas para o nervo renal. Além disso, o recrutamento de neurônios do DMH direito parece ser essencial na geração de respostas cronotrópicas mais amplas [ver anexo 1 (Xavier et al., 2009)]. Contudo, os mecanismos envolvidos na assimetria no controle cardíaco ainda necessitavam ser melhor investigados.

Nós observamos que a inibição da RP abole as diferenças na amplitude das respostas cronotrópicas e inotrópicas entre os lados direito e esquerdo do DMH. Tomando como base resultados anteriores (Xavier et al., 2009, Xavier et al., 2010) e atuais, acreditamos que a estimulação do DMH direito recruta mais neurônios pré-motores simpáticos cardíacos, atingindo assim maiores

amplitudes nas respostas inotrópicas e cronotrópicas cardíacas. Considerando que grupos de neurônios pré-motores simpáticos e nervos cardíacos diferem em termos de função (Randall et al., 1972, Schwartz et al., 1976, Kamosinska et al., 1989, Furukawa et al., 1990, Campos and McAllen, 1999), é plausível considerar que seu recrutamento diferencial poderia ser determinante da variabilidade individual nas respostas cardiovasculares. Nossos resultados pioneiros também demonstram que as respostas inotrópicas evocadas pela estimulação do DMH são independentes das simultâneas influências cronotrópicas e de pós-carga, como visto nos experimentos realizados sob bloqueio do marcapasso e dos receptores beta-adrenérgicos cardíacos. Eles permitem concluir que o DMH provoca alterações diretamente na função contrátil cardíaca, sendo o DMH direito responsável pela geração de respostas inotrópicas de maior magnitude, relacionadas a maior incidência de batimentos ectópicos (ver anexo 3).

A inibição de neurônios da RP, um núcleo central e único que recebe projeções do DMH, atenuou as respostas cardíacas e termogênicas, mas não as respostas pressoras causadas pela estimulação do DMH unilateral. Já havia sido previamente relatado que as respostas pressora e simpatoexcitatória renal são predominantemente governadas pelos neurônios pré-motores da RVLM (Fontes et al., 2001). Adicionalmente, foi visto que a resposta taquicárdica evocada pelo DMH não é alterada pela inibição da RVLM, mas sim pela inibição de neurônios da RP. Nossos resultados estendem estes achados prévios (Cao and Morrison, 2003, Cao et al., 2004), demonstrando que a inibição da RP abole as diferenças nas respostas cronotrópicas e inotrópicas cardíacas evocadas pelos lados direito e esquerdo do DMH. Isto pode estar

relacionado a diferenças anatômicas e funcionais na via DMH unilateral-RP. Apesar de claramente demonstrarem que o recrutamento de neurônios da RP é determinante das respostas assimétricas do DMH, estudos complementares ainda são necessários para melhor elucidar os mecanismos envolvidos nas respostas controladas pela via DMH-RP.

No presente estudo, um dos objetivos foi também avaliar a contribuição dos receptores EAA que existem no DMH e PAG para as respostas assimétricas e lateralizadas já descritas. Nós observamos que os receptores EAA estão envolvidos nestas respostas (ver anexo 4). Entretanto, somadas à dados anteriores (Soltis and DiMicco, 1992, da Silva et al., 2006, de Menezes et al., 2009), a nanoinjeção de NMDA e Kyn no DMH e PAG unilateral revelaram que os EAA no DMH direito são essenciais para atingir a performance cardíaca máxima durante estresse. Além disso, é possível sugerir que as respostas lateralizadas, mas não as assimétricas, evocadas pelo DMH poderiam passar pela PAG, uma vez que a estimulação unilateral da PAG revelou lateralidade semelhante ao DMH, mas não mostrou diferenças entre seus lados direito e esquerdo. É importante considerar que as respostas lateralizadas geradas tanto pelo DMH quanto pela PAG unilaterais poderiam ser controladas pelos pré-motores simpáticos PVH e RVLM, sabidamente recrutados para que se alcancem especialmente respostas vasomotoras (Lovick, 1993, Ter Horst et al., 1996, Farkas et al., 1998, Fontes et al., 2001, Vianna and Brandao, 2003, Zaretskaia et al., 2008).

Em uma revisão recente (Fontes et al., 2011), baseamos a redação da sessão que trata de assimetria cerebral e DMH em publicações de artigos e resumos com dados da presente tese (ver anexo 2), sumarizando nosso

entendimento sobre tais resultados. Nela, nós propomos que as respostas lateralizadas que partem do DMH são determinadas pelas projeções lateralizadas para núcleos integradores bilaterais (DMH-PAG-RVL e outros), seguindo também uma via lateralizada para o sistema de condução das eferências simpáticas. Por outro lado, a assimetria no controle cardíaco é governada pela via DMH-RP (ver figura 2 do paper Fontes et al., 2011 – Anexo 2).

É possível afirmar que a via DMH-PAG-RP está envolvida na organização de respostas cardiovasculares assimétricas e lateralizadas. Além disso, as diferenças na amplitude das respostas cardíacas evocadas pelo DMH unilateral é controlada pelos neurônios da RP. É possível sugerir que a abordagem utilizada pode auxiliar na compreensão da variabilidade individual das respostas cardiovasculares ao estresse e nos mecanismos centrais que participam de processos fisiopatológicos cardiovasculares.

7 – CONCLUSÃO

Nossos dados permitem concluir que: i) há assimetria no controle da performance miocárdica pelo hipotálamo dorsomedial; ii) a assimetria no controle cronotrópico cardíaco e lateralização no controle da atividade simpática pelo dorsomedial dependem dos receptores de aminoácido excitatório; iii) existe lateralização no controle da atividade simpática renal pelo hipotálamo dorsomedial e pela substância cinzenta periaquedutal; iv) a lateralização controle simpático renal pelo hipotálamo dorsomedial passa pela substância cinzenta periaquedutal; v) o recrutamento os neurônios da *raphe pallidus* é determinante de respostas cardíacas mais amplas evocadas pelo lado direito do hipotálamo dorsomedial. Estudos complementares são necessários para o melhor entendimento dos mecanismos neuroquímicos e anatômicos envolvidos na elaboração de respostas assimétricas e lateralizadas de diferentes amplitude.

8 – REFERÊNCIAS

Amendt K, Czachurski J, Dembowsky K, Seller H (1979) Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. J Auton Nerv Syst 1:103-107.

Apps R, Ruigrok TJ (2007) A fluorescence-based double retrograde tracer strategy for charting central neuronal connections. Nature protocols 2:1862-1868.

Bard P (1928) A diencephalic mechanism for the expression of rage with special reference to the sympathetic nervous system. Am J Physiol 84:490-515.

Blessing WW, Goodchild AK, Dampney RA, Chalmers JP (1981) Cell groups in the lower brain stem of the rabbit projecting to the spinal cord, with special reference to catecholamine-containing neurons. Brain Res 221:35-55.

Campos RR, McAllen RM (1999) Cardiac inotropic, chronotropic, and dromotropic actions of subretrofacial neurons of cat RVLM. Am J Physiol 276:R1102-1111.

Cannon WB (1929) Bodily changes in pain, hunger, fear and rage: an account of recent researches into the function of emotional excitement. Appleton, New York.

Cao WH, Fan W, Morrison SF (2004) Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience 126:229-240.

Cao WH, Morrison SF (2003) Disinhibition of rostral raphe pallidus neurons increases cardiac sympathetic nerve activity and heart rate. Brain Res 980:1-10.

Carter JR, Kupiers NT, Ray CA (2005) Neurovascular responses to mental stress. J Physiol 564:321-327.

Cechetto DF, Chen SJ (1990) Subcortical sites mediating sympathetic responses from insular cortex in rats. Am J Physiol 258:R245-255.

Cechetto DF, Shoemaker JK (2009) Functional neuroanatomy of autonomic regulation. Neuroimage 47:795-803.

Critchley HD, Taggart P, Sutton PM, Holdright DR, Batchvarov V, Hnatkova K, Malik M, Dolan RJ (2005) Mental stress and sudden cardiac death: asymmetric midbrain activity as a linking mechanism. Brain 128:75-85.

da Silva LG, de Menezes RC, dos Santos RA, Campagnole-Santos MJ, Fontes MA (2003) Role of periaqueductal gray on the cardiovascular response evoked by disinhibition of the dorsomedial hypothalamus. Brain Res 984:206-214.

da Silva LG, Jr., Menezes RC, Villela DC, Fontes MA (2006) Excitatory amino acid receptors in the periaqueductal gray mediate the cardiovascular response evoked by activation of dorsomedial hypothalamic neurons. Neuroscience 139:1129-1139.

Davies SJ, Ghahramani P, Jackson PR, Noble TW, Hardy PG, Hippisley-Cox J, Yeo WW, Ramsay LE (1999) Association of panic disorder and panic attacks with hypertension. Am J Med 107:310-316.

de Menezes RC, Zaretsky DV, Fontes MA, Dimicco JA (2006) Microinjection of muscimol into caudal periaqueductal gray lowers body temperature and attenuates increases in temperature and activity evoked from the dorsomedial hypothalamus. Brain Res 1092:129-137.

de Menezes RC, Zaretsky DV, Fontes MA, DiMicco JA (2009) Cardiovascular and thermal responses evoked from the periaqueductal grey require neuronal activity in the hypothalamus. J Physiol 587:1201-1215.

de Menezes RC, Zaretsky DV, Sarkar S, Fontes MA, Dimicco JA (2008) Microinjection of muscimol into the periaqueductal gray suppresses cardiovascular and neuroendocrine response to air jet stress in conscious rats. Am J Physiol Regul Integr Comp Physiol.

De Novellis V, Stotz-Potter EH, Morin SM, Rossi F, DiMicco JA (1995) Hypothalamic sites mediating cardiovascular effects of microinjected bicuculline and EAAs in rats. Am J Physiol 269:R131-140.

Diamant M, Kashtanov, S.I., Fodor, M., De Wied, D. (1992) Corticotropin-releasing factor induces differential behavioral and cardiovascular effects after intracerebroventricular and lateral hypothalamic/perifornical injections in rats. Neuroendocrinology 56:750-760.

DiFrancesco D (1994) Some properties of the UL-FS 49 block of the hyperpolarization-activated current (i(f)) in sino-atrial node myocytes. Pflugers Arch 427:64-70.

DiMicco JA, Abshire VM (1987) Evidence for GABAergic inhibition of a hypothalamic sympathoexcitatory mechanism in anesthetized rats. Brain Res 402:1-10.

DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV (2002) The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. Pharmacol Biochem Behav 71:469-480.

DiMicco JA, Stotz-Potter EH, Monroe AJ, Morin SM (1996) Role of the dorsomedial hypothalamus in the cardiovascular response to stress. Clin Exp Pharmacol Physiol 23:171-176.

Farkas E, Jansen AS, Loewy AD (1998) Periaqueductal gray matter input to cardiac-related sympathetic premotor neurons. Brain Res 792:179-192.

Folkow B (1982) Physiological aspects of primary hypertension. Physiol Rev 62:347-504.

Fontes MA, Tagawa T, Polson JW, Cavanagh SJ, Dampney RA (2001) Descending pathways mediating cardiovascular response from dorsomedial hypothalamic nucleus. Am J Physiol Heart Circ Physiol 280:H2891-2901.

Fontes MA, Xavier CH, de Menezes RC, Dimicco JA (2011) The dorsomedial hypothalamus and the central pathways involved in the cardiovascular response to emotional stress. Neuroscience 184:64-74.

Furukawa Y, Wallick DW, Martin PJ, Levy MN (1990) Chronotropic and dromotropic responses to stimulation of intracardiac sympathetic nerves to sinoatrial or atrioventricular nodal region in anesthetized dogs. Circ Res 66:1391-1399.

Gerin W, Chaplin W, Schwartz JE, Holland J, Alter R, Wheeler R, Duong D, Pickering TG (2005) Sustained blood pressure increase after an acute stressor: the effects of the 11 September 2001 attack on the New York City World Trade Center. J Hypertens 23:279-284.

Halliwill JR, Lawler LA, Eickhoff TJ, Dietz NM, Nauss LA, Joyner MJ (1997) Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. J Physiol 504 (Pt 1):211-220.

Harris CW, Edwards, J.L., Baruch, A., Riley, W.A., Pusse,r B.E., Rejeski, W.J., Herrington, D.M. (2000) Effects of mental stress on brachial artery flow-mediated vasodilation in healthy normal individuals. American Heart Journal 139:405-411.

Harris JA, Guglielmotti V, Bentivoglio M (1996) Diencephalic asymmetries. Neurosci Biobehav Rev 20:637-643.

Hayashi N, Someya N, Endo MY, Miura A, Fukuba Y (2006) Vasoconstriction and blood flow responses to in visceral arteries to mental task in humans. Experimental Physiology 91:215-220.

Horiuchi J, McAllen RM, Allen AM, Killinger S, Fontes MA, Dampney RA (2004) Descending vasomotor pathways from the dorsomedial hypothalamic nucleus: role of medullary raphe and RVLM. Am J Physiol Regul Integr Comp Physiol 287:R824-832.

Horiuchi J, McDowall LM, Dampney RA (2009) Vasomotor and respiratory responses evoked from the dorsolateral periaqueductal grey are mediated by the dorsomedial hypothalamus. J Physiol 587:5149-5162.

Hosoya Y, Ito R, Kohno K (1987) The topographical organization of neurons in the dorsal hypothalamic area that project to the spinal cord or to the nucleus raphe pallidus in the rat. Exp Brain Res 66:500-506.

Huang J, Chowhdury SI, Weiss ML (2002) Distribution of sympathetic preganglionic neurons innervating the kidney in the rat: PRV transneuronal tracing and serial reconstruction. Auton Neurosci 95:57-70.

Hunt JL, Zaretsky DV, Sarkar S, Dimicco JA (2010) Dorsomedial hypothalamus mediates autonomic, neuroendocrine, and locomotor responses evoked from

the medial preoptic area. Am J Physiol Regul Integr Comp Physiol 298:R130-140.

Hurley KM, Herbert H, Moga MM, Saper CB (1991) Efferent projections of the infralimbic cortex of the rat. J Comp Neurol 308:249-276.

Jansen AS, Wessendorf MW, Loewy AD (1995) Transneuronal labeling of CNS neuropeptide and monoamine neurons after pseudorabies virus injections into the stellate ganglion. Brain Res 683:1-24.

Julius S (1993) Corcoran Lecture. Sympathetic hyperactivity and coronary risk in hypertension. Hypertension 21:886-893.

Kamosinska B, Nowicki D, Szulczyk P (1989) Control of the heart rate by sympathetic nerves in cats. J Auton Nerv Syst 26:241-249.

Lane JD, Adcock RA, Burnett RE (1992a) Respiratory sinus arrhythmia and cardiovascular responses to stress. Psychophysiology 29:461-470.

Lane RD, Wallace JD, Petrosky PP, Schwartz GE, Gradman AH (1992b) Supraventricular tachycardia in patients with right hemisphere strokes. Stroke 23:362-366.

Lathers CM, Schraeder PL (2006) Stress and sudden death. Epilepsy Behav 9:236-242.

Leor J, Poole WK, Kloner RA (1996) Sudden cardiac death triggered by an earthquake. N Engl J Med 334:413-419.

Lisa M, Filippelli A, Marmo E, Wible JH, Jr., DiMicco JA (1989a) Microinjection of muscimol into posterior hypothalamus blocks cardiovascular response to experimental stress in rats. Pharmacol Res 21 Suppl 1:9-10.

Lisa M, Marmo E, Wible JH, Jr., DiMicco JA (1989b) Injection of muscimol into posterior hypothalamus blocks stress-induced tachycardia. Am J Physiol 257:R246-251.

Loewy AD (1981) Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. Brain Res 222:129-133.

Lovallo WR, Gerin W (2003) Psychophysiological reactivity: mechanisms and pathways to cardiovascular disease. Psychosom Med 65:36-45.

Lovick TA (1993) The periaqueductal gray-rostral medulla connection in the defence reaction: efferent pathways and descending control mechanisms. Behav Brain Res 58:19-25.

Nakamura K, Matsumura K, Hubschle T, Nakamura Y, Hioki H, Fujiyama F, Boldogkoi Z, Konig M, Thiel HJ, Gerstberger R, Kobayashi S, Kaneko T (2004) Identification of sympathetic premotor neurons in medullary raphe regions mediating fever and other thermoregulatory functions. J Neurosci 24:5370-5380.

Nakamura K, Morrison SF (2008) A thermosensory pathway that controls body temperature. Nature neuroscience 11:62-71.

Oppenheimer S (2006) Cerebrogenic cardiac arrhythmias: cortical lateralization and clinical significance. Clin Auton Res 16:6-11.

Parati G, Antonicelli R, Guazzarotti F, Paciaroni E, Mancia G (2001) Cardiovascular effects of an earthquake: direct evidence by ambulatory blood pressure monitoring. Hypertension 38:1093-1095.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York.

Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates. Amsterdam ; Boston: Elsevier Academic Press.

Queiroz EA, Okada MN, Fumega U, Fontes MA, Moraes MF, Haibara AS (2011) Excitatory amino acid receptors in the dorsomedial hypothalamus are involved in the cardiovascular and behavioural chemoreflex responses. Exp Physiol 96:73-84.

Randall WC, Armour JA, Geis WP, Lippincott DB (1972) Regional cardiac distribution of the sympathetic nerves. Fed Proc 31:1199-1208.

Salo LM, Nalivaiko E, Anderson CR, McAllen RM (2009) Control of cardiac rate, contractility, and atrioventricular conduction by medullary raphe neurons in anesthetized rats. Am J Physiol Heart Circ Physiol 296:H318-324.

Samuels BC, Zaretsky DV, DiMicco JA (2002) Tachycardia evoked by disinhibition of the dorsomedial hypothalamus in rats is mediated through medullary raphe. J Physiol 538:941-946.

Samuels BC, Zaretsky DV, DiMicco JA (2004) Dorsomedial hypothalamic sites where disinhibition evokes tachycardia correlate with location of raphe-projecting neurons. Am J Physiol Regul Integr Comp Physiol 287:R472-478.

Schmidt B, DiMicco JA (1984) Blockade of GABA receptors in periventricular forebrain of anesthetized cats: effects on heart rate, arterial pressure, and hindlimb vascular resistance. Brain Res 301:111-119.

Schwartz PJ, Stone HL, Brown AM (1976) Effects of unilateral stellate ganglion blockade on the arrhythmias associated with coronary occlusion. Am Heart J 92:589-599.

Sgoifo A, Stilli D, Medici D, Gallo P, Aimi B, Musso E (1996) Electrode positioning for reliable telemetry ECG recordings during social stress in unrestrained rats. Physiol Behav 60:1397-1401.

Smith OA, Devito, J.L., Astley, C.A. (1990) Neurons controlling cardiovascular responses to emotion are located in lateral hypothalamus-perifornical region. Am J Physiol 259:R943-R954.

Smith PA, Graham, L.N., Mackintosh, A.F., Stoker, J.B., Mary, D. (2004) Relationship Between Central Sympathetic Activity and Stages of Human Hypertension. American Journal of Hypertension 17:217-222.

Soltis RP, Cook JC, Gregg AE, Stratton JM, Flickinger KA (1998) EAA receptors in the dorsomedial hypothalamic area mediate the cardiovascular response to activation of the amygdala. Am J Physiol 275:R624-631.

Soltis RP, DiMicco JA (1991a) GABAA and excitatory amino acid receptors in dorsomedial hypothalamus and heart rate in rats. Am J Physiol 260:R13-20.

Soltis RP, DiMicco JA (1991b) Interaction of hypothalamic GABAA and excitatory amino acid receptors controlling heart rate in rats. Am J Physiol 261:R427-433.

Soltis RP, DiMicco JA (1992) Hypothalamic excitatory amino acid receptors mediate stress-induced tachycardia in rats. Am J Physiol 262:R689-697.

Stephan KE, Fink GR, Marshall JC (2007) Mechanisms of hemispheric specialization: insights from analyses of connectivity. Neuropsychologia 45:209-228.

Stotz-Potter EH, Morin SM, DiMicco JA (1996a) Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air

stress-induced neuroendocrine and cardiovascular changes in rats. Brain Res 742:219-224.

Stotz-Potter EH, Willis LR, DiMicco JA (1996b) Muscimol acts in dorsomedial but not paraventricular hypothalamic nucleus to suppress cardiovascular effects of stress. J Neurosci 16:1173-1179.

Tanaka M, McAllen RM (2008) Functional topography of the dorsomedial hypothalamus. Am J Physiol Regul Integr Comp Physiol 294:R477-486.

Taylor RB, Weaver LC (1992) Spinal stimulation to locate preganglionic neurons controlling the kidney, spleen, or intestine. Am J Physiol 263:H1026-1033.

Ter Horst GJ, Hautvast RW, De Jongste MJ, Korf J (1996) Neuroanatomy of cardiac activity-regulating circuitry: a transneuronal retrograde viral labelling study in the rat. Eur J Neurosci 8:2029-2041.

ter Horst GJ, Luiten PG (1986) The projections of the dorsomedial hypothalamic nucleus in the rat. Brain Res Bull 16:231-248.

Thompson RH, Canteras NS, Swanson LW (1996) Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. J Comp Neurol 376:143-173.

Toga AW, Thompson PM (2003) Mapping brain asymmetry. Nat Rev Neurosci 4:37-48.

Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32-58.

Vianna DM, Brandao ML (2003) Anatomical connections of the periaqueductal gray: specific neural substrates for different kinds of fear. Braz J Med Biol Res 36:557-566.

Wittling W (1997) The right hemisphere and the human stress response. Acta Physiol Scand Suppl 640:55-59.

Wittling W, Block A, Genzel S, Schweiger E (1998a) Hemisphere asymmetry in parasympathetic control of the heart. Neuropsychologia 36:461-468.

Wittling W, Block A, Schweiger E, Genzel S (1998b) Hemisphere asymmetry in sympathetic control of the human myocardium. Brain Cogn 38:17-35.

Xavier CH, Beig MI, Ianzer D, Fontes MA, Nalivaiko E (2010) Cardiac chronotropic and inotropic responses evoked from right or left sides of dorsomedial hypothalamus. FASEB Journal 24:1019.1020.

Xavier CH, Nalivaiko E, Beig MI, Menezes GB, Cara DC, Campagnole-Santos MJ, Fontes MA (2009) Functional asymmetry in the descending cardiovascular pathways from dorsomedial hypothalamic nucleus. Neuroscience 164:1360-1368.

Xavier CH, Villela DC, Fontes MA (2008) Influência do sistema nervoso na hipertensão arterial: vias centrais envolvidas na resposta cardiovascular ao estresse emocional. Hipertensão 11:65-70.

Yanowitz F, Preston JB, Abildskov JA (1966) Functional distribution of right and left stellate innervation to the ventricles. Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. Circ Res 18:416-428.

Zagon A, Smith AD (1993) Monosynaptic projections from the rostral ventrolateral medulla oblongata to identified sympathetic preganglionic neurons. Neuroscience 54:729-743.

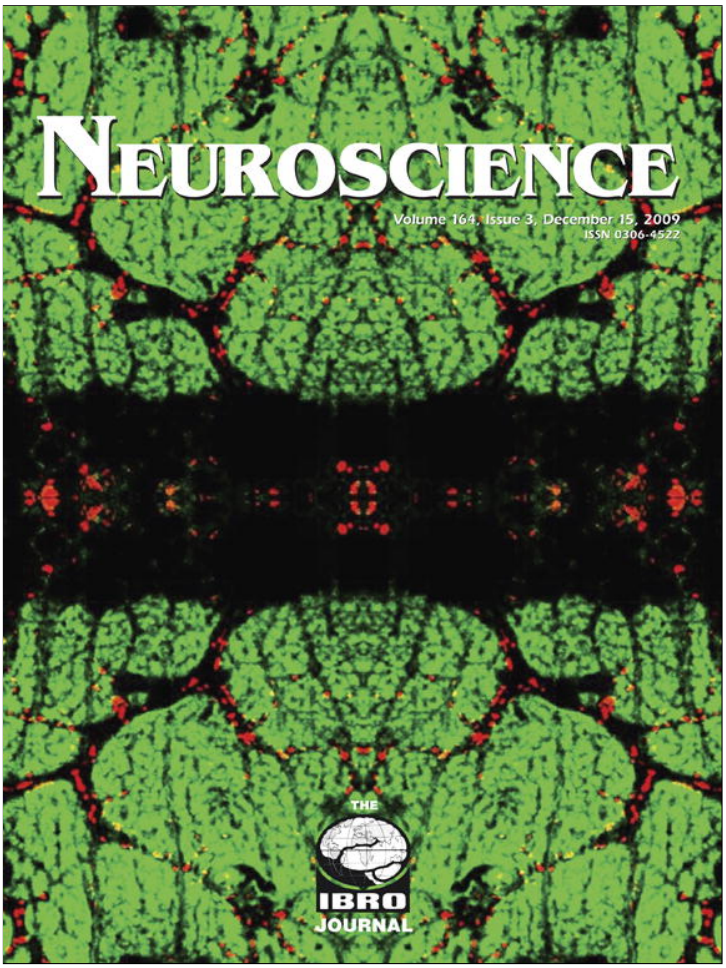
Zaretskaia MV, Zaretsky DV, Sarkar S, Shekhar A, DiMicco JA (2008) Induction of Fos-immunoreactivity in the rat brain following disinhibition of the dorsomedial hypothalamus. Brain Res 1200:39-50.

Zaretsky DV, Zaretskaia MV, Samuels BC, Cluxton LK, DiMicco JA (2003) Microinjection of muscimol into raphe pallidus suppresses tachycardia associated with air stress in conscious rats. J Physiol 546:243-250.

9 – ANEXOS

Artigos publicados ou submetidos para publicação contendo resultados da presente tese.

Anexo 1



Neuroscience 164 (2009) 1360–1368

FUNCTIONAL ASYMMETRY IN THE DESCENDING CARDIOVASCULAR PATHWAYS FROM DORSOMEDIAL HYPOTHALAMIC NUCLEUS

C. H. XAVIER,[a] E. NALIVAIKO,[b] M. I. BEIG,[b] G. B. MENEZES,[c] D. C. CARA,[d] M. J. CAMPAGNOLE-SANTOS[a] AND M. A. P. FONTES[a]*

[a]*Laboratório de Hipertensão, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil*

[b]*Neurocardiology Laboratory, School of Biomedical Sciences, University of Newcastle, Newcastle, New South Wales, Australia*

[c]*Laboratório de Neuroimunopatologia Experimental, Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil*

[d]*Laboratório do Sistema Linfóide e da Regeneração, Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil*

Abstract—Neurons in the dorsomedial hypothalamus (DMH) play a key role in mediating tachycardia elicited by emotional stress. DMH activation by microinjections of the $GABA_A$ antagonist evokes tachycardia and physiological changes typically seen in experimental stress. DMH inhibition abolishes the tachycardia evoked by stress. Based on anatomic evidences for lateralization in the pathways from DMH, we investigated a possible inter-hemispheric difference in DMH-evoked cardiovascular responses. In anesthetized rats we compared changes in heart rate (HR), renal sympathetic activity (RSNA), mesenteric blood flow (MBF) and tail vascular conductance produced by activation of right (R) and left (L) sides of the DMH. We also evaluated the tachycardia produced by air jet stress after inhibition of R or L DMH. There were always greater increases in RSNA when bicuculline was injected ipsilaterally to the side where these parameters were recorded (average ΔRSNA: L=+50% and R=+26%; $P<0.05$). Compared to pre-injection values, right DMH activation caused pronounced decrease (0.87±0.1% vs. 0.4±0.11%/mm Hg; $P<0.05$), whereas bicuculline methiodide (BMI) into left DMH produced no significant changes (0.95±0.09% vs. 1.04±0.25%/mm Hg) in tail vascular conductance. R or L DMH disinhibition produced decreases in MBF, but no differences in the range of these changes were observed. Activation of the right DMH caused greater tachycardia compared to the left DMH activation (average ΔHR: R=+92 bpm; L=+48 bpm; $P<0.05$). Tachycardia evoked by air jet stress was smallest after right DMH inhibition (average ΔHR: R=+57 bpm and L=+134 bpm; $P<0.05$). These results indicate that the descending cardiovascular pathways from DMH are predominantly lateralized and the right DMH might exert a prominent control on heart rate changes during emotional stress.

Key words: asymmetry, dorsomedial hypothalamus, emotional stress, laterality, tachycardia.

Stress is thought to contribute to the development of cardiovascular diseases such as hypertension (Esler et al., 2008) and cardiac arrhythmias (Rozanski et al., 1999), and revealing the relevant central mechanisms is a critical step to understand the pathogenesis of stress-related cardiovascular diseases.

The dorsomedial hypothalamus (DMH) coordinates neuroendocrine, autonomic and behavioral responses to emotional stress (DiMicco et al., 2002; Chou et al., 2003). Inhibition of DMH neurons abolishes the tachycardia evoked by experimental emotional stress (Stotz-Potter et al., 1996b; McDougall et al., 2004). In anesthetized rats, removal of the tonic inhibitory input to DMH neurons evokes increases in heart rate (HR), blood pressure (BP), renal (Fontes et al., 2001) and cardiac sympathetic nerve activity (Cao et al., 2004). In conscious rats, activation of DMH neurons increases HR, BP (da Silva et al., 2006) and plasma adrenocorticotropic hormone (ACTH) (Bailey and Dimicco, 2001), a hallmark neuroendocrine response to stress. In addition, DMH activation also elicits panic-like behavioral responses (Johnson and Shekhar, 2006). Given the striking similarity between the response to acute emotional stress in rats and the response evoked by chemical activation of DMH (DiMicco et al., 2002), this procedure has been used to investigate the descending cardiovascular pathways from DMH and therefore those mediating the cardiovascular response to stress (Fontes et al., 2001; Samuels et al., 2002; Cao et al., 2004; Horiuchi et al., 2004).

Left–right differences in the functional properties of bilateral nervous system regions are known as lateralization of function. This phenomenon has been observed in different brain structures and at different levels of neuraxis (Schwartz et al., 1976; Harris et al., 1996; Toga and Thompson, 2003). In some conditions, asymmetric function of brain regions might result in lateralized imbalance of autonomic input to the heart, possibly leading to cardiac arrhythmias (Lane and Schwartz, 1987), and this includes mental stress conditions (Critchley et al., 2005).

Considering the critical role of DMH in mediating the autonomic cardiovascular responses to emotional stress,

*Correspondence to: M. A. P. Fontes, Laboratório de Hipertensão, Departamento de Fisiologia e Biofísica-ICB, Universidade Federal de Minas Gerais, Antonio Carlos Avenue, 6627, Belo Horizonte, MG 31270-901, Brazil. Tel: +55-31-3409-2953; fax: +55-31-3409-2924.
E-mail address: peliky@icb.ufmg.br (M. A. P. Fontes).
All experimental procedures were performed by Xavier C. H. All authors were involved in, conception and design, drafting the article or revising it critically for important intellectual content, final approval of the version to be published.
Abbreviations: ACTH, adrenocorticotropic hormone; AV, atrioventricular; BMI, bicuculline methiodide; BP, blood pressure; DHA, dorsal hypothalamic area; DMH, dorsomedial hypothalamus; $GABA_A$, γ-aminobutyric acid receptor subtype A; HR, heart rate; L, left; MAP, mean arterial pressure; MBF, mesenteric blood flow; PAG, periaqueductal gray; PVN, paraventricular nucleus; R, right; RSNA, renal sympathetic nerve activity; SA, sinoatrial.

0306-4522/09 $ - see front matter © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2009.09.018

the question then arises as to whether the functional cardiovascular responses evoked by left or right DMH might be different. The present study was undertaken to systematically compare cardiovascular responses mediated by the right and left sides of DMH. The general strategy in these experiments was to test whether pharmacological activation or inhibition of the left or right sides of DMH differentially affect functional properties (sympathetic activity, heart rate, hemodynamic) in different target organs. Parts of these results have been published in abstract form (Fontes et al., 2008; Xavier et al., 2008).

EXPERIMENTAL PROCEDURES

All experiments were performed on male Wistar rats (250–320 g) bred at the animal facilities of the Biological Sciences Institute (CEBIO, UFMG, Belo Horizonte, MG, Brazil) and conducted in accordance with the guidelines established by our local institutional animal welfare committee (CETEA/UFMG protocol number 137/2006), and in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used. Animals were housed in individual home cages (47 cm ×31 cm×16 cm) and had free access to food and water.

Experiments in anesthetized rats

Rats were anesthetized with urethane (Sigma, USA) (1.2–1.4 g/kg i.p.), and the trachea was cannulated to maintain the airways opened. The adequacy of anesthesia was verified by the absence of a withdrawal response to nociceptive stimulation of a hindpaw. Supplemental doses of urethane (0.1 g/kg i.v.) were given when necessary. Body temperature was maintained in the range of 36.5–37.0 °C with a heating lamp. The head was placed in a stereotaxic frame (Stoelting, IL, USA) with the tooth bar fixed at −3.3 mm below the interaural line. A small craniotomy was made near the bregma to allow for later insertion of an injection needle into the DMH (3.2 mm posterior, 0.6 mm lateral, 8.5 mm ventral). These initial surgical procedures were performed in all rats before the specific surgery for recording of different cardiovascular variables as described below.

Experiment 1. Effects of DMH activation on heart rate (HR), mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA) and tail pulse pressure. Catheters were placed in a femoral artery and vein (to record AP/HR and for supplementary anesthesia, respectively). Using a retroperitoneal approach, the left renal nerve was isolated and covered with mineral oil, and put in contact with a silver bipolar electrode. RSNA signal was amplified by 10K, filtered (100–1000 Hz), displayed on an oscilloscope and monitored by means of an audio amplifier. The filtered nerve activity signal was rectified; integrated (resetting every 2 s), displayed online and acquired using Powerlab 4/20—Chart 5.0 (AD-Instruments, Sydney, Australia). All data were digitized at 1 kHz. The noise level of the RSNA recording system was determined postmortem and subtracted from initial RSNA values.

Aiming to compare the changes on HR, MAP and RSNA evoked by activation of the left DMH and the right DMH, we divided these experiments in two protocols. In protocol 1 (n=5), microinjection of bicuculline methiodide (BMI) (a $GABA_A$ receptor antagonist; 40 pmol/100 nl) was made into left DMH and 40 min later, after variables returned to the baseline, a second injection of BMI was made into the right DMH. In protocol 2 (n=5), using the same procedures, the sequence of microinjections into DMH was reverse, that is first into right DMH and 40 min later, into left DMH. This strategy was adopted in order to avoid misinterpretation of data caused by a possible spreading of BMI from one side to another and consequently interfering with the effect produced by the second microinjection.

In some animals used in protocols 1 and 2 (n=6), instead of RSNA, we assessed tail vascular conductance through the tail pulse pressure amplitude (a surrogate of tail blood flow), by placing a piezo-electric pulse transducer (MLT1010, ADInstruments, Sydney, Australia) around the proximal portion of the tail, as reported previously in rabbits (Nalivaiko and Blessing, 1999). The temperature of the animals was rigorously controlled and maintained as close as possible to 36.5 °C with a heating pad.

Experiment 2. Effects of DMH activation on the mesenteric blood flow (MBF). This study was conducted in a separate group of rats (n=5). A segment of jejunum was exteriorized through the abdominal incision, and the mesentery was draped over an optically clear viewing pedestal that allows for transillumination of tissue as previously described (Baker and Wayland, 1974; House and Lipowsky, 1987; Gaboury et al., 1995; Kubes et al., 1995, 2003). All exposed tissue was covered with saline-soaked gauze to minimize tissue dehydration. The temperature of the pedestal and mesentery were maintained at 37 °C. The exposed mesentery was suffused using a warmed bicarbonate-buffered saline (pH=7.4). The mesenteric preparation was then observed by using a microscope (Olympus BX-40) with a ×10 objective lens and a ×10 eyepiece. A videocamera (Sony, Japan) mounted on the microscope projected the image onto a color monitor, and the images were recorded for playback analysis using a color DVD recorder (LG, Brazil). To evaluate the flow velocity, unbranched mesenteric vennules (diameter 25–40 micrometers) were chosen. Red cells velocity (Vrbc) was measured on-line using an optical Doppler velocimeter (Microcirculation Research Institute, TX, USA) connected to a light microscope (Olympus BX-40). Venular blood flow (Vmean) was calculated from the product of cross-sectional area and mean Vrbc (Vmean=Vrbc/1.6), assuming cylindrical geometry. Baseline flow values were obtained before BMI injection into DMH.

As above described technique required supine position of animals, intra-DMH microinjections were made using guide cannulas implanted during the preliminary surgery performed 1 week prior to the experiment. In these experiments, which were performed in another laboratory without measuring arterial pressure, the procedures for microinjections were the same as in experiment 1, but only one sequence was made. The side for the first DMH injection chosen randomly. The second injection was made in the contralateral side.

Experiments in conscious rats

Experiment 3. Effects of unilateral DMH inhibition on stress-induced pressor and tachycardic responses. Under tribromoethanol (Sigma, USA) anesthesia (250 mg/kg i.p.), rats were placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA) and instrumented with bilateral stainless steel guide cannulas (22 gauge, 16 mm length) targeted to the DMH. The guide cannulas were fixed to the skull by dental acrylic cement anchored with stainless steel screws. After surgical procedures, animals were allowed to recover in their home cages for at least 3 days. Subsequently, they were anesthetized again with tribromoethanol (250 mg/kg i.p.), and polyethylene catheter (0.011 ID, Clay Adams, Parsippany, NJ, USA) filled with heparinized saline and sealed with a stylet was inserted in the abdominal aorta through the femoral artery for recording of BP and HR. The catheter was routed s.c. to the nape of the neck where it was exteriorized and secured. Rats were then allowed to recover in their home cages for at least 24 h before experiments began. All animals remained in good health conditions throughout the course of surgical procedures and experimental protocol as assessed by appearance, behavior, and maintenance of body weight.

On the day of experiment, after 20 min of baseline heart rate and blood pressure monitoring, a $GABA_A$ agonist muscimol (1 nmol/100 nl) was injected unilaterally into either left or right DMH. Rats were then placed into a plastic restrainer (60-mm inner diameter) and subjected to a 10-min air jet stress—a stream of air (10 l/min) directed to the head. After the air jet, the animals remained in the

restrainer for additional 10 min. Air jet stress was repeated on three consecutive days. In five animals, the right DMH was inhibited prior to the first stress exposure, and the left DMH was inhibited prior to the second one (protocol 1); in five other rats the sequence was reverse (protocol 2). On the third day, in all rats used in protocols 1 and 2, the air jet stress was repeated after bilateral microinjection of vehicle (NaCl 0.9%, 100 nl) into the DMH.

Microinjections and drugs

The drugs employed were the $GABA_A$ receptor antagonist, BMI (Sigma; 40 pmol/100 nl), the $GABA_A$ agonist, muscimol (Sigma; 1 nmol/100 nl), or vehicle (NaCl 0.9%, 100 nl). Microinjections were made into DMH with a 30-gauge injection needle connected to a Hamilton syringe (5 μl) as described previously (da Silva et al., 2003). The coordinates for injections were −3.2 mm posterior and ±0.6 mm lateral to the bregma and at a depth of −8.6 mm below the dura, as determined by the atlas of Paxinos and Watson (Paxinos and Watson, 1986).

Histology

At the end of experiments, all rats were euthanized by an overdose of anesthetic, and a microinjection of Alcian Blue dye 2% (100 nl) was made into injection sites for subsequent histological confirmation according to the methodology described previously (da Silva et al., 2003). The atlas of Paxinos and Watson (Paxinos and Watson, 1986) was used as a reference for histological confirmation of the injection sites in the DMH.

Analysis

Comparisons between responses evoked by the first and second microinjections of BMI into DMH were determined by a paired *t*-test. In air jet stress experiments, comparisons between groups were performed with two-way analysis of variance (factors drug and time) followed by Bonferroni's post hoc test. For computing a surrogate for tail vascular conductance, we divided normalized pulse pressure amplitude by corresponding value of the MAP. Significance was taken at $P<0.05$. Data are expressed as mean±SEM.

RESULTS

Comparison of the cardiovascular effects evoked by disinhibition of left or right sides of DMH in anesthetized animals

There were no differences in the basal values of HR and MAP, recorded in different groups of rats (Table 1). Fig. 1 shows the effect on HR, MAP, RSNA and tail vascular conductance produced by microinjection of BMI into the left or right sides of DMH. Unilateral disinhibition of the DMH produced marked increases in HR and RSNA accompanied by increases in MAP (Fig. 1) as has been reported previously (Fontes et al., 2001). These responses began within 30 s after microinjection of BMI into DMH, reached peak levels within 5–10 min and were sustained for at least 10 min before returning to control levels. Pressor responses produced by right- and left-side hypothalamic activation did not differ (Fig. 2A). No significant differences in the magnitude of the increases in MAP was observed between groups (mean maximum changes: +12±3 mm Hg; averaged for all groups). In contrast, activation of the right DMH always caused a greater tachycardia when compared with left side (protocol 1: L=+46±8 bpm vs. R=+102±16 bpm; protocol 2: R=+81±9 bpm vs. L=+50±6 bpm; $P<0.05$; Fig. 2B). In both protocols, there were always greater increases in RSNA when BMI was injected into the left DMH, that is, ipsilateral to the side where RSNA was recorded (average RSNA: L=+50% and R=+26.5%; $P<0.05$; Fig. 2C).

Compared to the pre-injection values, administration of BMI into left DMH caused no significant changes in the tail vascular conductance (0.95±0.09 vs. 1.04±0.25%/mm Hg), whereas BMI into right DMH produced pronounced decrease (0.87±0.1 vs. 0.4±0.11%/mm Hg; $P<0.05$) (Fig. 2D).

Effect of microinjection of BMI into DMH on the mesenteric blood flow was assessed in five rats. Disinhibition of left or right DMH induced pronounced falls in the mesenteric blood flow (Fig. 3, see supplementary data). Approximately 50% reduction of the blood flow occurred in the first 2 min, and the response reached minimum (25–30% of basal level) at 8–10 min post-injection. This effect was reversible, returning to the baseline approximately 20 min after microinjection BMI into DMH. There were no differences between effects evoked from left and right DMH.

Effects of DMH inhibition on cardiovascular effects produced by air jet stress. Fig. 4. shows results of experiments conducted in conscious animals. Baseline HR and MAP were not significantly different between two groups of

Table 1. Basal and maximal values of mean arterial pressure (MAP) and heart rate (HR) from anesthetized and non-anesthetized rats before and after microinjections into right and left DMH on both protocols (1 and 2); or vehicle bilaterally into DMH of non-anesthetized rats

Protocols	Anesthetized				Non-anesthetized			
	Mean arterial pressure (mm Hg)		Heart rate (bpm)		Mean arterial pressure (mm Hg)		Heart rate (bpm)	
	Basal	Maximal	Basal	Maximal	Basal	Maximal	Basal	Maximal
1 - Left	90±3	104±3†	345±8	396±5†	103±2	115±3†	335±2	461±26†
1 - Right	89±4	102±5†	336±6	441±18†,*	107±4	120±3†	342±4	404±21†,*
2 - Right	95±3	106±4†	339±12	423±4†,#	107±3	116±5†	344±6	406±15†,#
2 - Left	95±2	106±3†	355±10	398±6†	107±5	120±4†	348±4	462±21†
Bilateral vehicle	—	—	—	—	105±4	116±3†	369±9	485±12†

Results showed as mean ±SE.

* $P<0.05$ – protocol 1 (L vs. R).

† $P<0.05$ (baseline vs. maximal changes).

\# $P<0.05$ – protocol 2 (R vs. L).

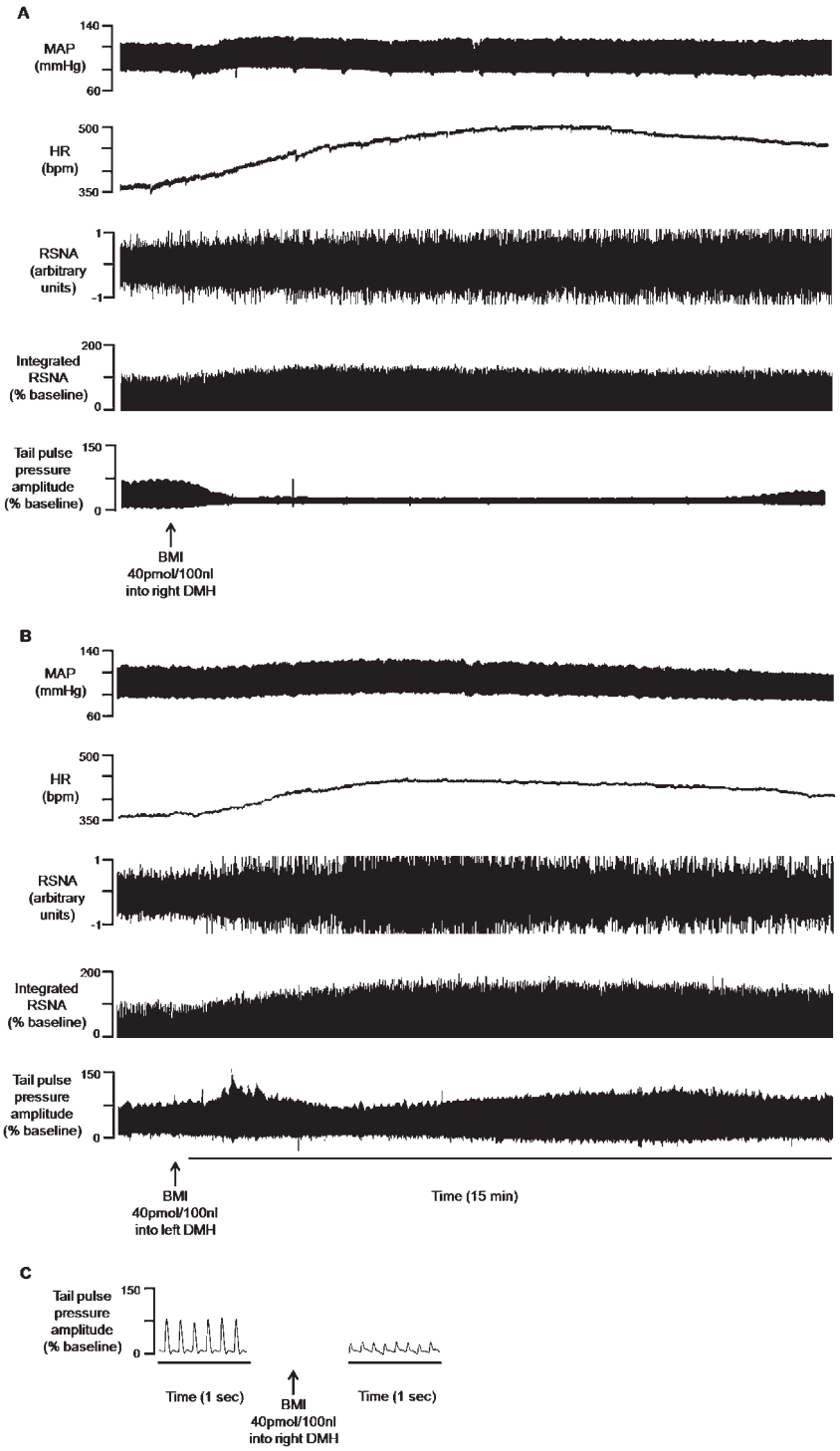


Fig. 1. Effects on HR, MAP, RSNA and tail pulse pressure amplitude produced by microinjection of BMI into the right (A) or left (B) sides of DMH. (C) tail pulse pressure amplitude in a fast time scale (1 s) showing the pulse shape before and after microinjection into DMH.

rats studied. After microinjection of saline into DMH, air jet stress evoked marked tachycardia and variable pressor responses as reported previously (Stotz-Potter et al., 1996a,b). Heart rate rose at the onset of air stress to a plateau that was maintained for the duration of the stress period and returned gradually to baseline after its termination.

Microinjection of muscimol into right DMH caused a marked attenuation in the tachycardia evoked by the airjet stress whereas microinjection into the left DMH was without effect (ΔHR: protocol 1 L=+143±17 bpm vs. R=+58±20 bpm; protocol 2 R=+56±13 bpm vs. L=+124±11 bpm; $P<0.05$ for both protocols). This attenuation was observed

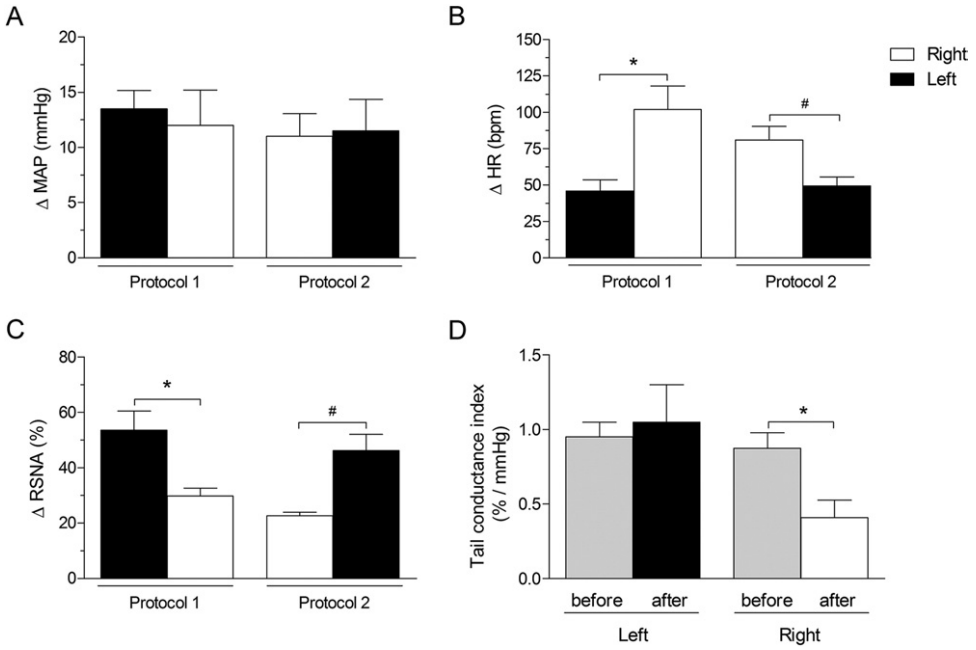


Fig. 2. Maximal changes in cardiovascular parameters (±SEM) from baseline evoked by unilateral microinjection of BMI into right (white bars) and left DMH (black bars) on both protocols at the first series of experiments. (A) mean arterial pressure (MAP); (B) heart rate (HR); (C) renal sympathetic nerve activity (RSNA) sampled from left renal nerve and (D) tail vascular conductance. * $P<0.05$—protocol 1 (L vs. R); # $P<0.05$—protocol 2 (R vs. L).

throughout the air stress trial. Also, there were no changes when comparing the range of responses evoked by air stress after microinjection of bilateral vehicle and muscimol into left DMH ($P>0.05$ for both protocols).

After inhibition of the right DMH, the HR during much of the post stress (recovery) period was also lower than that seen after treatment with saline or with muscimol injection into the left DMH side (Fig. 4).

The air jet stress evoked significant increases in mean arterial pressure (Fig. 4, Table 1). The DMH inhibition with muscimol caused no significant changes in the range of the hypertensive response provoked by the stress, when compared to the responses after microinjection of saline into DMH (mean maximum changes in MAP: protocol 1 L=115±3 mm Hg and $R=+120\pm3$ mm Hg; protocol 2 R=116±5 mm Hg and L=120±4 mm Hg; bilateral saline=116±3 mm Hg; Table 1).

Post-mortem histology confirmed that injection sites in all microinjection experiments for which data were analyzed were located in the DMH, extending from 2.8 to 3.6 mm caudal to bregma. Fig. 5 illustrates examples of typical injection sites in the DMH.

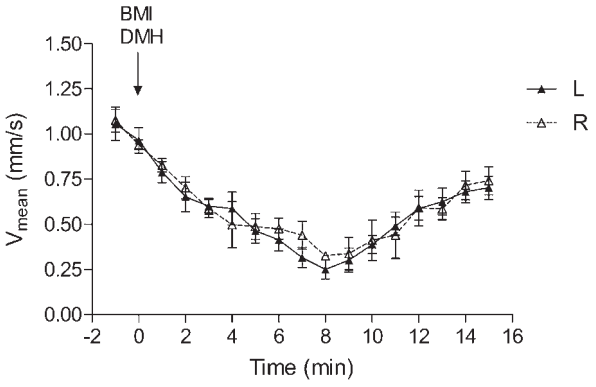


Fig. 3. Changes in mesenteric blood flow (MBF) evoked by unilateral microinjection of BMI into right (R – dashed line and open symbol) and left DMH (L – solid line and filled symbol) sampled from the end portion of jejunum at the second series of experiments.

DISCUSSION

Our two major findings are: (i) right-side dominance of the DMH in controlling cardiac cycle and tail (cutaneous) vascular bed; and (ii) predominantly ipsilateral effects of DMH on the renal sympathetic nerve activity.

DMH asymmetry in cardiac control

We observed, in anesthetized animals, that the right DMH activation causes greater tachycardia compared to the increases in HR produced by the left DMH activation. This is in good accord with our finding that the right DMH inhibition in conscious animals virtually abolished stress-induced tachycardia. Taken together, these results strongly suggest that the right DMH dominates over the left one in the control of the heart rate/cardiac cycle.

The idea of brain functional asymmetry in cardiovascular control is not new. In their pioneering study, Fang and Wang (Fang and Wang, 1962) found that electrical stimulation of the right side of hypothalamus induced greater chronotropic effect compared to the left side. Speaking more generally, there is an evidence that most components of the stress response are influenced asymmetrically by the cerebral hemispheres (Wittling, 1997), and lateralization of the function during emotional

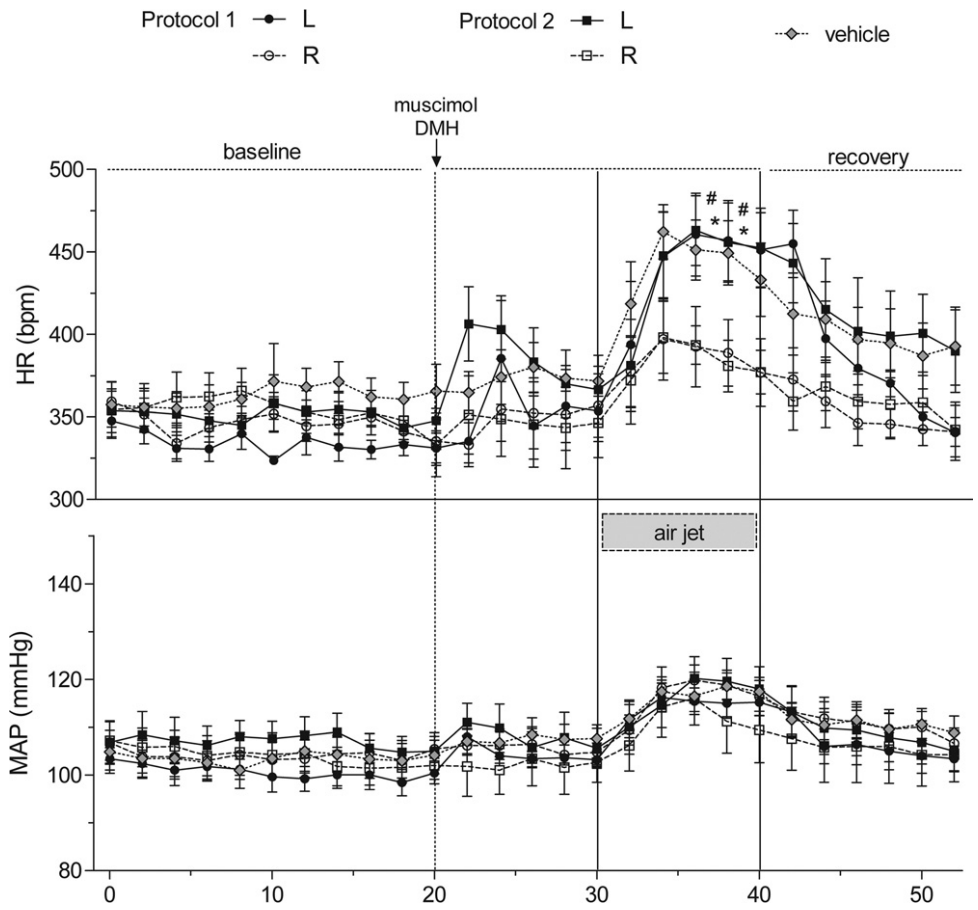


Fig. 4. Mean (±SEM) heart rate before and after injection of muscimol into right (open symbols and dashed line) and left DMH (filled symbols and solid line) or bilateral vehicle into DMH (gray symbol) on protocols of non-anesthetized rats submitted to air jet stress at the third series of experiments.

arousal is described as component in the induction of these responses in sympathetic input to heart (Lane and Schwartz, 1987).

Tachycardia evoked from the DMH results from an increase in cardiac sympathetic nerve activity (Cao et al., 2004) that is completely abolished by beta adrenergic blockade (Fontes et al., 2001). Considering this, it is possible that activation of right DMH neurons could, through its descending pathways and respective synaptic relays, mostly drive right-sided cardiac preganglionic neurons in the spinal cord. This would increase the activity of right-side cardiac sympathetic nerve. As the left and right cardiac sympathetic nerves differ in their actions on the heart, with the sinoatrial node driven principally by the right sympathetic nerve (Yanowitz et al., 1966; Randall et al., 1972; Kamosinska et al., 1989; Furukawa et al., 1990), this would result in pronounced tachycardia. Thus, ipsilateral projection from the DMH to the spinal sympathetic neurons could alone explain the asymmetry in cardiac control described above. There is currently lack of neuroanatomical data for either proving or disproving this hypothesis. The DMH contains very few neurons that project directly to the spinal cord (ter Horst and Luiten, 1986; Hosoya et al., 1987; Thompson et al., 1996), and recent studies have shown that the cardiovascular responses evoked from DMH depend on different synaptic relay nuclei, such as the rostral ventrolateral medulla (Fontes et al., 2001; Cao et al., 2004), raphe pallidus (Samuels et al., 2002; Cao et al., 2004; Horiuchi et al., 2004) and periaqueductal gray (PAG) (da Silva et al., 2003; de Menezes et al., 2006, 2008, 2009). Descending cardiovascular pathways from the DMH relay in the medulla in a differential manner: those for controlling vascular tone in different beds (except cutaneous one) pass via rostral ventrolateral medulla (RVLM) (Dampney et al., 2002) whereas those controlling cardiac chronotropic function and cutaneous vasculature descend via raphe/parapyramidal area (Nalivaiko and Blessing, 2001; Samuels et al., 2002). Retrograde viral tracing from the heart demonstrated that location of cardiac presympathetic neurons in the ventral medulla is not limited to the midline raphe zone but rather spread across the ventral surface (Ter Horst et al., 1996). Interestingly, retrograde tracing with viral injections into the right stellate ganglion did reveal more infected neurons in the ipsilateral part of the raphe/parapyramidal region (Farkas et al., 1998). This suggests that medullary presympathetic neurons controlling stress-induced tachycardia may have right-side prevalence, similar to

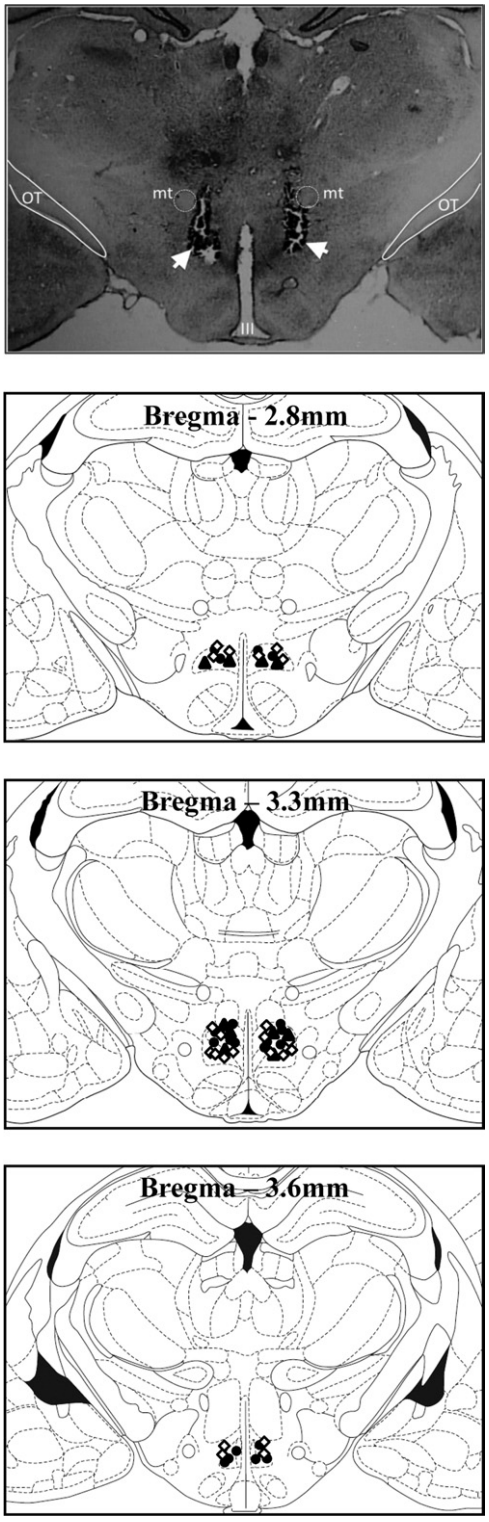


Fig. 5. Schematic coronal sections of rat brain at level of DMH eliciting approximate sites of microinjection of BMI (40 pmol/100 nl) from all series of experiments. First series (●-circles); second series (▲-triangles); third series (◇-diamonds).

medullary presympathetic neurons involved in homeostatic cardiac control (Campos and McAllen, 1999).

DMH asymmetry in the control of cutaneous vascular bed

Falls in tail pulse pressure amplitude reflect vasoconstriction in the tail vascular bed (that is predominantly cutaneous). The method used here was previously described and used in rabbits (Nalivaiko and Blessing, 1999), and correlated well with direct measurements of the blood flow. Cutaneous vasoconstriction is an integral part of arousing/defense response; it also plays a critical role in thermoregulation (Dimicco and Zaretsky, 2007; Morrison et al., 2008). Cutaneous blood flow could be readily measured in rabbit ear and rat tail; in both species potent vasoconstrictor responses may be elicited from the DMH (Nalivaiko and Blessing, 2001; Tanaka and McAllen, 2008), as was also found in the current study. Our new observation is that the right-side DMH dominates in eliciting this reaction. As evidenced by our data, left-side stimulation caused much smaller responses, or even sometimes increases rather than decreases in pulse amplitude. The latter was likely the consequence of elevated systemic arterial pressure. We do not have any potential mechanistic explanations of lateralized control of tail blood flow. In two studies that examined sympathetic innervation of rat tail with retrograde viral tracing, no lateralization in distribution of infected neurons was described in the medulla, but higher brain levels were not studied (Nakamura et al., 2004; Toth et al., 2006). It is worth noting, however, that future experiments using a direct measurement of tail blood flow or tail temperature, are necessary to further strength our current observations regarding this lateralized vasoconstrictor response elicited from right-side DMH.

DMH and renal sympathetic outflow

Unilateral (left-side) DMH activation caused greater increases in ipsilateral RSNA. This indicates that in contrast to right-side DMH dominance in controlling sympathetic outflow to the heart and the tail vasculature, there is no such dominance in the control of renal sympathetic outflow—at least left-side one. This, in turn, is in a good accord with reported here lack of right-DMH dominance in provoking generalized pressor responses, as descending pathways to spinal sympathetic neurons relay in the RVLM (see also Conclusion). Our results suggest that unilateral DMH neurons exert predominant autonomic control over ipsilateral preganglionic spinal sympathetic neurons that target kidneys. These functional findings are in agreement with previous neuroanatomical tracing studies that showed that the descending pathways from DMH are predominantly ipsilateral (ter Horst and Luiten, 1986; Thompson et al., 1996). The descending contralateral pathways appear to mirror the ipsilateral pathway but are far less dense (Thompson et al., 1996). In addition, a recent report by Zaretskaia and colleagues (Zaretskaia et al., 2008) showed that disinhibition of the DMH neurons resulted in greater increases in Fos expression in brain regions ipsilateral to the injection site in the DMH. Therefore, the pronounced physiological changes in the renal nerve ipsilateral to the injection side observed in our current study

are likely due to a greater ipsilateral activation of the descending pathways from DMH. In this regard, it is known that air-jet stress causes marked increases in renal sympathetic nerve activity (Burke and Head, 2003; Head and Burke, 2004). Future experiments in conscious rats are necessary in order to investigate the functional implications of these findings during acute stress.

In contrast to substantial right/left side differences in cardiac, cutaneous and renal sympathetic outflows, both sides of the DMH were equipotent in elevating systemic arterial pressure and in provoking mesenteric vasoconstriction.

CONCLUSION

Our most intriguing finding is that sympathetic tone in some target end-organs (heart, tail) is controlled predominantly by the right DMH whereas for others (mesentery), both sides of the DMH are equipotent. It is of major interest in this regard that descending pathways from the DMH controlling HR and tail vascular bed relay in the medullary raphe, whereas descending pressor pathways relay in the RVLM. Our data thus indicates that activation of the right DMH is critical for the expression of full cardiac response observed during acute stress. In contrast, bilateral DMH activation might be necessary for full expression of pressor responses.

Acknowledgments—We thank Jose Roberto Silva for excellent technical support. Financial support, FAPEMIG (APQ-4804/07 PPM) and CNPq (PQ M.A.P.F. and Edital Universal 472749/2007–2.). Xavier CH was recipient of CNPq master fellowship at the postgraduation program in Biological Sciences (Physiology and Pharmacology, ICB, UFMG).

REFERENCES

Bailey TW, Dimicco JA (2001) Chemical stimulation of the dorsomedial hypothalamus elevates plasma ACTH in conscious rats. Am J Physiol Regul Integr Comp Physiol 280:R8–R15.

Baker M, Wayland H (1974) On-line volume flow rate and velocity profile measurement for blood in microvessels. Microvasc Res 7:131–143.

Burke SL, Head GA (2003) Method for in vivo calibration of renal sympathetic nerve activity in rabbits. J Neurosci Methods 127:63–74.

Campos RR, McAllen RM (1999) Cardiac inotropic, chronotropic, and dromotropic actions of subretrofacial neurons of cat RVLM. Am J Physiol 276:R1102–R1111.

Cao WH, Fan W, Morrison SF (2004) Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience 126:229–240.

Chou TC, Scammell TE, Gooley JJ, Gaus SE, Saper CB, Lu J (2003) Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. J Neurosci 23:10691–10702.

Critchley HD, Taggart P, Sutton PM, Holdright DR, Batchvarov V, Hnatkova K, Malik M, Dolan RJ (2005) Mental stress and sudden cardiac death: asymmetric midbrain activity as a linking mechanism. Brain 128:75–85.

da Silva LG, de Menezes RC, Dos Santos RA, Campagnole-Santos MJ, Fontes MA (2003) Role of periaqueductal gray on the cardiovascular response evoked by disinhibition of the dorsomedial hypothalamus. Brain Res 984:206–214.

da Silva LG Jr, Menezes RC, Villela DC, Fontes MA (2006) Excitatory amino acid receptors in the periaqueductal gray mediate the cardiovascular response evoked by activation of dorsomedial hypothalamic neurons. Neuroscience 139:1129–1139.

Dampney RA, Coleman MJ, Fontes MA, Hirooka Y, Horiuchi J, Li YW, Polson JW, Potts PD, Tagawa T (2002) Central mechanisms underlying short- and long-term regulation of the cardiovascular system. Clin Exp Pharmacol Physiol 29:261–268.

de Menezes RC, Zaretsky DV, Fontes MA, Dimicco JA (2006) Microinjection of muscimol into caudal periaqueductal gray lowers body temperature and attenuates increases in temperature and activity evoked from the dorsomedial hypothalamus. Brain Res 1092: 129–137.

de Menezes RC, Zaretsky DV, Fontes MA, DiMicco JA (2009) Cardiovascular and thermal responses evoked from the periaqueductal grey require neuronal activity in the hypothalamus. J Physiol 587:1201–1215.

de Menezes RC, Zaretsky DV, Sarkar S, Fontes MA, Dimicco JA (2008) Microinjection of muscimol into the periaqueductal gray suppresses cardiovascular and neuroendocrine response to air jet stress in conscious rats. Am J Physiol Regul Integr Comp Physiol 295:R881–R890.

DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV (2002) The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. Pharmacol Biochem Behav 71:469–480.

Dimicco JA, Zaretsky DV (2007) The dorsomedial hypothalamus: a new player in thermoregulation. Am J Physiol Regul Integr Comp Physiol 292:R47–R63.

Esler M, Eikelis N, Schlaich M, Lambert G, Alvarenga M, Dawood T, Kaye D, Barton D, Pier C, Guo L, Brenchley C, Jennings G, Lambert E (2008) Chronic mental stress is a cause of essential hypertension: presence of biological markers of stress. Clin Exp Pharmacol Physiol 35:498–502.

Fang HS, Wang SC (1962) Cardioaccelerator and cardioaugmentor points in hypothalamus of the dog. Am J Physiol 203:147–150.

Farkas E, Jansen AS, Loewy AD (1998) Periaqueductal gray matter input to cardiac-related sympathetic premotor neurons. Brain Res 792:179–192.

Fontes MA, Tagawa T, Polson JW, Cavanagh SJ, Dampney RA (2001) Descending pathways mediating cardiovascular response from dorsomedial hypothalamic nucleus. Am J Physiol Heart Circ Physiol 280:H2891–H2901.

Fontes MAP, Campagnole-Santos MJ, Xavier CH (2008) Evaluation of the heart rate changes evoked by air jet stress after inhibition of the right or left sides of dorsomedial hypothalamus. Experimental biology meeting abstracts. FASEB J 22:1170:1174.

Furukawa Y, Wallick DW, Martin PJ, Levy MN (1990) Chronotropic and dromotropic responses to stimulation of intracardiac sympathetic nerves to sinoatrial or atrioventricular nodal region in anesthetized dogs. Circ Res 66:1391–1399.

Gaboury JP, Johnston B, Niu XF, Kubes P (1995) Mechanisms underlying acute mast cell-induced leukocyte rolling and adhesion in vivo. J Immunol 154:804–813.

Harris JA, Guglielmotti V, Bentivoglio M (1996) Diencephalic asymmetries. Neurosci Biobehav Rev 20:637–643.

Head GA, Burke SL (2004) Sympathetic responses to stress and rilmenidine in 2K1C rabbits: evidence of enhanced nonvascular effector mechanism. Hypertension 43:636–642.

Horiuchi J, McAllen RM, Allen AM, Killinger S, Fontes MA, Dampney RA (2004) Descending vasomotor pathways from the dorsomedial hypothalamic nucleus: role of medullary raphe and RVLM. Am J Physiol Regul Integr Comp Physiol 287:R824–R832.

Hosoya Y, Ito R, Kohno K (1987) The topographical organization of neurons in the dorsal hypothalamic area that project to the spinal cord or to the nucleus raphe pallidus in the rat. Exp Brain Res 66:500–506.

House SD, Lipowsky HH (1987) Leukocyte-endothelium adhesion: microhemodynamics in mesentery of the cat. Microvasc Res 34:363–379.

Johnson PL, Shekhar A (2006) Panic-prone state induced in rats with GABA dysfunction in the dorsomedial hypothalamus is mediated by NMDA receptors. J Neurosci 26:7093–7104.

Kamosinska B, Nowicki D, Szulczyk P (1989) Control of the heart rate by sympathetic nerves in cats. J Auton Nerv Syst 26:241–249.

Kubes P, Heit B, van Marle G, Johnston JB, Knight D, Khan A, Power C (2003) In vivo impairment of neutrophil recruitment during lentivirus infection. J Immunol 171:4801–4808.

Kubes P, Jutila M, Payne D (1995) Therapeutic potential of inhibiting leukocyte rolling in ischemia/reperfusion. J Clin Invest 95:2510–2519.

Lane RD, Schwartz GE (1987) Induction of lateralized sympathetic input to the heart by the CNS during emotional arousal: a possible neurophysiologic trigger of sudden cardiac death. Psychosom Med 49:274–284.

McDougall SJ, Widdop RE, Lawrence AJ (2004) Medial prefrontal cortical integration of psychological stress in rats. Eur J Neurosci 20:2430–2440.

Morrison SF, Nakamura K, Madden CJ (2008) Central control of thermogenesis in mammals. Exp Physiol 93:773–797.

Nakamura K, Matsumura K, Hubschle T, Nakamura Y, Hioki H, Fujiyama F, Boldogkoi Z, Konig M, Thiel HJ, Gerstberger R, Kobayashi S, Kaneko T (2004) Identification of sympathetic premotor neurons in medullary raphe regions mediating fever and other thermoregulatory functions. J Neurosci 24:5370–5380.

Nalivaiko E, Blessing WW (1999) Synchronous changes in ear and tail blood flow following salient and noxious stimuli in rabbits. Brain Res 847:343–346.

Nalivaiko E, Blessing WW (2001) Raphe region mediates changes in cutaneous vascular tone elicited by stimulation of amygdala and hypothalamus in rabbits. Brain Res 891:130–137.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. 2nd ed. New York: Academic Press.

Randall WC, Armour JA, Geis WP, Lippincott DB (1972) Regional cardiac distribution of the sympathetic nerves. Fed Proc 31:1199–1208.

Rozanski A, Blumenthal JA, Kaplan J (1999) Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 99:2192–2217.

Samuels BC, Zaretsky DV, DiMicco JA (2002) Tachycardia evoked by disinhibition of the dorsomedial hypothalamus in rats is mediated through medullary raphe. J Physiol 538:941–946.

Schwartz PJ, Stone HL, Brown AM (1976) Effects of unilateral stellate ganglion blockade on the arrhythmias associated with coronary occlusion. Am Heart J 92:589–599.

Stotz-Potter EH, Morin SM, DiMicco JA (1996a) Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air stress-induced neuroendocrine and cardiovascular changes in rats. Brain Res 742:219–224.

Stotz-Potter EH, Willis LR, DiMicco JA (1996b) Muscimol acts in dorsomedial but not paraventricular hypothalamic nucleus to suppress cardiovascular effects of stress. J Neurosci 16:1173–1179.

Tanaka M, McAllen RM (2008) Functional topography of the dorsomedial hypothalamus. Am J Physiol Regul Integr Comp Physiol 294:R477–R486.

Ter Horst GJ, Hautvast RW, De Jongste MJ, Korf J (1996) Neuroanatomy of cardiac activity-regulating circuitry: a transneuronal retrograde viral labelling study in the rat. Eur J Neurosci 8:2029–2041.

ter Horst GJ, Luiten PG (1986) The projections of the dorsomedial hypothalamic nucleus in the rat. Brain Res Bull 16:231–248.

Thompson RH, Canteras NS, Swanson LW (1996) Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. J Comp Neurol 376:143–173.

Toga AW, Thompson PM (2003) Mapping brain asymmetry. Nat Rev Neurosci 4:37–48.

Toth IE, Toth DE, Boldogkoi Z, Hornyak A, Palkovits M, Blessing WW (2006) Serotonin-synthesizing neurons in the rostral medullary raphe/parapyramidal region transneuronally labelled after injection of pseudorabies virus into the rat tail. Neurochem Res 31:277–286.

Wittling W (1997) The right hemisphere and the human stress response. Acta Physiol Scand Suppl 640:55–59.

Xavier CH, Campagnole-Santos MJ, Fontes MAP (2008) Comparison of the cardiovascular responses evoked by chemical activation of the left and right sides of dorsomedial hypothalamus. Experimental biology meeting abstracts FASEB Journal 22.

Yanowitz F, Preston JB, Abildskov JA (1966) Functional distribution of right and left stellate innervation to the ventricles. Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. Circ Res 18:416–428.

Zaretskaia MV, Zaretsky DV, Sarkar S, Shekhar A, DiMicco JA (2008) Induction of Fos-immunoreactivity in the rat brain following disinhibition of the dorsomedial hypothalamus. Brain Res 1200:39–50.

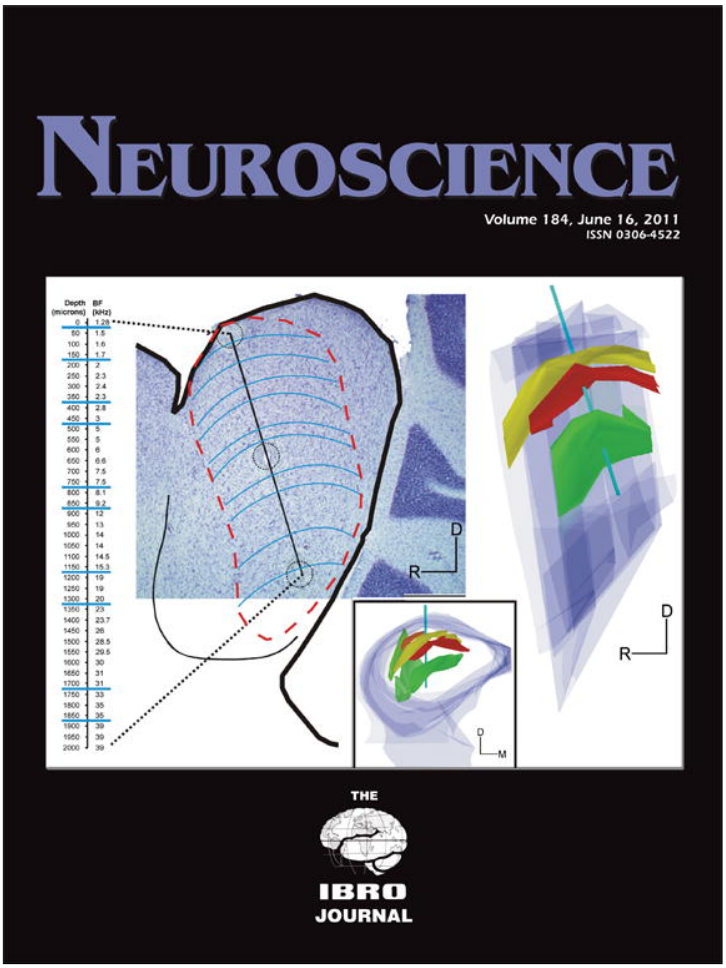
APPENDIX

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroscience.2009.09.018.

(Accepted 8 September 2009)
(Available online 15 September 2009)

Anexo 2



Neuroscience 184 (2011) 64–74

THE DORSOMEDIAL HYPOTHALAMUS AND THE CENTRAL PATHWAYS INVOLVED IN THE CARDIOVASCULAR RESPONSE TO EMOTIONAL STRESS

M. A. P. FONTES,[a]* C. H. XAVIER,[a] R. C. A. DE MENEZES[b] AND J. A. DIMICCO[c]

[a]*Laboratório de Hipertensão, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG), Minas Gerais, Brazil*

[b]*Departamento de Ciências Biológicas, Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto (UFOP), Ouro Preto, Brazil*

[c]*Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN, USA*

Abstract—Psychological stress elicits increases in sympathetic activity accompanied by a marked cardiovascular response. Revealing the relevant central mechanisms involved in this phenomenon could contribute significantly to our understanding of the pathogenesis of stress-related cardiovascular diseases, and the key to this understanding is the identification of the nuclei, pathways and neurotransmitters involved in the organization of the cardiovascular response to stress. The present review will focus specifically on the dorsomedial hypothalamus, a brain region now known to play a primary role in the synaptic integration underlying the cardiovascular response to emotional stress. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: dorsomedial hypothalamus, stress, cardiovascular system, central pathways.

Contents

*Corresponding author. Tel: +55-31-3409-2953; fax: +55-31-3409-2924.
E-mail address: peliky@icb.ufmg.br (M. A. Peliky Fontes).

Abbreviations: BMI, bicuculline methiodide; BP, blood pressure; DMH, dorsomedial hypothalamus; DMN, dorsomedial hypothalamic nucleus; HR, heart rate; l/dlPAG, lateral/dorsolateral region of PAG; NTS, nucleus tractus solitarius; PAG, periaqueductal gray region; PVN, paraventricular nucleus; RPa, raphe pallidus; RVLM, rostral ventrolateral medulla.

STRESS: IMPACT ON THE CARDIOVASCULAR SYSTEM

Psychological stress elicits increases in sympathetic activity that result in changes in the level of cardiac function and vascular resistance with consequent redistribution of blood flow. This physiological strategy enhanced the probability of survival for mammals faced with a physical threat in nature. However, with one-half of the world's population living in the cities (Ginkel, 2008), the impact of psychosocial stress has undoubtedly been a challenge for the cardiovascular system and body homeostasis. Indeed, psychological stress is considered a component of the so called cardiovascular risk (Lloyd-Jones et al., 2009), and examples of such stressors in modern society are numerous. Mittleman and colleagues reported that the relative risk of acute myocardial infarction in the 2 h after an episode of anger was more than double compared with no anger (Mittleman et al., 1995). The number of sudden deaths resulting from cardiac causes sharply increased on the day of the Northridge earthquake that struck the Los Angeles area in 1994 (Leor et al., 1996). Signs of elevated sympathetic activity are commonly observed in patients with white coat hypertension (Smith et al., 2004), a phenomenon in which patients exhibit elevated blood pressure (BP) that is likely a consequence of increased anxiety in a clinical setting. These examples illustrate the potential contribution of emotional stress in precipitating adverse cardiovascular events.

According to the reactivity hypothesis, persistently exaggerated psychological stress responses might be a marker of individuals or subgroups with increased risk of cardiovascular disease (Lovallo and Gerin, 2003). Although the potential causes of the individual differences in reactivity remain poorly understood, the possibility that prolonged stress might cause perpetuated changes in critical groups of neurons in the CNS, resulting in sympathetic overreactivity, overactivity or autonomic imbalance is plausible. Thus, to understand how psychological stress affects the cardiovascular system, it is necessary first to identify the nuclei involved and the central pathways that control the cardiac and vascular sympathetic outflows. The present brief review summarizes our current understanding of a central circuit that integrates the cardiovascular response to acute stress. The focus is the region of dorsomedial hypothalamus (DMH), which plays a key role within this circuit.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.03.018

DMH: ANATOMICAL ORGANIZATION

As functional studies involving the human hypothalamus are rare, comparison of the structural organization of the human hypothalamus with the hypothalamus of other species could provide a meaningful reference for extrapolating physiological findings obtained in studies involving hypothalamus of experimental animals to humans. In this regard, the human hypothalamus is now known to be significantly more homologous to the hypothalamus of the rat than was previously thought, and this seems to be particularly true regarding the DMH (Koutcherov et al., 2003, 2004). In this review, we refer to DMH to indicate a region of the hypothalamus that includes the dorsomedial hypothalamic nucleus (DMN) but also adjoining areas, particularly dorsal and posterior to the nucleus itself as well as laterally including the medial part of the perifornical area. In the rat, the DMH lies adjacent to the third ventricle, caudal and ventral to the hypothalamic paraventricular nucleus (PVN), dorsal to the ventromedial hypothalamic nucleus (VMH) and ventral to the mamillothalamic tract. Laterally, the DMH is bounded by the fornix and the lateral hypothalamic area (Fig. 1). Its caudal border is far less distinct and is loosely delimited with the posterior hypothalamic area. The DMN itself is subdivided in two distinct portions, a poorly defined diffuse portion and a cell dense compact portion or zona compacta (Paxinos and Watson, 1986), the latter being clearly delimited in the posterior part of the DMH. Since this subcompartmental organization is homologous to that found in monkeys and humans (Koutcherov et al., 2004), the DMH seems to be highly conserved during the course of the mammalian evolution. This observation fuels speculation that the same may be true for its functional role in the cardiovascular response to emotional stress.

DMH: A KEY REGION IN THE CARDIOVASCULAR RESPONSE TO STRESS

The DMH plays a key role in coordinating the neuroendocrine, autonomic and behavioral responses to emotional stress (DiMicco et al., 2002). Similarly, the DMH has also been implicated as a key component of the “panic circuit”. Chronic disruption of GABAergic inhibition in the DMH leads to panic-like responses in rats (Johnson and Shekhar, 2006; Shekhar et al., 2006). In the pioneering exper-

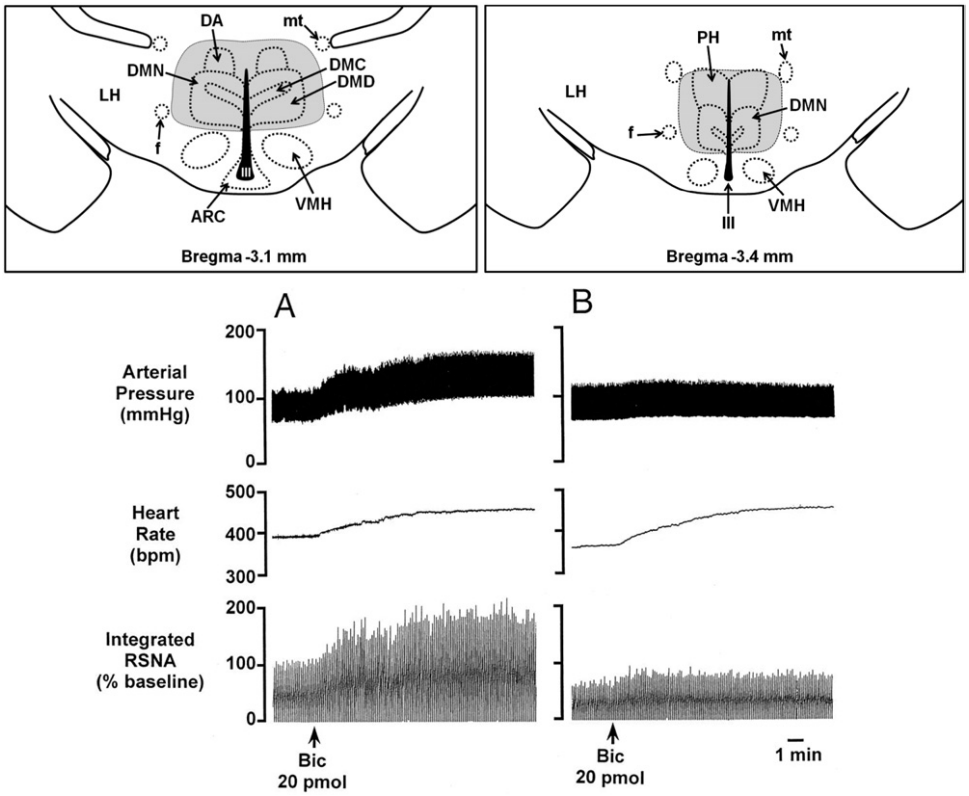


Fig. 1. Upper panel: Gray shading indicates the dorsomedial hypothalamus as referred in this review (3.1–3.4 mm posterior to Bregma according to atlas of Paxinos and Watson, 1986). Bottom panel: Example of the cardiovascular response evoked by microinjection of bicuculline methiodide into a site in the dorsomedial hypothalamic nucleus before (A) and after (B) unilateral microinjection of muscimol (1 nmol) into the rostral ventrolateral medulla pressor region (Fontes et al., 2001). Phenylephrine was infused continuously after the bilateral injections of muscimol to maintain baseline arterial pressure close to the control level. Note that after bilateral inhibition of the RVLM, bicuculline injection into the dorsomedial hypothalamic nucleus still evokes a tachycardic response, whereas the renal sympathetic and vasomotor responses are completely abolished. 3V, third ventricle; DMN, dorsomedial hypothalamic nucleus; DMC, compact portion of dorsomedial hypothalamic nucleus; DMD, diffuse portion of the dorsomedial hypothalamic nucleus; PH, posterior hypothalamic area; VMH, ventromedial hypothalamic nucleus; f, fornix; mt, mamillothalamic tract; LH, lateral hypothalamus; DA, dorsal hypothalamic area; ARC, arcuate hypothalamic nucleus. Bottom panel taken from Fontes et al., 2001, Am J Physiol Heart Circ Physiol. Am Physiol Soc, used with permission.

iments demonstrating a crucial role of DMH neurons in the cardiovascular response to acute stress, Lisa and colleagues (Lisa et al., 1989a) demonstrated that inhibition of DMH neurons with the $GABA_A$ agonist, muscimol, failed to influence baroreflex-induced tachycardia but abolished the increases in heart rate (HR) normally seen in an air stress paradigm (Lisa et al., 1989a). The site of action for muscimol was demonstrated to be specifically the DMH and not the paraventricular nucleus, another region potentially involved in the physiological responses to stress (Stotz-Potter et al., 1996b).

Conversely, DiMicco and colleagues demonstrated that microinjection of bicuculline methiodide (BMI), a $GABA_A$ receptor antagonist, into the DMH of conscious rats evokes marked increases in heart rate and pressor responses in conscious rats in a pattern that mimics the cardiovascular response to emotional stress (DiMicco et al., 2002). Thus, DMH neurons are under powerful GABAergic inhibition. Injections of BMI at doses ranging from 0.1 to 40 pmol that targeted the region of DMH evoke dose-related increases in mean arterial pressure (MAP), HR and renal sympathetic nerve activity (Horiuchi et al., 2004b). Moreover, injections of excitatory aminoacids (EEA) into the DMH also produce increases in HR and blood pressure (Soltis and DiMicco, 1991a, 1992; De Novellis et al., 1995). In addition, blockade of EEA receptors in the DMH suppresses the cardiovascular response evoked by BMI injections into the same region (Soltis and DiMicco, 1991b). These findings suggest that the response caused by blockade of GABAergic inhibition in the DMH of the rat is dependent on activation of local EAA receptors.

Although several studies have reported that disinhibition or excitation of DMH neurons can produce changes in blood pressure or heart rate similar to the ones seen during emotional stress, the precise location of these sites were not well characterized. Nonetheless, Samuels and colleagues (2004) have shown that injections (2 pmol/5 nl) of BMI into a specific area, dorsal to the DMN, called dorsal hypothalamic area (DA, Fig. 1) evoke increases in HR that are significantly greater than the increases observed after injection into the DMN itself (Samuels et al., 2004). They have also observed that the site of these injections corresponded to the location of neurons which project directly to the RP, which has been shown to be fundamental to the tachycardia evoked during stress (Zaretsky et al., 2003b). These data were later confirmed by Tanaka and McAllen (2008), who demonstrated that injections of D,L-homocysteic acid (50 mM in 15 nl) in the DA and dorsal parts of the DMN produced increases in heart rate, however, injections in the ventral parts of the DMN did not change it (Tanaka and McAllen, 2008). On the other hand, increases in blood pressure could be achieved by activating different areas of the DMH. Hence, even with high spatial resolution data, it is still not possible to determine the exact sites responsible for the changes in specific physiological variables evoked by DMH (or DA) activation.

It could be speculated that during acute psychological stress, the sensory input from the environment overcomes or reduces the tonic inhibition of neurons in the DMH, resulting in a characteristic cardiovascular response. Although the DMH receives inputs from several forebrain regions including amygdala (Soltis et al., 1998), that play roles in the physiological responses to stress (Fig. 2), the origin of the GABAergic input to neurons in the DMH remains unknown. The medial preoptic area (mPOA) may represent a significant source of inhibitory tone to key neurons in the DMH (Hunt et al., 2010). Another possibility is the medial prefrontal cortex (mPFC) which is a limbic structure involved in the regulation of cognitive and emotional information (Bush et al., 2000) and in the regulation of stress-induced neural activity (Amat et al., 2005). The DMH also receives projections from neurons in the mPFC, specifically in the infralimbic division (Hurley et al., 1991; Vertes, 2004). However, the only two functional studies offering a clue about a possible mPFC-DMH inhibitory projection contain divergent findings (McDougall et al., 2004; Radley et al., 2009).

ROSTRAL VENTROLATERAL MEDULLA AND THE VASOMOTOR COMPONENT OF THE RESPONSE TO ACTIVATION OF THE DMH

In the past decade, much has been learned about the descending pathways that mediate the sympathoexcitatory response evoked from the DMH. Previous anatomic studies indicated that the DMH contains no or very few neurons that project directly to the spinal cord (ter Horst and Luiten, 1986; Hosoya et al., 1987; Thompson et al., 1996). Therefore, the descending sympathoexcitatory pathway from the DMH should include one or more synaptic connections in supraspinal regions, such as the rostral ventrolateral medulla (Fontes et al., 2001), since this brainstem region contains sympathetic premotor neurons involved in the maintenance of vasomotor sympathetic activity and blood pressure (Dampney et al., 2000). The pressor and sympathoexcitatory responses, but not the tachycardic response evoked by activation of neurons in the DMH with BMI were greatly reduced after bilateral microinjection of muscimol into the rostral ventrolateral medulla (Fig. 1B). Thus, as shown in Fig. 2 (pathway 7), the vasomotor component of the response evoked from DMH is dependent on neuronal activity in the rostral ventrolateral medulla (RVLM) (Fontes et al., 2001). Indeed, in response to activation of the DMH, the firing rate of RVLM neurons could be increased by as much as 400% (Horiuchi et al., 2004b).

RAPHE PALLIDUS AND THE CARDIAC COMPONENT OF THE RESPONSE TO ACTIVATION OF THE DMH

The findings discussed above suggested that the pathway mediating the cardiac stimulation evoked by activation of the DMH was independent of the RVLM (Fontes et al., 2001). A search for the synaptic relay mediating the increase in heart rate caused by activation of DMH led to the raphe pallidus (RPa) as a potential candidate. Neurons in the RPa send direct projections to the upper thoracic intermediolateral cell column at those levels containing car-

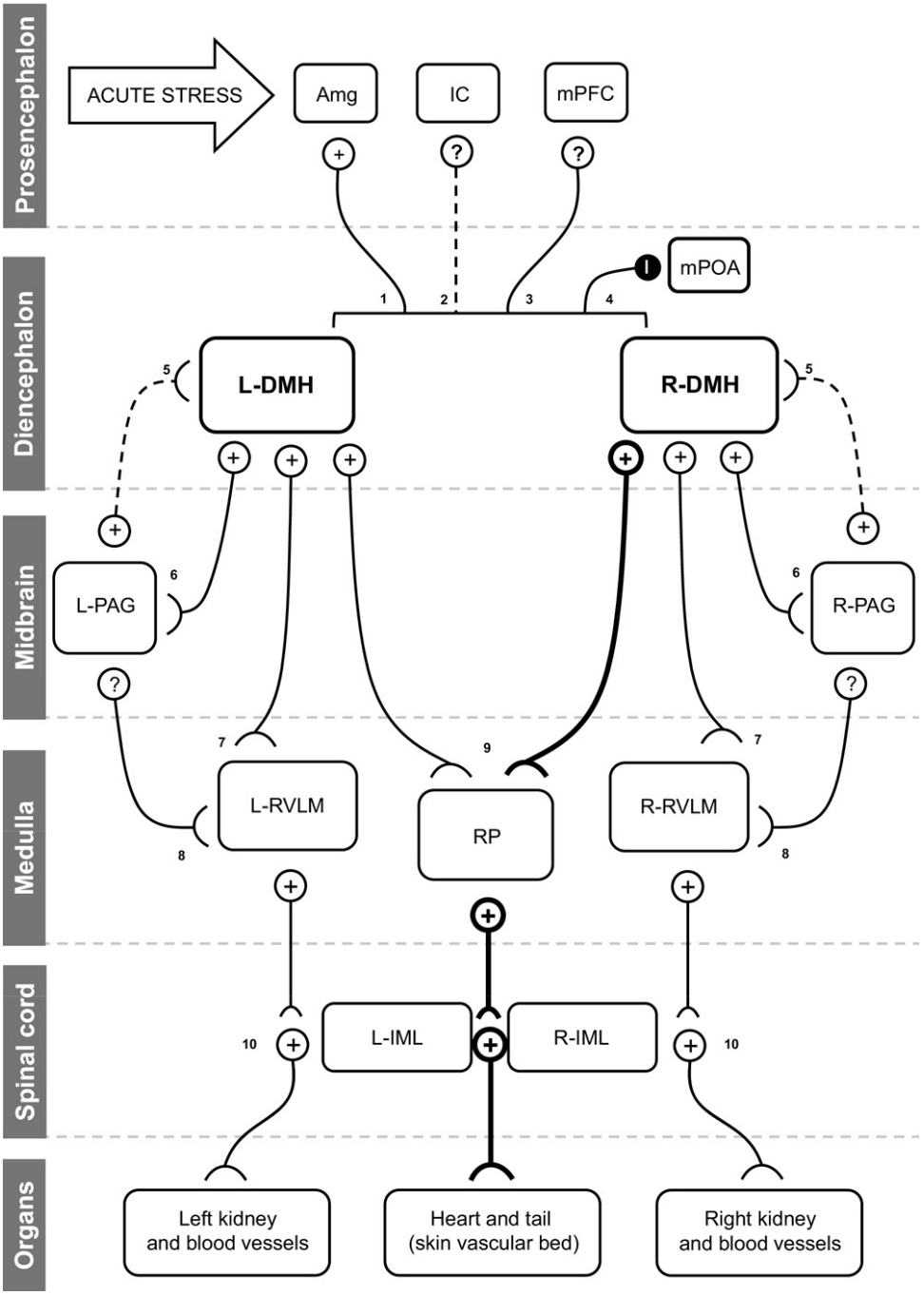


Fig. 2. Schematic diagram based on functional and anatomic studies showing descending pathways involved in the organization of the cardiovascular response to emotional stress at different levels of the neuraxis. The DMH is showed as a key integrative region in this response (Lumb, 1990; Thompson and Swanson, 1998; DiMicco et al., 2002), which also involves higher and lower brain regions (Kober et al., 2008; Cechetto and Shoemaker, 2009). From the DMH, descending pathways are represented bilaterally to better illustrate our recent functional findings and hypothesis (see text for details). 1—Amygdala/DMH (Nalivaiko and Blessing, 2001; Quirk and Gehlert, 2003; Quirk et al., 2003); 2—Insular cortex/DMH (Cechetto and Chen, 1990; Oppenheimer and Cechetto, 1990; Yasui et al., 1991; Butcher and Cechetto, 1998); 3—Medial prefrontal cortex/DMH (Vertes, 2004, 2006; Hoover and Vertes, 2007); 4—Medial preoptic area/DMH (Okamura et al., 1990; Zaretskaia et al., 2003; Yoshida et al., 2009; Hunt et al., 2010); 5 and 6—Periaqueductal gray/DMH and vice-versa (da Silva et al., 2003, 2006; de Menezes et al., 2006, 2008, 2009; Horiuchi et al., 2009; Villela et al., 2009); 7—DMH/rostral ventrolateral medulla (Fontes et al., 2001; Cao et al., 2004); 8—Periaqueductal gray/rostral ventrolateral medulla (Hudson and Lumb, 1996; Farkas et al., 1998); 9—DMH/raphe pallidus (Hosoya et al., 1987; Nalivaiko and Blessing, 2001; Zaretsky et al., 2003b; Horiuchi et al., 2004b; Samuels et al., 2004); 10—Rostral ventrolateral medulla and raphe pallidus/spinal cord/target organs (Loewy, 1981; Taylor and Weaver, 1992; Jansen et al., 1995; McAllen et al., 1995; Campos and McAllen, 1999; Morrison et al., 1999; Huang et al., 2002; Blessing, 2003; Cao and Morrison, 2003; Dampney et al., 2003; Zaretsky et al., 2003a,b; Horiuchi et al., 2004a,b; Nalivaiko et al., 2005). Dashed lines represent unknown and/or indirect projections. Thick line from DMH to RP, and from RP to target organs illustrates a hypothetical descending pathway illustrating the asymmetric functional responses evoked from DMH (see text for details).

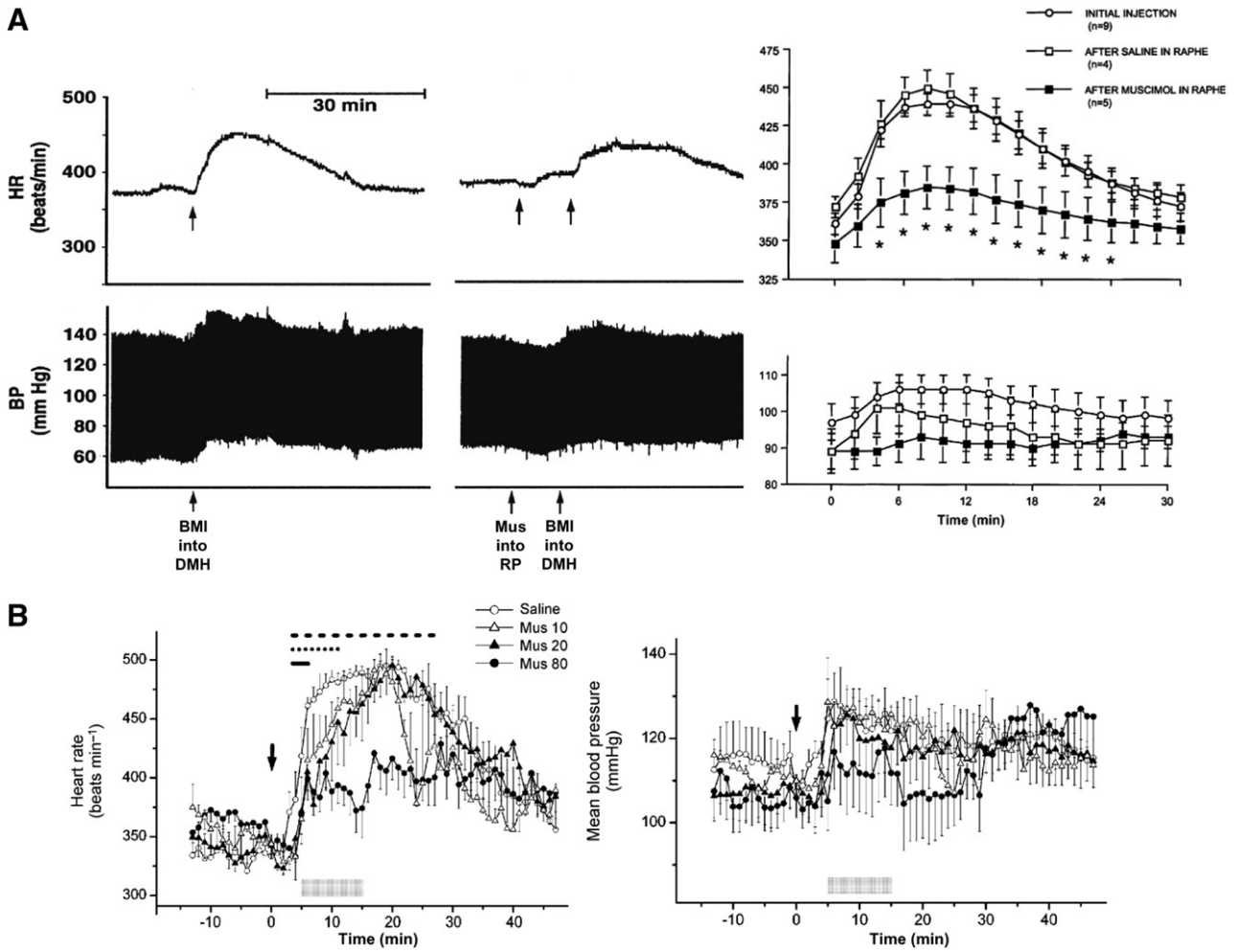


Fig. 3. Raphe neurons are a crucial relay in the pathway responsible for stress-induced tachycardia. (A) Inhibition of raphe neurons, through microinjection of the $GABA_A$ receptor agonist muscimol, reduces the increase in HR, but not in BP, evoked by disinhibition of the DMH (by microinjection of the $GABA_A$ receptor antagonist bicuculline). Left: Example of an original tracing from a representative experiment. Right: Grouped data depicting the changes in HR and BP induced by disinhibition of the DMH after microinjection of muscimol or saline into the RP. * $P<0.05$. (B) Inhibition of raphe neurons, by microinjection of muscimol, reduces the increase in HR, but not in BP, evoked by air-jet stress. Injections were performed a $t=0$ min (arrows), and rats were subjected to air-jet stress during stress trial between $t=+5$ min and $t=+15$ min (shaded bars). Horizontal lines (top) indicate significant differences from corresponding values after treatment with saline: dashes, 80 pmol; dots, 20 pmol; continuous,10 pmol. BMI, bicuculline methiodide; DMH, dorsomedial hypothalamus; Mus, muscimol; RP, raphe pallidus. Data presented at this figure taken from Samuels et al., J Physiol 538:941-946, 2002 (Panel A) and Zaretsky et al., J Physiol 546:243-250, 2003 (Panel B).

diac sympathetic preganglionic neurons (Amendt et al., 1979; Loewy, 1981; Ter Horst et al., 1996). As demonstrated by Samuels and colleagues (2002), the tachycardia evoked by activation of neurons in the DMH with BMI was markedly suppressed after inhibition of neurons in the RPa with muscimol (Fig. 3A). Subsequent experiments in conscious rats demonstrated that inhibition of the RPa virtually abolished the tachycardia evoked by acute stress (Fig. 3B) but failed to influence the tachycardia produced by baroreceptor unloading (Zaretsky et al., 2003b). Further support for the involvement of neurons in the RPa in the tachycardia evoked by stress comes from experiments showing that direct injection of BMI into RPa neurons evokes tachycardia of similar magnitude as that evoked by activation of neurons in the DMH (Samuels et al., 2002; Cao and Morrison, 2003). Interestingly, inhibition of the RPa in conscious rats has no effect on baseline HR (Zaretsky et al., 2003b), but blockade of $GABA_A$ receptors in the RPa produces sustained increases in cardiac sympathetic activity and in HR even after complete suppression of activity in sympathoexcitatory neurons in the RVLM with muscimol (Cao and Morrison, 2003). Therefore, the cardiac sympathoexcitation and tachycardia evoked by activation of neurons in the RPa can occur independently of excitation of sympathetic premotor neurons in the RVLM that normally provide the excitatory drive to support basal cardiac sympathetic activity and HR. As proposed by Cao and Morrison (2003), dorsomedial hypothalamic neurons apparently act to reduce or overcome the tonic inhibition of these RPa neurons, which in turn provide an excitatory drive to spinal cardiac sympathetic

preganglionic neurons to augment cardiac sympathetic activity and HR (Fig. 2, pathway 9).

PERIAQUEDUCTAL GRAY: A SOURCE OF EXCITATORY INPUT TO NEURONS IN THE DMH?

Ultimately, a model that relies on the regulation of neuronal activity through disinhibition must include a mechanism responsible for excitation of the neuronal population being studied (Morrison, 2004). As is seen after acute stress or disinhibition of neurons in the DMH with BMI, a tachycardic response can also be induced by stimulating neurons in the DMH with agonists of EAA receptors (Soltis and DiMicco, 1991a, 1992; Tanaka and McAllen, 2008). The first structure evaluated as a source of excitatory input to DMH neurons was the amygdala, a structure well-known to be involved in stress and anxiety (LeDoux, 2007). Chemical stimulation of the amygdala results in cardiovascular changes that are abolished after blockade of glutamatergic receptors in the DMH (Soltis et al., 1998). However, recent attempts to reveal the descending cardiovascular connections from DMH led us also to consider the periaqueductal gray region (PAG) (da Silva et al., 2003, 2006) (Fig. 2 pathway 6).

Our findings that increases in HR and MAP seen in air jet stress were reduced by microinjection of muscimol into the lateral/dorsolateral region of PAG (l/dlPAG) (de Menezes et al., 2008), in the same manner that the inhibition of DMH neurons alters the cardiovascular response to air jet stress (Stotz-Potter et al., 1996b), suggested that neurons in the l/dlPAG constitute downstream effectors for cardiovascular changes evoked from the DMH. Surprisingly, however, we also observed that microinjection of muscimol into the l/dlPAG reduced the increases in plasma adrenocorticotropic hormone (ACTH) evoked by air jet stress. Increases in plasma ACTH seen in this paradigm represent activation of the hypothalamic-pituitary-adrenal axis, a hallmark of the response to stress, and have been proposed to be mediated in large part through a direct projection from neurons in the DMH to the hypothalamic paraventricular nucleus [PVN; for review, see (DiMicco et al., 2002)]. On the other hand, neurons in the l/dlPAG do not project to the PVN (Cameron et al., 1995).

This hypothesis that the PAG is a source of excitatory input to neurons in the DMH during stress was validated by demonstrating that the increases in HR, BP and core body temperature produced by microinjection of the excitatory amino acid (NMDA) into l/dlPAG in conscious rats were markedly attenuated either by neuronal inhibition (microinjection of muscimol) or by blockade of glutamate transmission (microinjection of NBQX+Ap5) within the DMH, but not within the PVN (de Menezes et al., 2009). Likewise, microinjection of muscimol into the DMH of anaesthetized rats reduced the increases in BP as well as the increases in phrenic and renal sympathetic nerve activity produced by the activation of the dlPAG (Horiuchi et al., 2009).

Taken together, these data demonstrated that the physiological responses produced by activation of the l/dlPAG depend on neuronal activity in the DMH. Thus, the l/dlPAG may represent one of several regions that provide glutamatergic excitation to neurons in the DMH (Fig. 2, pathway 5) whose activation is ultimately responsible for physiological changes seen in experimental stress. Previous data from anatomical studies are consistent with this notion. For instance, it is known that neurons in the l/dlPAG send axonal projections to neurons located in the region of the DMH (Shaikh et al., 1987; Cameron et al., 1995; Siegel et al., 1997). Also, chemical or electrical stimulation of the l/dlPAG increases the expression of c-fos, a marker for neuronal activation (Dragunow and Faull, 1989), in the DMH, where the terminals of projections from the l/dlPAG can also be found (de Oliveira et al., 2000; Borelli et al., 2006). It important to observe that, in the study of de Oliveira, the increase in c-fos expression was restricted to the dorsomedial nucleus and occurred mainly on the side ipsilateral to the stimulation site in the dlPAG. This fact suggests that the increase in c-fos expression within the DMH was due to the specific activation of the neurons in the PAG and not to the generalized behavioral arousal that was also produced. Thus, during stress, afferents from neurons in the l/dlPAG, perhaps along with those from other regions, might act to excite neurons in the DMH. On the other hand, the tonic inhibitory drive that is present under resting conditions might at the same time be withdrawn, thus changing the balance between GABAergic and glutamatergic transmission that occurs in the DMH [see (DiMicco et al., 2002)]. These shifts would then lead to activation of (1) CRH-containing neurons in the PVN to stimulate the secretion of ACTH, and (2) autonomic centers in the brainstem to increase HR, MAP, temperature and respiratory rate. It is important to consider that the projections through which the l/dlPAG influences the DMH do not necessarily have to be direct. For example, the dlPAG has major projections to the cuneiform nucleus and to the superior lateral parabrachial nucleus in the pons (Lisa et al., 1989b; Carrive, 1993; Krout et al., 1998), and these in turn have projections to the hypothalamus, including the DMH (Bester et al., 1997; Lam et al., 1997).

The results of the studies showing that the physiological changes produced by the activation of the l/dlPAG neurons depend on neuronal activity in the DMH (de Menezes et al., 2009; Horiuchi et al., 2009), combined with data from the earlier studies showing that the changes evoked by disinhibition of the DMH are, also, dependent on the activation of l/dlPAG neurons (da Silva et al., 2003, 2006; de Menezes et al., 2006), requires alternative explanations to the hypothesis presented above. In this regard, de Menezes and colleagues (2009) proposed two distinct hypotheses that are consistent with the data concerning the relationship between the DMH and the l/dlPAG. One possibility is that these regions are reciprocally connected to form a functional network involved in the stress response. In this case, the stimulation of either region would facilitate the activation of the neurons in the other region. Another possibility is that this critical facilitation from neurons in the DMH and in the PAG converges on common medullary targets related to the physiological responses

evoked from either region. Once again, loss of either source of background facilitation may effectively weaken responses evoked from the other.

NUCLEUS TRACTUS SOLITARIUS: STRESS, DMH AND BAROREFLEX MODULATION

Acute psychological stress and stimulation of the DMH can both generate physiological and behavioral responses, as described above, with the main cardiovascular effect being increases in HR and BP (Stotz-Potter et al., 1996a,b; Fontes et al., 2001; DiMicco et al., 2002; da Silva et al., 2003; de Menezes et al., 2006). In addition to these changes, it is known that both stress and stimulation of the DMH can modulate the baroreceptor reflex (Kunos and Varga, 1995; Hatton et al., 1997; Schadt and Hasser, 1998; Sevoz-Couche et al., 2003; McDowall et al., 2006). This modulation during the defense reaction is necessary to ensure that the changes in HR and BP can occur simultaneously, without compromise of either response (i.e. increases in HR or BP). Studies in conscious animals indicate that during stress the increases in HR and BP are accompanied by resetting of the baroreceptor reflex (Hatton et al., 1997; Schadt and Hasser, 1998). In these studies, the reflex control of HR was reset to higher levels of arterial pressure without any changes in the gain. In addition, recent evidence by Kanbar and colleagues (2007) demonstrated that the baroreflex control of sympathetic activity is reset and sensitized during emotional stress (Kanbar et al., 2007).

The exact nature of DMH influence in the baroreceptor reflex remains to be determined. An early report by Kunos and Varga showed that ipsilateral intra-nucleus tractus solitarius (NTS) injection of BMI or 2-OH-saclofen ($GABA_B$ antagonist), attenuated the tachycardia elicited by BMI injection into the DMH. The tachycardia was also inhibited by intra-NTS administration of EEA receptor channel blockers. Authors concluded that the descending input from the DMH to the NTS releases GABA via glutamate acting on ionotropic glutamate receptors located on GABAergic interneurons (Kunos and Varga, 1995). This mechanism would inhibit baroreflex bradycardia during activation of DMH. Similarly, a study by Sevoz-Couche and colleagues (2003) demonstrated that electrical stimulation of the DMH in anaesthetized rats also inhibits baroreflex bradycardia. However, a recent report by McDowal and colleagues showed that chemical disinhibition of DMH neurons resets the baroreflex to higher levels of arterial pressure, in the same way stress does (Hatton et al., 1997; Schadt and Hasser, 2001; Kanbar et al., 2007), with the baroreflex remaining effective and without losing sensitivity (McDowall et al., 2006).

BRAIN FUNCTIONAL ASYMMETRY AND DMH

Left-right differences in the functional properties of bilateral nervous system regions are known as lateralization of function. This phenomenon has been observed at different levels of the neuraxis (Toga and Thompson, 2003; Stephan et al., 2007), including the hypothalamus from several species (Harris et al., 1996). Studies revealed that, under some conditions, stress may generate lateralized and imbalanced autonomic outflow (Critchley, 2005). This asymmetric autonomic activity may cause cardiac arrhythmias, (Lane et al., 1992a,b). Wittling et al. found a functional division between the cerebral hemispheres with the left dominant in generating parasympathetic activity to the heart while the right plays a greater role in generating the sympathetic activity to the myocardium (Wittling et al., 1998a,b). If this division is physiological, it may provide a substrate for described cardiac arrhythmias. Pathways from the DMH are predominantly lateralized such that most neurons on one side do not project contralaterally, but rather they are organized as ipsilateral “mirrors” (ter Horst and Luiten, 1986; Thompson et al., 1996). Additionally, anatomic projections from other nuclei involved in autonomic control to sympathetic preganglionic neurons in the intermediolateral column are also lateralized (Amendt et al., 1979; Blessing et al., 1981; Zagon and Smith, 1993).

In the hypothalamus, the hypothesis of functional asymmetry was first reported based upon the observation that electrical stimulation of the right hypothalamus evokes greater tachycardia than that evoked by identical stimulation of the left (Fang and Wang, 1962). Recently, we demonstrated that unilateral disinhibition of neurons in the

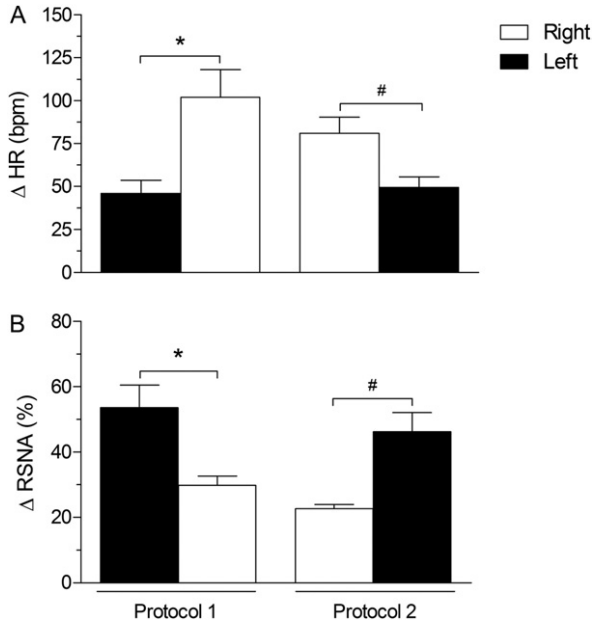


Fig. 4. Maximal changes in cardiovascular parameters from baseline evoked by unilateral microinjection of BMI into right (white bars) and left DMH (black bars) on two experimental protocols. Sequence for injections into unilateral (R or L) DMH followed two different orderliness: Protocol 1—First microinjection was done into L-DMH and the second was into R-DMH; Protocol 2—Sequence for injections was reverse of that used in protocol 1, for example, first into R-DMH and second into L-DMH. (A) Heart rate (HR) and (B) renal sympathetic nerve activity (RSNA) sampled from left renal nerve. * $P<0.05$—protocol 1 (L vs. R); # $P<0.05$—protocol 2 (R vs. L). (A) shows asymmetry with clear predominance of R-DMH in provoking greater positive chronotropy. (B) shows lateralization in the pathways from unilateral DMH which controls ipsilateral RSNA. Data taken from Xavier et al., Neuroscience 164:1360-1368, 2009.

DMH with BMI evokes Fos expression in different nuclei, including the DMH itself, the midline rostral RP, and the lateral septal nucleus, the parvocellular and magnocellular subdivisions of the PVN, the NTS, and the ventrolateral medulla. In the latter bilateral regions, labeling was increased on both sides but was markedly greater ipsilateral to the site of DMH stimulation (Zaretskaia et al., 2008). In determining if cardiac sympathoexcitation evoked by activation of neurons on one side of the DMH is transmitted preferentially through ipsilateral relays, we found that disinhibition of the right DMH evokes a greater tachycardia than that evoked from the left DMH (Xavier et al., 2009) (Fig. 4). Additionally, disinhibition of the right DMH evokes substantially larger changes in cardiac contractility compared to those evoked from left DMH. This effect is independent of the simultaneous changes in heart rate and afterload and so might be interpreted as a direct positive inotropic effect. Interestingly, in the same study we detected a greater number of ectopic beats during the 10 min following injections of BMI into the right DMH (Xavier et al., 2010). This finding prompted us to speculate that recruiting the right DMH during stress exposure might improve the range of cardiac responses and increase the risk of arrhythmic episodes.

The possibility that descending input to the DMH from brain structures, such as the insular cortex, may be asymmetric should also be considered (see Fig. 2). First, there is anatomical and functional evidence for connections between the insular cortex and the hypothalamus (Cechetto and Chen, 1990). Second, neural activity in the insular cortex may have an arrythmogenic role according to past findings. In rats, the cardiac effects of stimulation of the insular cortex mimic the repolarization and structural changes that occur with catecholamine-induced cardiomyopathy seen under certain clinical circumstances, including death following extreme and prolonged stress, and these effects are likely associated with sympathetic neural activation of the ventricular myocardium (Oppenheimer, 2007). Similarly, in humans, insular damage may produce effects on cardiac repolarization (Sander and Klingelhofer, 1994). Third, there is evidence suggesting lateralization and specialization of cardioregulatory function within the insular cortex. The right insular cortex has been primarily implicated with modulation of cardiac sympathetic nerve activity and the left with effects primarily on cardiac vagal activity. Interestingly, patients with stroke lateralized to the left insular cortex reportedly exhibit impaired sympathovagal balance, with one third of the stroke patients developing sinus tachycardia even in the absence of significant coronary disease (Oppenheimer, 2007). Whether or not insular cortical imbalance might result in consequent asymmetric activity of DMH neurons for triggering adverse cardiac outcomes remains to be determined.

CONCLUSION AND PERSPECTIVES

In conclusion, although many of the details regarding the role of the dorsomedial hypothalamus in the cardiovascular response to emotional stress remain to be determined, considerable progress has been made in the past few years in determining the central pathways involved. Undoubtedly, a critical step is to further investigate the implications of the lateralization observed in the descending pathways from the DMH. The role of the DMH in adverse cardiac events observed after cortical stimulation or damage deserves extensive investigation. Elucidating the functional organization of this network could provide a framework for understanding how, in some conditions, stress results in autonomic imbalance resulting in cardiovascular risk.

Acknowledgments—Authors would like to thank the financial support provided by: (1) Conselho Nacional de Desenvolvimento Cientifico e Tecnologico do Brazil (CNPq) and Fundacao de Amparo a Pesquisa do Estado de Minas Gerais (Fapemig-PPM), Brazil; (2) USPHS Grant NS 19883, USA.

REFERENCES

Amat J, Baratta MV, Paul E, Bland ST, Watkins LR, Maier SF (2005) Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. Nat Neurosci 8: 365–371.

Amendt K, Czachurski J, Dembowsky K, Seller H (1979) Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. J Auton Nerv Syst 1:103–107.

Bester H, Besson JM, Bernard JF (1997) Organization of efferent projections from the parabrachial area to the hypothalamus: a *Phaseolus vulgaris*-leucoagglutinin study in the rat. J Comp Neurol 383:245–281.

Blessing WW (2003) Lower brainstem pathways regulating sympathetically mediated changes in cutaneous blood flow. Cell Mol Neurobiol 23:527–538.

Blessing WW, Goodchild AK, Dampney RA, Chalmers JP (1981) Cell groups in the lower brain stem of the rabbit projecting to the spinal cord, with special reference to catecholamine-containing neurons. Brain Res 221:35–55.

Borelli KG, Ferreira-Netto C, Brandao ML (2006) Distribution of Fos immunoreactivity in the rat brain after freezing or escape elicited by inhibition of glutamic acid decarboxylase or antagonism of GABA-A receptors in the inferior colliculus. Behav Brain Res 170:84–93.

Bush G, Luu P, Posner MI (2000) Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 4:215–222.

Butcher KS, Cechetto DF (1998) Receptors in lateral hypothalamic area involved in insular cortex sympathetic responses. Am J Physiol 275:H689–H696.

Cameron AA, Khan IA, Westlund KN, Cliffer KD, Willis WD (1995) The efferent projections of the periaqueductal gray in the rat: a *Phaseolus vulgaris*-leucoagglutinin study. I. Ascending projections. J Comp Neurol 351:568–584.

Campos RR, McAllen RM (1999) Cardiac inotropic, chronotropic, and dromotropic actions of subretrofacial neurons of cat RVLM. Am J Physiol 276:R1102–R1111.

Cao WH, Fan W, Morrison SF (2004) Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience 126:229–240.

Cao WH, Morrison SF (2003) Disinhibition of rostral raphe pallidus neurons increases cardiac sympathetic nerve activity and heart rate. Brain Res 980:1–10.

Carrive P (1993) The periaqueductal gray and defensive behavior: functional representation and neuronal organization. Behav Brain Res 58:27–47.

Cechetto DF, Chen SJ (1990) Subcortical sites mediating sympathetic responses from insular cortex in rats. Am J Physiol 258:R245–R255.

Cechetto DF, Shoemaker JK (2009) Functional neuroanatomy of autonomic regulation. Neuroimage 47:795–803.
Critchley HD (2005) Neural mechanisms of autonomic, affective, and cognitive integration. J Comp Neurol 493:154–166.
da Silva LG, de Menezes RC, dos Santos RA, Campagnole-Santos MJ, Fontes MA (2003) Role of periaqueductal gray on the cardiovascular response evoked by disinhibition of the dorsomedial hypothalamus. Brain Res 984:206–214.
da Silva LG Jr, Menezes RC, Villela DC, Fontes MA (2006) Excitatory amino acid receptors in the periaqueductal gray mediate the cardiovascular response evoked by activation of dorsomedial hypothalamic neurons. Neuroscience 139:1129–1139.
Dampney RA, Horiuchi J, Tagawa T, Fontes MA, Potts PD, Polson JW (2003) Medullary and supramedullary mechanisms regulating sympathetic vasomotor tone. Acta Physiol Scand 177:209–218.
Dampney RA, Tagawa T, Horiuchi J, Potts PD, Fontes M, Polson JW (2000) What drives the tonic activity of presympathetic neurons in the rostral ventrolateral medulla? Clin Exp Pharmacol Physiol 27:1049–1053.
de Menezes RC, Zaretsky DV, Fontes MA, Dimicco JA (2006) Microinjection of muscimol into caudal periaqueductal gray lowers body temperature and attenuates increases in temperature and activity evoked from the dorsomedial hypothalamus. Brain Res 1092: 129–137.
de Menezes RC, Zaretsky DV, Fontes MA, DiMicco JA (2009) Cardiovascular and thermal responses evoked from the periaqueductal grey require neuronal activity in the hypothalamus. J Physiol 587:1201–1215.
de Menezes RC, Zaretsky DV, Sarkar S, Fontes MA, Dimicco JA (2008) Microinjection of muscimol into the periaqueductal gray suppresses cardiovascular and neuroendocrine response to air jet stress in conscious rats. Am J Physiol Regul Integr Comp Physiol 295:R881–R890.
De Novellis V, Stotz-Potter EH, Morin SM, Rossi F, DiMicco JA (1995) Hypothalamic sites mediating cardiovascular effects of microinjected bicuculline and EAAs in rats. Am J Physiol 269:R131–R140.
de Oliveira RW, Del Bel EA, Guimaraes FS (2000) Behavioral and c-fos expression changes induced by nitric oxide donors microinjected into the dorsal periaqueductal gray. Brain Res Bull 51:457–464.
DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV (2002) The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. Pharmacol Biochem Behav 71:469–480.
Dragunow M, Faull R (1989) The use of c-fos as a metabolic marker in neuronal pathway tracing. J Neurosci Methods 29:261–265.
Fang HS, Wang SC (1962) Cardioaccelerator and cardioaugmentor points in hypothalamus of the dog. Am J Physiol 203:147–150.
Farkas E, Jansen AS, Loewy AD (1998) Periaqueductal gray matter input to cardiac-related sympathetic premotor neurons. Brain Res 792:179–192.
Fontes MA, Tagawa T, Polson JW, Cavanagh SJ, Dampney RA (2001) Descending pathways mediating cardiovascular response from dorsomedial hypothalamic nucleus. Am J Physiol Heart Circ Physiol 280:H2891–H2901.
van Ginkel HJ (2008). Urban future. Nature 456:32–33.
Harris JA, Guglielmotti V, Bentivoglio M (1996) Diencephalic asymmetries. Neurosci Biobehav Rev 20:637–643.
Hatton DC, Brooks V, Qi Y, McCarron DA (1997) Cardiovascular response to stress: baroreflex resetting and hemodynamics. Am J Physiol 272:R1588–R1594.
Hoover WB, Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149–179.
Horiuchi J, Killinger S, Dampney RA (2004a) Contribution to sympathetic vasomotor tone of tonic glutamatergic inputs to neurons in the RVLM. Am J Physiol Regul Integr Comp Physiol 287:R1335–R1343.
Horiuchi J, McAllen RM, Allen AM, Killinger S, Fontes MA, Dampney RA (2004b) Descending vasomotor pathways from the dorsomedial hypothalamic nucleus: role of medullary raphe and RVLM. Am J Physiol Regul Integr Comp Physiol 287:R824–R832.
Horiuchi J, McDowall LM, Dampney RA (2009) Vasomotor and respiratory responses evoked from the dorsolateral periaqueductal grey are mediated by the dorsomedial hypothalamus. J Physiol 587:5149–5162.
Hosoya Y, Ito R, Kohno K (1987) The topographical organization of neurons in the dorsal hypothalamic area that project to the spinal cord or to the nucleus raphe pallidus in the rat. Exp Brain Res 66:500–506.
Huang J, Chowhdury SI, Weiss ML (2002) Distribution of sympathetic preganglionic neurons innervating the kidney in the rat: PRV transneuronal tracing and serial reconstruction. Auton Neurosci 95:57–70.
Hudson PM, Lumb BM (1996) Neurones in the midbrain periaqueductal grey send collateral projections to nucleus raphe magnus and the rostral ventrolateral medulla in the rat. Brain Res 733:138–141.
Hunt JL, Zaretsky DV, Sarkar S, Dimicco JA (2010) Dorsomedial hypothalamus mediates autonomic, neuroendocrine, and locomotor responses evoked from the medial preoptic area. Am J Physiol Regul Integr Comp Physiol 298:R130–R140.
Hurley KM, Herbert H, Moga MM, Saper CB (1991) Efferent projections of the infralimbic cortex of the rat. J Comp Neurol 308: 249–276.
Jansen AS, Wessendorf MW, Loewy AD (1995) Transneuronal labeling of CNS neuropeptide and monoamine neurons after pseudorabies virus injections into the stellate ganglion. Brain Res 683:1–24.
Johnson PL, Shekhar A (2006) Panic-prone state induced in rats with GABA dysfunction in the dorsomedial hypothalamus is mediated by NMDA receptors. J Neurosci 26:7093–7104.
Kanbar R, Orea V, Chapuis B, Barres C, Julien C (2007) A transfer function method for the continuous assessment of baroreflex control of renal sympathetic nerve activity in rats. Am J Physiol Regul Integr Comp Physiol 293:R1938–R1946.
Kober H, Barrett LF, Joseph J, Bliss-Moreau E, Lindquist K, Wager TD (2008) Functional grouping and cortical-subcortical interactions in emotion: a meta-analysis of neuroimaging studies. Neuroimage 42:998–1031.
Koutcherov Y, Mai JK, Ashwell KW, Paxinos G (2004) Organisation of the human dorsomedial hypothalamic nucleus. Neuroreport 15: 107–111.
Koutcherov Y, Mai JK, Paxinos G (2003) Hypothalamus of the human fetus. J Chem Neuroanat 26:253–270.
Krout KE, Jansen AS, Loewy AD (1998) Periaqueductal gray matter projection to the parabrachial nucleus in rat. J Comp Neurol 401: 437–454.
Kunos G, Varga K (1995) The tachycardia associated with the defense reaction involves activation of both GABAA and GABAB receptors in the nucleus tractus solitarii. Clin Exp Hypertens 17:91–100.
Lam W, Gundlach AL, Verberne AJ (1997) Neuronal activation in the forebrain following electrical stimulation of the cuneiform nucleus in the rat: hypothalamic expression of c-fos and NGFI-A messenger RNA. Neuroscience 78:1069–1085.
Lane JD, Adcock RA, Burnett RE (1992a) Respiratory sinus arrhythmia and cardiovascular responses to stress. Psychophysiology 29:461–470.
Lane RD, Wallace JD, Petrosky PP, Schwartz GE, Gradman AH (1992b) Supraventricular tachycardia in patients with right hemisphere strokes. Stroke 23:362–366.
LeDoux J (2007) The amygdala. Curr Biol 17:R868–R874.
Leor J, Poole WK, Kloner RA (1996) Sudden cardiac death triggered by an earthquake. N Engl J Med 334:413–419.
Lisa M, Filippelli A, Marmo E, Wible JH Jr, DiMicco JA (1989a) Microinjection of muscimol into posterior hypothalamus blocks cardio-

vascular response to experimental stress in rats. Pharmacol Res 21 (Suppl 1):9–10.

Lisa M, Marmo E, Wible JH Jr, DiMicco JA (1989b) Injection of muscimol into posterior hypothalamus blocks stress-induced tachycardia. Am J Physiol 257:R246–R251.

Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, Ford E, Furie K, Go A, Greenlund K, Haase N, Hailpern S, Ho M, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott M, Meigs J, Mozaffarian D, Nichol G, O'Donnell C, Roger V, Rosamond W, Sacco R, Sorlie P, Stafford R, Steinberger J, Thom T, Wasserthiel-Smoller S, Wong N, Wylie-Rosett J, Hong Y (2009) Heart disease and stroke statistics—2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 119:480–486.

Loewy AD (1981) Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. Brain Res 222:129–133.

Lovallo WR, Gerin W (2003) Psychophysiological reactivity: mechanisms and pathways to cardiovascular disease. Psychosom Med 65:36–45.

Lumb BM (1990) Hypothalamic influences on viscero-somatic neurones in the lower thoracic spinal cord of the anaesthetized rat. J Physiol 424:427–444.

McAllen RM, May CN, Shafton AD (1995) Functional anatomy of sympathetic premotor cell groups in the medulla. Clin Exp Hypertens 17:209–221.

McDougall SJ, Widdop RE, Lawrence AJ (2004) Medial prefrontal cortical integration of psychological stress in rats. Eur J Neurosci 20:2430–2440.

McDowall LM, Horiuchi J, Killinger S, Dampney RA (2006) Modulation of the baroreceptor reflex by the dorsomedial hypothalamic nucleus and perifornical area. Am J Physiol Regul Integr Comp Physiol 290:R1020–R1026.

Mittleman MA, Maclure M, Sherwood JB, Mulry RP, Tofler GH, Jacobs SC, Friedman R, Benson H, Muller JE (1995) Triggering of acute myocardial infarction onset by episodes of anger. Determinants of Myocardial Infarction Onset Study Investigators. Circulation 92:1720–1725.

Morrison SF (2004) Central pathways controlling brown adipose tissue thermogenesis. News Physiol Sci 19:67–74.

Morrison SF, Sved AF, Passerin AM (1999) GABA-mediated inhibition of raphe pallidus neurons regulates sympathetic outflow to brown adipose tissue. Am J Physiol 276:R290–R297.

Nalivaiko E, Blessing WW (2001) Raphe region mediates changes in cutaneous vascular tone elicited by stimulation of amygdala and hypothalamus in rabbits. Brain Res 891:130–137.

Nalivaiko E, Ootsuka Y, Blessing WW (2005) Activation of 5-HT1A receptors in the medullary raphe reduces cardiovascular changes elicited by acute psychological and inflammatory stresses in rabbits. Am J Physiol Regul Integr Comp Physiol 289:R596–R604.

Okamura H, Abitbol M, Julien JF, Dumas S, Berod A, Geffard M, Kitahama K, Bobillier P, Mallet J, Wiklund L (1990) Neurons containing messenger RNA encoding glutamate decarboxylase in rat hypothalamus demonstrated by *in situ* hybridization, with special emphasis on cell groups in medial preoptic area, anterior hypothalamic area and dorsomedial hypothalamic nucleus. Neuroscience 39:675–699.

Oppenheimer S (2007) Cortical control of the heart. Cleve Clin J Med 74 (Suppl 1):S27–S29.

Oppenheimer SM, Cechetto DF (1990) Cardiac chronotropic organization of the rat insular cortex. Brain Res 533:66–72.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic Press.

Quirk GJ, Gehlert DR (2003) Inhibition of the amygdala: key to pathological states? Ann N Y Acad Sci 985:263–272.

Quirk GJ, Likhtik E, Pelletier JG, Pare D (2003) Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. J Neurosci 23:8800–8807.

Radley JJ, Gosselink KL, Sawchenko PE (2009) A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. J Neurosci 29:7330–7340.

Samuels BC, Zaretsky DV, DiMicco JA (2002) Tachycardia evoked by disinhibition of the dorsomedial hypothalamus in rats is mediated through medullary raphe. J Physiol 538:941–946.

Samuels BC, Zaretsky DV, DiMicco JA (2004) Dorsomedial hypothalamic sites where disinhibition evokes tachycardia correlate with location of raphe-projecting neurons. Am J Physiol Regul Integr Comp Physiol 287:R472–R478.

Sander D, Klingelhofer J (1994) Changes of circadian blood pressure patterns after hemodynamic and thromboembolic brain infarction. Stroke 25:1730–1737.

Schadt JC, Hasser EM (1998) Hemodynamic effects of acute stressors in the conscious rabbit. Am J Physiol 274:R814–R821.

Schadt JC, Hasser EM (2001) Defense reaction alters the response to blood loss in the conscious rabbit. Am J Physiol Regul Integr Comp Physiol 280:R985–R993.

Sevoz-Couche C, Comet MA, Hamon M, Laguzzi R (2003) Role of nucleus tractus solitarius 5-HT3 receptors in the defense reaction-induced inhibition of the aortic baroreflex in rats. J Neurophysiol 90:2521–2530.

Shaikh MB, Barrett JA, Siegel A (1987) The pathways mediating affective defense and quiet biting attack behavior from the midbrain central gray of the cat: an autoradiographic study. Brain Res 437:9–25.

Shekhar A, Johnson PL, Sajdyk TJ, Fitz SD, Keim SR, Kelley PE, Gehlert DR, DiMicco JA (2006) Angiotensin-II is a putative neurotransmitter in lactate-induced panic-like responses in rats with disruption of GABAergic inhibition in the dorsomedial hypothalamus. J Neurosci 26:9205–9215.

Siegel A, Schubert KL, Shaikh MB (1997) Neurotransmitters regulating defensive rage behavior in the cat. Neurosci Biobehav Rev 21:733–742.

Smith PA, Graham LN, Mackintosh AF, Stoker JB, Mary DA (2004) Relationship between central sympathetic activity and stages of human hypertension. Am J Hypertens 17:217–222.

Soltis RP, Cook JC, Gregg AE, Stratton JM, Flickinger KA (1998) EAA receptors in the dorsomedial hypothalamic area mediate the cardiovascular response to activation of the amygdala. Am J Physiol 275:R624–R631.

Soltis RP, DiMicco JA (1991a) GABAA and excitatory amino acid receptors in dorsomedial hypothalamus and heart rate in rats. Am J Physiol 260:R13–R20.

Soltis RP, DiMicco JA (1991b) Interaction of hypothalamic GABAA and excitatory amino acid receptors controlling heart rate in rats. Am J Physiol 261:R427–R433.

Soltis RP, DiMicco JA (1992) Hypothalamic excitatory amino acid receptors mediate stress-induced tachycardia in rats. Am J Physiol 262:R689–R697.

Stephan KE, Fink GR, Marshall JC (2007) Mechanisms of hemispheric specialization: insights from analyses of connectivity. Neuropsychologia 45:209–228.

Stotz-Potter EH, Morin SM, DiMicco JA (1996a) Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air stress-induced neuroendocrine and cardiovascular changes in rats. Brain Res 742:219–224.

Stotz-Potter EH, Willis LR, DiMicco JA (1996b) Muscimol acts in dorsomedial but not paraventricular hypothalamic nucleus to suppress cardiovascular effects of stress. J Neurosci 16:1173–1179.

Tanaka M, McAllen RM (2008) Functional topography of the dorsomedial hypothalamus. Am J Physiol Regul Integr Comp Physiol 294:R477–R486.

Taylor RB, Weaver LC (1992) Spinal stimulation to locate preganglionic neurons controlling the kidney, spleen, or intestine. Am J Physiol 263:H1026–H1033.

Ter Horst GJ, Hautvast RW, De Jongste MJ, Korf J (1996) Neuroanatomy of cardiac activity-regulating circuitry: a transneuronal retrograde viral labelling study in the rat. Eur J Neurosci 8:2029–2041.

ter Horst GJ, Luiten PG (1986) The projections of the dorsomedial hypothalamic nucleus in the rat. Brain Res Bull 16:231–248.

Thompson RH, Canteras NS, Swanson LW (1996) Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. J Comp Neurol 376:143–173.

Thompson RH, Swanson LW (1998) Organization of inputs to the dorsomedial nucleus of the hypothalamus: a reexamination with fluorogold and PHAL in the rat. Brain Res Brain Res Rev 27:89–118.

Toga AW, Thompson PM (2003) Mapping brain asymmetry. Nat Rev Neurosci 4:37–48.

Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32–58.

Vertes RP (2006) Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience 142:1–20.

Villela DC, da Silva LG Jr, Fontes MA (2009) Activation of 5-HT receptors in the periaqueductal gray attenuates the tachycardia evoked from dorsomedial hypothalamus. Auton Neurosci 148: 36–43.

Wittling W, Block A, Genzel S, Schweiger E (1998a) Hemisphere asymmetry in parasympathetic control of the heart. Neuropsychologia 36:461–468.

Wittling W, Block A, Schweiger E, Genzel S (1998b) Hemisphere asymmetry in sympathetic control of the human myocardium. Brain Cogn 38:17–35.

Xavier CH, Beig MI, Ianzer D, Fontes MA, Nalivaiko E (2010) Cardiac chronotropic and inotropic responses evoked from right or left sides of dorsomedial hypothalamus. FASEB J 24:1019–1020.

Xavier CH, Nalivaiko E, Beig MI, Menezes GB, Cara DC, Campagnole-Santos MJ, Fontes MA (2009) Functional asymmetry in the descending cardiovascular pathways from dorsomedial hypothalamic nucleus. Neuroscience 164:1360–1368.

Yasui Y, Breder CD, Saper CB, Cechetto DF (1991) Autonomic responses and efferent pathways from the insular cortex in the rat. J Comp Neurol 303:355–374.

Yoshida K, Li X, Cano G, Lazarus M, Saper CB (2009) Parallel preoptic pathways for thermoregulation. J Neurosci 29:11954–11964.

Zagon A, Smith AD (1993) Monosynaptic projections from the rostral ventrolateral medulla oblongata to identified sympathetic preganglionic neurons. Neuroscience 54:729–743.

Zaretskaia MV, Zaretsky DV, DiMicco JA (2003) Role of the dorsomedial hypothalamus in thermogenesis and tachycardia caused by microinjection of prostaglandin E2 into the preoptic area in anesthetized rats. Neurosci Lett 340:1–4.

Zaretskaia MV, Zaretsky DV, Sarkar S, Shekhar A, DiMicco JA (2008) Induction of Fos-immunoreactivity in the rat brain following disinhibition of the dorsomedial hypothalamus. Brain Res 1200:39–50.

Zaretsky DV, Zaretskaia MV, DiMicco JA (2003a) Stimulation and blockade of GABA(A) receptors in the raphe pallidus: effects on body temperature, heart rate, and blood pressure in conscious rats. Am J Physiol Regul Integr Comp Physiol 285: R110–R116.

Zaretsky DV, Zaretskaia MV, Samuels BC, Cluxton LK, DiMicco JA (2003b) Microinjection of muscimol into raphe pallidus suppresses tachycardia associated with air stress in conscious rats. J Physiol 546:243–250.

(Accepted 7 March 2011)
(Available online 22 March 2011)

Anexo 3

From: jjones@the-aps.org
Subject: R-00401-2012 Manuscript Received
Date: 5 de setembro de 2012 10:44:28 BRT
To: fisioch@gmail.com

Dear Mr. Xavier:

R-00401-2012, "Asymmetry in the control of cardiac performance by dorsomedial hypothalamus" has been submitted to American Journal of Physiology - Regulatory, Integrative and Comparative Physiology on 5th Sep 2012. Authors are:
Carlos Xavier, Mirza Beig, Danielle Ianzer, Marco Fontes, and Eugene Nalivaiko

If you have not already done so, please complete the Mandatory Submission Form. A copy of this form is located at:
http://ajpregu.msubmit.net/cgi-bin/main.plex?el=A7Dn6VTi4A3Bint2G5A9mqvvv4XldIyVfPdO2kmywZ

(Press/Click on the above link to be automatically sent to the web page.)

The form must be signed and returned by fax to +301-634-7243.

Thank you for submitting your manuscript to the journal.

Regards,
Editorial Staff

Asymmetry in the control of cardiac performance by dorsomedial hypothalamus

Carlos Henrique Xavier[1,2,3], Mirza Irfan Beig[2], Danielle Ianzer[4], Marco Antônio Peliky Fontes[1] and Eugene Nalivaiko[2]

[1]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil;
[2]School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, New South Wales, Australia;
[3]Departamento de Ciências Biológicas, Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil;
[4]Laboratório Especial de Toxinologia Aplicada / CAT-CEPID, Instituto Butantan, Sao Paulo, SP, Brazil.

Authors contribution: Xavier CH – performed experiments, analyzed data, drafted the manuscript; Beig MI – performed experiments; Iazner D – performed experiments; Fontes MAP – designed the study, drafted the manuscript; Nalivaiko E – designed the study, performed experiments, analyzed data, drafted the manuscript.

Running head: Control of cardiac contractility by dorsomedial hypothalamus

Corresponding author:
A/Prof Eugene Nalivaiko
School of Biomedical Sciences and Pharmacy
University of Newcastle
Callaghan NSW 2308, Australia.
Tel.: 61 2 4921 5620
Email:Eugene.Nalivaiko@newcastle.edu.au

ABSTRACT:

Dorsomedial hypothalamus (DMH) plays a key role in integrating cardiovascular responses to stress. We have recently reported greater heart rate responses following disinhibition of the right side of the DMH (R-DMH) in anesthetized rats and greater suppression of stress-induced tachycardia following inhibition of the R-DMH in conscious rats (both compared to similar intervention in the left DMH), suggesting existence of right/left side asymmetry in controlling cardiac chronotropic responses by the DMH. The aim of the present study was to determine whether similar asymmetry is present for controlling cardiac contractility. In anesthetized rats, microinjections of the $GABA_A$ antagonist bicuculline methiodide (BMI, 40pmol/100nl) into the DMH evoked increases in heart rate (HR), left ventricular pressure (LVP), myocardial contractility (LVdP/dt), arterial pressure and respiratory rate. DMH disinhibition also precipitated multiple ventricular and supraventricular ectopic beats. DMH-induced increases in HR, LVP, LVdP/dt and in the number of ectopic beats depended on the side of stimulation, with R-DMH provoking larger responses. In contrast, pressor and respiratory responses did not depend on the side of stimulation. Newly described DMH-induced inotropic responses were rate-, preload- and (largely) afterload-independent; they were mediated by sympathetic cardiac pathway as revealed by their sensitivity to beta-adrenergic blockade. We conclude that recruitment of DMH neurons causes sympathetically-mediated positive chronotropic and inotropic effects, and that there is an asymmetry, at the level of the DMH, in the potency to elicit these effects, with R-DMH>L-DMH.

KEYWORDS: cardiac contractility; dorsomedial hypothalamus; asymmetry.

INTRODUCTION

Psychological stress may cause cardiovascular disturbances, including potentially fatal cardiac arrhythmias (29). The end-organ (cardiac) mechanisms precipitating stress-induced arrhythmias are relatively well understood; they comprise excessive release of noradrenaline from the cardiac sympathetic terminals (20, 22, 28). Meanwhile, mechanistic link between mental events and activation of cardiac sympathetic neurons still remains unclear, and many details of central pathways that lead to their activation during stress are unknown. Animal experiments conducted during the two last decades provided the basis for understanding these pathways, and it is now firmly established that the dorsomedial hypothalamus (DMH) is a key region in orchestrating neurally-mediated cardiovascular responses to stress. Indeed, integrity of synaptic transmission in the DMH is essential for the expression of stress-induced tachycardic and pressor responses (15). These functional studies are well supported by neuroanatomical data: the DMH receives synaptic inputs from many cortical and subcortical areas that are activated during stress and are involved in emotional processing, including the amygdala, the medial prefrontal cortex and the insular cortex (7, 18, 34, 41). DMH descending projections, after relaying in the medullary raphe, can excite cardiomotor spinal sympathetic neurons and thus enhance cardiac sympathetic outflow [for review, see (15)]. It must be acknowledged here that with the exception of two earlier works employing electrical stimulation of the hypothalamic area (11, 21), the knowledge about DMH involvement in the heart control was accumulated using just heart rate as an index of an effect. Changes in heart rate however are not sufficient for making conclusion about autonomic influences on the myocardial performance as there are examples of dissociation between neurally-induced inotropic and chronotropic effects [eg. (25)]. Consequently, it remains entirely unknown whether DMH activation could lead to changes in myocardial contractility and/or to cardiac arrhythmias.

In 2005 Critchley at al. (8) have provided the first human brain imaging data showing robust positive relationship between midbrain activity and potentially pro-arrhythmic abnormalities in ventricular repolarization during psychological and physical stresses. These cardiac effects were attributed to the increase of sympathetic outflow to the ventricular myocardium, suggesting that relevant cardiac sympathetic neurons were activated concurrently with, and maybe from the midbrain. While authors carefully avoided using more specific anatomical terms, from their Figs. 2&3 it is evident that their midbrain region included the DMH. Of particular interest in this work was also the finding that the brain/heart correlation was observed exclusively for the right hemisphere. Based on previous evidence for right-side cortical dominance in cardiac control (43, 44), authors made a suggestion that "right-lateralized shift in midbrain activity reflects dysfunction, during stress, of a system that translates cortical activity into bilateral autonomic responses in the periphery". This human study triggered our interest towards the lateralization of cardiac control in the brain, and in our recent work we attempted to determine whether this phenomena is present in experimental animals. We found that pharmacological inhibition of the right DMH (R-DMH) performed in rats prior to air-jet stress nearly completely abolished tachycardia caused by this stress whereas inhibition of the left DMH (L-DMH) was without effect (47). In anesthetized rats, disinhibition of the R-DMH provoked more potent tachycardic responses compared to the L-DMH. In contrast, pressor responses elicited from right or left DMH did not differ (13, 46, 47). In this previous work we did not assess DMH-induced effects on myocardial contractility, and the principal aim of our current work was to determine whether such effects exist, and if so – whether there is an inter-hemispheric asymmetry in their origin. To this end, in anesthetized rats we recorded ECG and measured changes in the left ventricular pressure (LVP) and contractility (LVdP/dt) elicited by unilateral (right and left) disinhibition of the DMH with $GABA_A$ antagonist bicuculline. Our additional aims were to determine whether DMH activation could provoke cardiac

arrhythmias, and whether, if present, DMH-induced inotropic and pro-arrhythmic effects are sympathetically mediated. Part of our results have been published in abstract form (45).

METHODS

Animals and surgery

Experiments were performed on adult male Hooded Wistar rats (300-350 g) bred at the animal facilities at the School of Biomedical Sciences and Pharmacy, University of Newcastle. All experimental procedures were approved by the Animal Ethics and Care Committee of the University of Newcastle, and conformed to the National Health and Medical Research Council guidelines. Experiments were conducted under combined α-chloralose (60 mg/kg, i.p.) and urethane (600 mg/kg, i.p.) anesthesia. Its adequacy was verified by the absence of a withdrawal response to a nociceptive stimulation of a hindpaw. Supplemental doses of urethane were given when necessary. Following anesthesia induction, an endotracheal tube was inserted and connected to a line supplying humidified 100% oxygen. Animals were breathing spontaneously throughout the experiment. End-tidal CO_2 was monitored using Normocap CO_2 monitor (Datex, Helsinki, Finland). Polyethylene catheters were placed into the femoral artery and vein for recordings arterial pressure (AP) and for drug injections, respectively. Left ventricular pressure (LVP) was measured using a microtip pressure transducer (SPR-249, Millar Instruments, Houston, TX, USA) inserted into the left ventricle through the right common carotid artery. LVdP/dt (a measure of contractility) was computed online as a first derivative of LVP. Left ventricular end-diastolic pressure (LVEDP) was also calculated from LVP traces. Three-lead electrocardiagram (ECG) was recorded using subcutaneous stainless steel electrodes connected a BioAmp (ADInstruments, Sydney, Australia). Subsequently, the animals were positioned on a heating pad in a prone position, and their head was placed in a stereotaxic frame (Stoelting, IL, USA), with the tooth bar fixed at -3.3 mm below the interaural line. A small craniotomy was

made bilaterally near the Bregma level to allow later insertion of a glass micropipette into the DMH. Body temperature was monitored using rectal thermometer and maintained in the range of 37-37.5° C with a heating pad.

Experimental design

Experiment 1: Effects of DMH disinhibition on cardiovascular and respiratory parameters in unpaced preparation.

This experiment conducted in seven rats consisted of four steps. We initially performed the first microinjection of bicuculline methiodide (BMI; a $GABA_A$ receptor antagonist; 40 pmol/100 nl) into the right or left side DMH, randomly chosen. After physiological variables returned to the baseline, a second injection of BMI was made into the contralateral side of the DMH. In the second step, the rats underwent i.v. administration of zatebradine (1 mg/kg), a blocker of Na pacemaker current (I_f) (10). We then repeated injections of BMI into right and left DMH. The purpose of using zatebradine was two-fold: i) to reduce, as much as possible, rate-dependent effects on cardiac contractility, and ii) to prolong vulnerable diastolic period, in anticipation that this may facilitate occurrence of ventricular arrhythmic events during DMH activation (26). In the third step we evaluated whether DMH-elicited cardiac responses were due to sympatho-excitation. For this purpose, β-adrenergic receptor blocker atenolol was administered i.v. (2 mg/kg) prior to repeating microinjections of bicuculline into the left and right DMH. Finally, the aim of the fourth step was to determine the contribution of afterload-dependent contractility rise in the DMH-induced responses; for this purpose, a bolus i.v. injection of the α-adrenergic agonist phenylephrine (10 μg/kg) was performed (see below for details).

Experiment 2 - Effects of unilateral DMH disinhibition on cardiovascular parameters during cardiac pacing.

In this series of experiments (n=4), our objective was to completely eliminate rate-dependent effects on contractility when studying DMH-elicited cardiac effects. For this purpose, after placing tracheal tube, arterial and venous lines, LVP catheter and ECG electrodes (as described above), a midline thoracotomy was performed, and animals were artificially ventilated using Dual-Mode TOPO ventilator (Kent Scientific, Torrington, CT, USA). Two stainless-still pacing electrodes were attached to the atria, and connected to a constant-current stimulator triggered from the PowerLab (ADInstruments, Sydney, Australia) output. The animals were placed in the stereotaxic apparatus and prepared for the injections into the DMH as described above. After stabilization of all parameters, zatebradine (1 mg/kg) was administered i.v. to decrease the heart rate to about 270 bpm. The heart was then paced at 360 bpm (the peak value achieved in zatebradine-treated rats without pacing). Pacing was started before the brain microinjections and lasted for the next 15 minutes past injection. The first microinjection of bicuculline methiodide was made in either right or left DMH, randomly selected; 30 min later the second microinjection was made into the contralateral DMH.

Microinjections and drugs

Microinjections of bicuculline were made into the DMH (100 nl/side) with a fine-tip (50 μm) glass micropipette. Injected volume was controlled by observing meniscus in a calibrated glass capillary. The coordinates were 3.2 mm posterior and 0.6 mm lateral to the bregma, at a depth of 8.5 mm below the dura (27). Bicuculline methiodide, atenolol and phenylephrine were from Sigma (USA); zatebradine [1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-][2-(3,4-dimethoxyphenyl)ethyl] methylimino]propyl]-2H-3-benzazepin-2-on hydrochloride], a blocker of I_f pacemaker (10) was a gift from Otsuka Pharmaceuticals (Tokyo, Japan).

Data acquisition and analysis.

All analog signals except ECG were sampled at 100 Hz; ECG was digitized at 1 kHz. Data were acquired using PowerLab 8/20 and LabChart 7.0 (ADInstruments, Sydney, Australia) and displayed online. Online computations were performed for the heart rate (from the ECG), respiratory rate (from the CO_2 signal) and LVdP/dT (by differentiating the LVP signal).

In order to control for the afterload-induced contractility changes, we compared the AP-independency contractility index computed from values obtained during DMH activation to that from values obtained during phenyleprine administration. The index was calculated according to the following equation: $I = (\Delta dP/dt)/(\Delta MAP)$ where ΔMAP and $\Delta dP/dt$ represent difference between the basal level and the maximal values for each variable obtained after either DMH activation or after phenylephrine.

Comparisons between responses evoked by the first and second microinjections of BMI into DMH were determined by a paired *t* test. For multiple comparisons, we used one-way ANOVA followed by Newman Keuls's post hoc test. Comparisons between the ranges of the responses following left or right DMH disinhibition were performed with two-way ANOVA (factors drug and time) followed by Bonferroni's post hoc test. Significance was taken at $P<0.05$. Data are expressed as mean ± SEM.

Histological verification of injection sites.

At the end of experiments rats were euthanized by an overdose of Lethabarb, and a microinjection of alcian blue dye 2% (100 nl) was made into the DMH injection sites for subsequent histological confirmation. Rats were then perfused transcardially with 50 ml of saline followed by 50 ml of phosphate-buffered paraformaldehyde (4%). The brains were removed, stored in paraformaldehyde (4%) for 24 hours and immersed in a sucrose solution (30%) for at least 48 hours. Brain slices (50 µm) were then cut in a freezing microtome. The

atlas of Paxinos & Watson (27) was used as a reference for histological confirmation of the injection sites in the DMH.

RESULTS

Effects of unilateral DMH disinhibition on cardiovascular and respiratory parameters and on the ECG.

Fig. 1 is an example of a coronal brain section depicting microinjection sites in the DMH. Unilateral microinjections of bicuculline into the DMH increased heart rate, arterial blood pressure, LVP, LVdP/dt and respiratory rate. We also noted that DMH activation raised the frequency of respiratory events that we interpreted as sighs or "augmented breaths" (see Discussion). Representative traces and data values for all parameters are presented in Fig. 2. Changes became noticeable within 1 min post-injection, peaked at 5-7 min and then gradually returned to the basal level within the next 25-30 min. Tachycardic and contractile (LVP and LVdP/dt) responses evoked from the right-side DMH were significantly higher compared to those evoked from the left side (Fig. 2, bar graphs). In contrast, the magnitude of pressor and tachypnoeic responses did not depend on the side of injection. There was also no difference in the incidence of sigh-like events evoked from the left and right sides.

In accord with presented above effects on the heart rate, DMH activation caused shortening of the ECG RR intervals, with more potent effect provoked from right side (R: -28±9 vs. L: -15±4 msec; $P<0.05$). No further differences were observed in other ECG parameters between right and left DMH (data not shown). Importantly, DMH activation consistently provoked supraventricular and ventricular ectopic beats (Fig. 3), with significantly higher incidence for both subtypes after disinhibition of R-DMH. Such ectopic beats were never observed at baseline.

Effects of I_f blockade on basal cardiovascular and respiratory parameters, and on the DMH-elicited responses.

Pharmacological blockade of the cardiac pacemaker current caused marked and sustained fall in HR (Δ HR -104±4 bpm, $P<0.05$ *vs.* baseline), without enduring effects on other parameters. During the initial phase of this fall we observed slight transient increases in $LVdP/dt_{peak}$ (Δ $LVdP/dt_{peak}$ = +1293±496 mmHg/s *vs.* baseline, $P<0.05$). This effect lasted less than 3 minutes, and at the 5th minute following injection the parameters stabilized at the levels not different from the pre-injection values (Fig. 4A).

Unilateral DMH activation performed under zatebradine blockade caused increases in MAP, HR and ventricular contractility indices (Fig. 5; mean data values shown near corresponding traces). Right-side dominance of the DMH influences on the cardiac performance was preserved after zatebradine (Fig. 5). The drug also substantially reduced the incidence of supraventricular ectopic beats triggered by the activation of right DMH (7±2 *vs.* 2±1 SVEB/10min; P<0.05); in contrast, the incidence of ventricular ectopics was not affected (L=3±2 *vs*. 2±1 and R= 9±4 *vs*. 6±2 VEB/10min; before *vs*. after zatebradine, respectively).

The magnitude of tachypnoeic responses did not differ from pre-zatebradine condition, and was similar for both sides of injection (ΔRespRate L= 47±8 *vs*. 43±9 and R= 54±9 *vs*. 47±8cpm; before *vs*. after zatebradine, respectively). There was also no difference in the incidence of sighs evoked from the left and right sides (Δsighs = 16±2 *vs*. 17±2 sighs/10min, L *vs*. R respectively).

Effects of atenolol on basal cardiovascular and respiratory parameters and on DMH-induced responses

Beta-adrenergic blockade with atenolol caused falls in MAP (-6±2mmHg), HR (-33±5bpm), LVP (-15±4mmHg) and LVdP/dt (-432±517mmHg/s) (Fig. 4B). Atenolol

completely blocked cardiac chronotropic responses evoked by microinjection of bicuculline into either side of the DMH (Fig. 6). However, DMH activation after atenolol still caused significant pressor responses associated with small but significant rises in the ventricular contractility; there was no lateral asymmetry in eliciting these responses (Fig.6). Atenolol completely abolished supraventricular ectopic beats during activation of either side of the DMH; a small number of ventricular ectopics (3±1 and 6±4 VEB/10min; L and R respectively) was however still present in 3 of 7 animals.

Effect of afterload on ventricular contractility

In order to determine whether DMH-elicited increases in ventricular contractility were secondary to increases in afterload (i.e. due to rises in the peripheral vascular resistance), we compared the (ΔLVdP/dt)/(ΔMAP) ratio determined during DMH activation to that determined during administration of phenylephrine. Result of this analysis is presented in Fig. 7. Both left and right DMH activation provoked pronounced increases LVdP/dt, with similar slope. In contrast, responses caused by phenylephrine elicited quite substantial increases in MAP, moderately affecting contractility. It is evident from this analysis that pressure rises provoked from the DMH (+15-20 mmHg) could account for only minor fraction of associated positive inotropic effect. Fig. 7 also illustrates our presented above finding that activation of the DMH provoked similar pressor effect but side-dependent (R>L) inotropic effect. The values of (ΔLVdP/dt)/(ΔMAP) ratios for DMH-induced responses obtained under beta-adrenergic blockade (L=84±16 and R=79±16 s^{-1}) did not differ from the phenylephrine-induced effect (61±9 s^{-1}).

Effects of unilateral DMH disinhibition on cardiovascular parameters during cardiac pacing.

Changes in HR, LVP and LVdp/dt during this experimental series are exemplified in Fig. 8. In unpaced preparations, pharmacological blockade of cardiac pacemaker current by zatebradine provoked enduring bradycardia and transient contractility changes similarly to those described above. Unilateral disinhibition of the DMH during cardiac pacing caused rises in LVP and LVdp/dt; as observed in the other experiments of this study, inotropic effects elicited from R-DMH were significantly larger compared to those triggered from L-DMH (mean data values are shown near corresponding traced in Fig. 8).

DISCUSSION

Our principal findings are: i) evidence that the DMH activation causes positive inotropic effects and alters myocardial excitability in a pro-arrhythmic manner; and ii) right-side dominance of the DMH to evoke these effects. These findings confirm and extend previous reports showing that right side of the hypothalamus dominates in controlling cardiac function (12, 47).

Methodological issues.

Maximal rate of LVP raise (LVdP/dt) is a well-accepted index of myocardial contractility, and we used it here to measure sympathetically-induced inotropic effects in anesthetized rats. In addition of being under autonomic control, LVdP/dt depends on several intrinsic parameters, including heart rate (Bowditch effect, treppe or frequency-dependent inotropy), preload (Frank-Starling mechanisms) and afterload (von Anrep effect) (1, 16, 42). We thus employed different approaches to exclude potential confounding effects of these influences. Firstly, we used zatebradine (a blocker of Na pacemaker current) to prevent/attenuate sympathetically-induced tachycardia. Indeed, after zatebradine administration, the maximal HR values following DMH activation were not greater than the pre-drug baseline. However, post-zatebradine DMH-induced tachycardic responses were still

quite substantial, and this strategy alone was not enough to exclude the rate-dependent effects. We have resolved this problem by reproducing DMH-induced inotropic effect in the situation when the heart was paced at a fixed rate. Secondly, in our experiments we assessed preload by measuring LVEDP. Lack of changes of this index during DMH-induced rises in LVP and LVdP/dt suggests that contractility changes were not preload-induced. It must be acknowledged that LVEDP is an indirect estimate of the left ventricular volume and could be used as an index of preload only if the ventricular compliance remains constant. Finally, as LVdP/dt in the rat heart is strongly affected by afterload (24, 42), we have controlled for this confound by comparing Δ(LVdP/dt)/ΔMAP ratio for DMH-induced responses to that provoked by administration of α-adrenergic agonist phenylephrine (30). As expected, phenylephrine provoked both pressor and contractile effect, but the magnitude of this afterload-induced contractile response was substantially smaller compared to DMH-induced inotropic effect. An additional argument in favor of afterload-independence of DMH-induced inotropic effects is that they did depend on the side of activation whereas pressor effects did not.

Analysis of our data from phenylephrine experiment clearly indicates that afterload could contribute to the DMH-induced inotropic response, but this contribution was relatively small. Indeed, as could be seen in Fig. 7A, rise of MAP by about 20 mmHg evoked by phenylephrine (a usual range of DMH-induced pressor effect) could account for not more than 25% of contractility rise evoked from the L-DMH and even less for the R-DMH-induced response. Thus, while a fraction of DMH-induced rise in cardiac contractility certainly was a consequence of increased afterload, we are confident that cardiac sympathetic nerves mediated the major part of this response. This suggestion was fully confirmed by its sensitivity to beta-blockade. Small rises in contractility that were still present post-atenonlol could be most likely attributed to the described above effect of afterload. This suggestion is supported by the fact that Δ(LVdP/dt)/ΔMAP ratios computed

for DMH-induced responses elicited under beta-blockade were identical to those computed for responses evoked by phenylephrine.

Stable physiological state of our preparation is evidenced by stability of pressor responses (control/post-zarebradine/post-atenolol) in the course of experiment.

DMH-induced chronotropic and inotropic responses are sympathetically-mediated.

Our data showing right-side DMH dominance for controlling HR confirms our recent findings where we have demonstrated such dominance in both anesthetized and conscious animals (46, 47). Current data also extends these findings to cardiac contractile function, showing that myocardial contractility is affected by removal tonic Gabaergic input on DMH neurons. Indirectly, our findings indicate that DMH neurons controlling heart rate and myocardial contractility are under tonic inhibitory influences.

We present evidence suggesting that presympathetic cardiomotor neurons controlling HR and contractility are predominantly segregated in the R-DMH. This appears to be quite a robust observation as in none of experiments reported here did we encounter L-DMH dominance in cardiac responses. Likewise, no cases of left-side dominance were observed in our previous study (46, 47).

Substantial reduction of basal HR by zatebradine is in full accord with the view that sympathetic influences in the pacemaker cell are mediated via activation of I_f, a Na pacemaker current (10, 39). Zatebradine had no effect on basal contractile indices (with the exception of brief transitional periods where HR was changing) or contractile responses, in agreement with established pharmacological profile of this drug (2, 9, 40).

Beta-adrenergic blockade resulted in the reductions of basal cardiac contractile indices and HR. As in rat heart force-frequency relation is negative (24), it is likely that the magnitude of the negative inotropic effects was underestimated. Our results thus indicate that in anesthetized rats, there is a substantial basal sympathetic outflow to the ventricular

myocardium. Suppresion by atenolol of DMH-induced tachycardic resposes confirms our previous finding that they are sympathetically mediated (15). Finally, the fact that atenolol suppressed afterload-independent component (see below) of DMH-induced contractile response is a solid proof that it was also mediated via cardiac sympathetic outflow.

Small contractile responses that persisted following beta-adrenergic blockade were no more depended on the side of DMH stimulation, and thus were likely elicited by different mechanisms. Two arguments support the idea that these responses were the consequence of increased afterload (Anrep effect): firstly, the slope of contractility/MAP ratio (or AP-independence index) was substantially less steep compared to the control condition but did not differ from that computed following phenylephrine test. Secondly, the time course of these contractile responses closely resembled time course of pressor changes whereas in control condition temporal differences between the two were quite obvious.

DMH activation affects cardiac excitability.

Activation of the DMH consistently elicited supraventricular and ventricular ectopic beats. While these cardiac arrhythmic events are not considered malignant clinically, it must be acknowledged that our study was performed in animals with normal (not predisposed) myocardium. Thus ectopic beats found in our study indicate that DMH activation resulted in observable changes in the excitability of cardiac tissues. This finding is new and relevant for elucidation of mechanisms of cardiac arrhythmias precipitated by psychological stresses (26). Excessive release of noradrenaline from cardiac sympathetic terminals is a well-established peripheral mechanism of these arrhythmias (22, 23). Providing a documented involvement of DMH into the sympathetically mediated responses to stressors (15) and its central role in generating vascular and cardiac sympathetic activity (including our current contractility data), DMH-induced cardiac arrhythmias are not an unexpected finding. Of interest is the fact that supraventricular, but not the ventricular ectopic beats evoked from

DMH were prevented by zatebradine, a blocker of the pacemaker sodium current. This suggests that supraventricular ectopics were originating in atrial conducting system that, in contrast to the myocardium, does express relevant sodium channels (33). Their sensitivity to beta-adrenergic stimulation is well established, and our data suggests that excessive release of noradrenaline in atria, in addition to physiological positive chronotropic effects, may provoke arrhythmic events by activating I_f.

Zatebradine had no effect on the number of ventricular ectopic beats; the incidence of the latter was reduced, but not totally eliminated, by beta-blocker atenolol. This residual ectopy could persist either due to activation of alpha-adrenoreceptors, or due to incomplete beta-blockade.

DMH-induced respiratory effects.

In contrast to human studies, where close association between respiratory indices and affective states is firmly established, studies in conscious animals are mainly limited to homeostatic (rhythm-generating and chemoreflex-related) ponto-medullary mechanisms. We have recently presented the first documented evidence that in rats, psychological stressors cause rise in the respiratory rate associated with elevated incidence of sighs (augmented breaths) (19). DMH-induced tachypnoea observed in the current and several previous studies (17, 35) could well be the principal mechanism underlying these stress-related respiratory effects. We also present preliminary evidence that DMH activation could lead to an increase of sighing frequency. We acknowledge that we did not measure tidal volume (an essential condition for identifying sigh); we made this serendipitous finding when reviewing the end-tidal CO_2 signal that was used for computing respiratory rate. We believe that discrete respiratory events that we observed could be classified as sighs based on the following: i) their fairly regular occurrence; ii) a delay of the "sigh"-related peak of CO_2 signal (compared to pre-sigh regular respiratory rate) was likely due to the fact that sigh-related inspiration

normally occurs at the end of a previous inspiratory phase, so that the expiratory phase is delayed; and iii) post-sigh apnea that is a distinctive feature of sighs (19).

Neural pathways mediating DMH-induced effects.

Beta-adrenergic blockade completely prevented tachycardia evoked from the DMH, indicating that it was sympathetically mediated. This is in full accord with a number of studies in conscious and anesthetized animals where stress- and/or DMH-induced tachycardia could be prevented by beta-blockade or inhibition of the medullary raphe that relays descending projections from the DMH to the spinal cardiac sympathetic neurons (5, 14, 31, 32). Beta-blockade dramatically reduced DMH-elicited inotropic responses, suggesting that they are also largely mediated by sympathetic pathways. To the best of our knowledge, this is the first description of functional cardiac inotropic responses induced from the supra-medullary level, and it is currently unknown whether signals generated in the DMH for controlling heart rate and myocardial contractility share the same descending pathway (5, 14). In discussing this possibility, we would like to consider two medullary regions: the rostral ventro-lateral medulla (RVLM) and the medullary raphe/parapyramidal area. In rats, chemical activation of either of these areas causes sympathetically mediated tachycardia and rise in cardiac contractility (3, 4, 6, 30). Furthermore, anatomical tracing studies have demonstrated that both regions receive direct projections from the DMH (14, 37), and that both of them in turn project to the heart, including to the ventricular myocardium (36, 38, 48). Current consensus regarding medullary control of HR is that the RVLM mediates homeostatic (baroreflex) control and that DMH-RVLM projection carries signals for vascular but nor cardiac presympathetic neurons (14). In contrast, raphe region is involved in cardiac responses to environmental stressors (see above). Whether presymapthetic pathways for the control of cardiac inotropic function are arranged in a similar way remains an open question. Lack of changes in cardiac sympathetic activity, HR

or contractile responses to microinjection of GABA into the raphe area indicates that raphe-spinal cardiomotor neurons are not tonically active (5, 30), at least in anesthetized animals. For elaborated discussion of functional specialization of neurons controlling cardiac function (see 30).

Perspectives and conclusion

One intriguing question that now remains open is how right-side dominance for controlling both HR and contractility at the midbrain level gets converted into preferentially left-side control of contractility and preferentially right-side control of HR by cardiac sympathetic nerves. In addition, it would be most interesting to test whether DMH stimulation can trigger malignant ventricular tachyarrhythmias in animals with predisposed myocardium (eg. ischemic or post-infarction).

In conclusion, activation of R-DMH provokes larger positive inotropic and chronotropic effects compared to L-DMH. In conjunction with earlier cortical data, this may mean that there exists right-hemispheric dominance in controlling cardiac sympatho-excitatory effects. Our findings are relevant for identifying mechanisms of stress-induced cardiac diseases.

GRANTS

This study was funded by the NHMRC Project Grant #569299 (EN). CHX was recipient of PhD fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (143005/2008-1).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. **Bowditch HP.** Uber die Eigenth¨uemlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens zeigen. *Verh K Sachs GenWochenschr Leipzig Math Phys Cl* 23: 652-669, 1871.

2. **Breall JA, Watanabe J, Grossman W.** Effect of zatebradine on contractility, relaxation and coronary blood flow. *J Am Coll Cardiol* 21: 471-477, 1993.

3. **Campos RR McAllen RM.** Cardiac inotropic, chronotropic, and dromotropic actions of subretrofacial neurons of cat RVLM. *Am J Physiol* 276: R1102-1111, 1999.

4. **Campos RR McAllen RM.** Cardiac sympathetic premotor neurons. *Am J Physiol* 272: R615-620, 1997.

5. **Cao WH, Fan W, Morrison SF.** Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. *Neuroscience* 126: 229-240, 2004.

6. **Cao WH Morrison SF.** Disinhibition of rostral raphe pallidus neurons increases cardiac sympathetic nerve activity and heart rate. *Brain Res* 980: 1-10, 2003.

7. **Cechetto DF Chen SJ.** Subcortical sites mediating sympathetic responses from insular cortex in rats. *Am J Physiol* 258: R245-255, 1990.

8. **Critchley HD, Taggart P, Sutton PM, Holdright DR, Batchvarov V, Hnatkova K, Malik M, Dolan RJ.** Mental stress and sudden cardiac death: asymmetric midbrain activity as a linking mechanism. *Brain* 128: 75-85, 2005.

9. **DiFrancesco D.** Cardiac pacemaker I(f) current and its inhibition by heart rate-reducing agents. *Curr Med Res Opin* 21: 1115-1122, 2005.

10. **DiFrancesco D.** Some properties of the UL-FS 49 block of the hyperpolarization-activated current (i(f)) in sino-atrial node myocytes. *Pflugers Arch* 427: 64-70, 1994.

11. **Fang HS, Wang SC.** Cardioaccelerator and cardioaugmentor points in hypothalamus of the dog. *Am J Physiol* 203: 147-150, 1962.

12. **Fang HS, Wang SC.** Cardioaccelerator and cardioaugmentor points in hypothalamus of the dog. *Am J Physiol* 203: 147-150, 1962.

13. **Fontes MA, Campagnole-Santos MJ, and Xavier CH** Evaluation of the heart rate changes evoked by air jet stress after inhibition of the right or left sides of dorsomedial hypothalamus. *FASEB J* 22: 1170.1174, 2008.

14. **Fontes MA, Tagawa T, Polson JW, Cavanagh SJ, Dampney RA.** Descending pathways mediating cardiovascular response from dorsomedial hypothalamic nucleus. *Am J Physiol Heart Circ Physiol* 280: H2891-2901, 2001.

15. **Fontes MA, Xavier CH, de Menezes RC, Dimicco JA.** The dorsomedial hypothalamus and the central pathways involved in the cardiovascular response to emotional stress. *Neuroscience* 184: 64-74, 2011.

16. **Hallback M, Isaksson O, Noresson E.** Consequences of myocardial structural adaptation on left ventricular compliance and the Frank-Starling relationship in spontaneously hypertensive rats. *Acta Physiol Scand* 94: 259-270, 1975.

17. **Horiuchi J, McDowall LM, Dampney RA.** Vasomotor and respiratory responses evoked from the dorsolateral periaqueductal grey are mediated by the dorsomedial hypothalamus. *J Physiol* 587: 5149-5162, 2009.

18. **Hunt JL, Zaretsky DV, Sarkar S, Dimicco JA.** Dorsomedial hypothalamus mediates autonomic, neuroendocrine, and locomotor responses evoked from the medial preoptic area. *Am J Physiol Regul Integr Comp Physiol* 298: R130-140, 2010.

19. **Kabir MM, Beig MI, Baumert M, Trombini M, Mastorci F, Sgoifo A, Walker FR, Day TA, Nalivaiko E.** Respiratory pattern in awake rats: effects of motor activity and of alerting stimuli. *Physiol Behav* 101: 22-31, 2010.

20. **Lampert R, Jain D, Burg MM, Batsford WP, McPherson CA.** Destabilizing effects of mental stress on ventricular arrhythmias in patients with implantable cardioverter-defibrillators. *Circulation* 101: 158-164, 2000.

21. **Manning JW, Peiss CN.** Cardiovascular responses to electrical stimulation in the diencephalon. *Am J Physiol* 198: 366-370, 1960.

22. **Mittleman MA, Maclure M, Sherwood JB, Mulry RP, Tofler GH, Jacobs SC, Friedman R, Benson H, Muller JE.** Triggering of acute myocardial infarction onset by episodes of anger. Determinants of Myocardial Infarction Onset Study Investigators. *Circulation* 92: 1720-1725, 1995.

23. **Myles RC, Wang L, Kang C, Bers DM, Ripplinger CM.** Local beta-adrenergic stimulation overcomes source-sink mismatch to generate focal arrhythmia. *Circ Res* 110: 1454-1464, 2012.

24. **Nalivaiko E, Antunes VR, Paton JF.** Control of cardiac contractility in the rat working heart-brainstem preparation. *Exp Physiol* 95: 107-119, 2010.

25. **Nalivaiko E, De Pasquale CG, Blessing WW.** Ventricular arrhythmias triggered by alerting stimuli in conscious rabbits pre-treated with dofetilide. *Basic research in cardiology* 99: 142-151, 2004.

26. **Nalivaiko E, Mastorci F, Sgoifo A.** 8-OH-DPAT prevents cardiac arrhythmias and attenuates tachycardia during social stress in rats. *Physiol Behav* 96: 320-327, 2009.

27. **Paxinos G, Watson C.** *The rat brain in stereotaxic coordinates*. New York, 1986.

28. **Reich P, DeSilva RA, Lown B, Murawski BJ.** Acute psychological disturbances preceding life-threatening ventricular arrhythmias. *JAMA* 246: 233-235, 1981.

29. **Rozanski A, Blumenthal JA, Kaplan J.** Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 99: 2192-2217, 1999.

30. **Salo LM, Nalivaiko E, Anderson CR, McAllen RM.** Control of cardiac rate, contractility, and atrioventricular conduction by medullary raphe neurons in anesthetized rats. *Am J Physiol Heart Circ Physiol* 296: H318-324, 2009.

31. **Samuels BC, Zaretsky DV, DiMicco JA.** Dorsomedial hypothalamic sites where disinhibition evokes tachycardia correlate with location of raphe-projecting neurons. *Am J Physiol Regul Integr Comp Physiol* 287: R472-478, 2004.

32. **Samuels BC, Zaretsky DV, DiMicco JA.** Tachycardia evoked by disinhibition of the dorsomedial hypothalamus in rats is mediated through medullary raphe. *J Physiol* 538: 941-946, 2002.

33. **Schram G, Pourrier M, Melnyk P, Nattel S.** Differential distribution of cardiac ion channel expression as a basis for regional specialization in electrical function. *Circ Res* 90: 939-950, 2002.

34. **Soltis RP, Cook JC, Gregg AE, Stratton JM, and Flickinger KA.** EAA receptors in the dorsomedial hypothalamic area mediate the cardiovascular response to activation of the amygdala. *Am J Physiol* 275: R624-631, 1998.

35. **Tanaka M, McAllen RM.** Functional topography of the dorsomedial hypothalamus. *Am J Physiol Regul Integr Comp Physiol* 294: R477-486, 2008.

36. **Ter Horst GJ, Hautvast RW, De Jongste MJ, Korf J.** Neuroanatomy of cardiac activity-regulating circuitry: a transneuronal retrograde viral labelling study in the rat. *Eur J Neurosci* 8: 2029-2041, 1996.

37. **ter Horst GJ Luiten PG.** The projections of the dorsomedial hypothalamic nucleus in the rat. *Brain Res Bull* 16: 231-248, 1986.

38. **Ter Horst GJ, Van den Brink A, Homminga SA, Hautvast RW, Rakhorst G, Mettenleiter TC, De Jongste MJ, Lie KI, Korf J.** Transneuronal viral labelling of rat heart left ventricle controlling pathways. *Neuroreport* 4: 1307-1310, 1993.

39. **Van Bogaert PP, Goethals M.** Blockade of the pacemaker current by intracellular application of UL-FS 49 and UL-AH 99 in sheep cardiac Purkinje fibers. *European journal of pharmacology* 229: 55-62, 1992.

40. **Van Bogaert PP, Goethals M, Simoens C.** Use- and frequency-dependent blockade by UL-FS 49 of the if pacemaker current in sheep cardiac Purkinje fibres. *European journal of pharmacology* 187: 241-256, 1990.

41. **Vertes RP.** Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse* 51: 32-58, 2004.

42. **von Anrep G.** On the part played by the suprarenals in the normal vascular reactions of the body. *J Physiol* 45: 307-317, 1912.

43. **Wittling W, Block A, Genzel S, Schweiger E.** Hemisphere asymmetry in parasympathetic control of the heart. *Neuropsychologia* 36: 461-468, 1998.

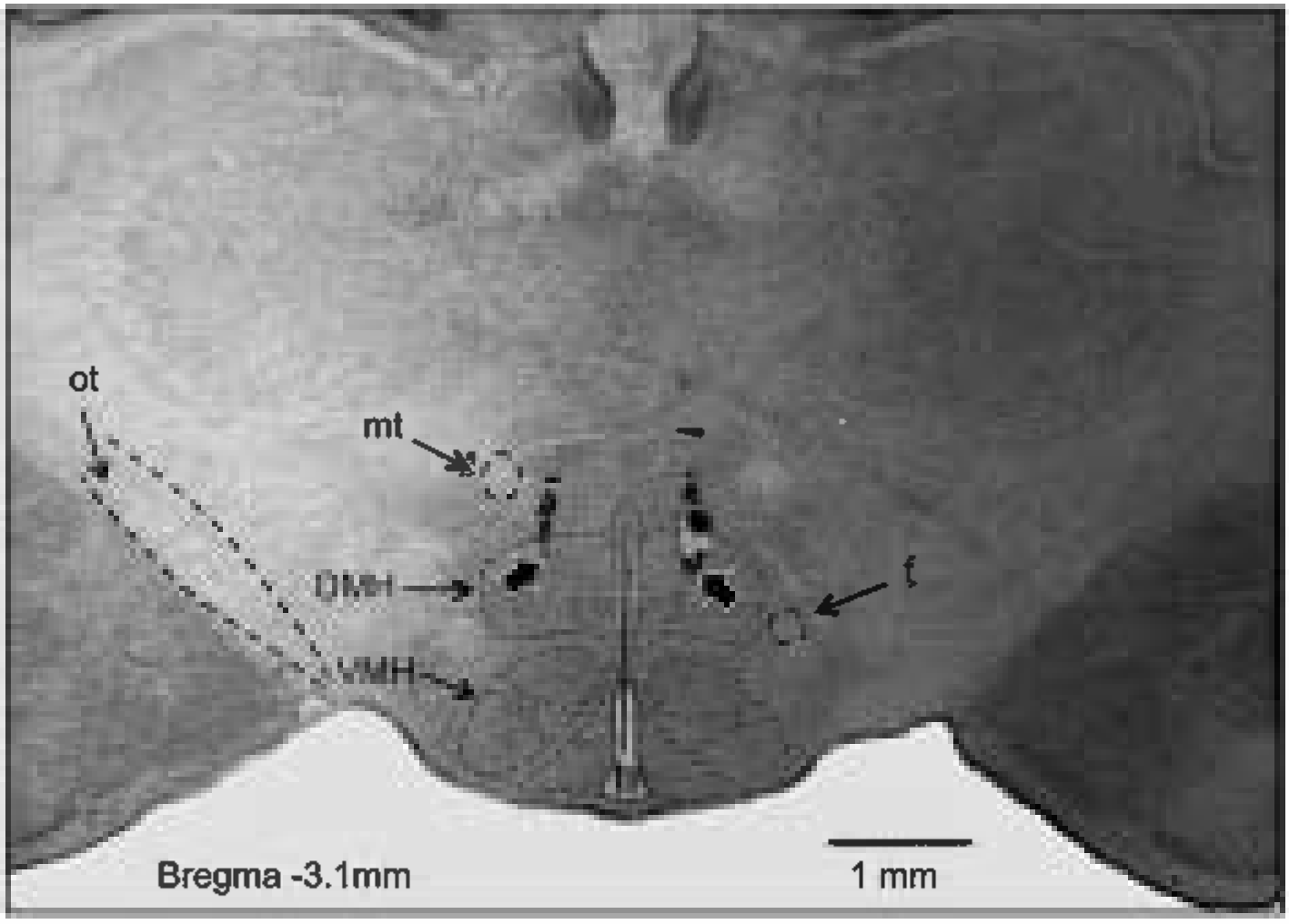
44. **Wittling W, Block A, Schweiger E, Genzel S.** Hemisphere asymmetry in sympathetic control of the human myocardium. *Brain Cogn* 38: 17-35, 1998.

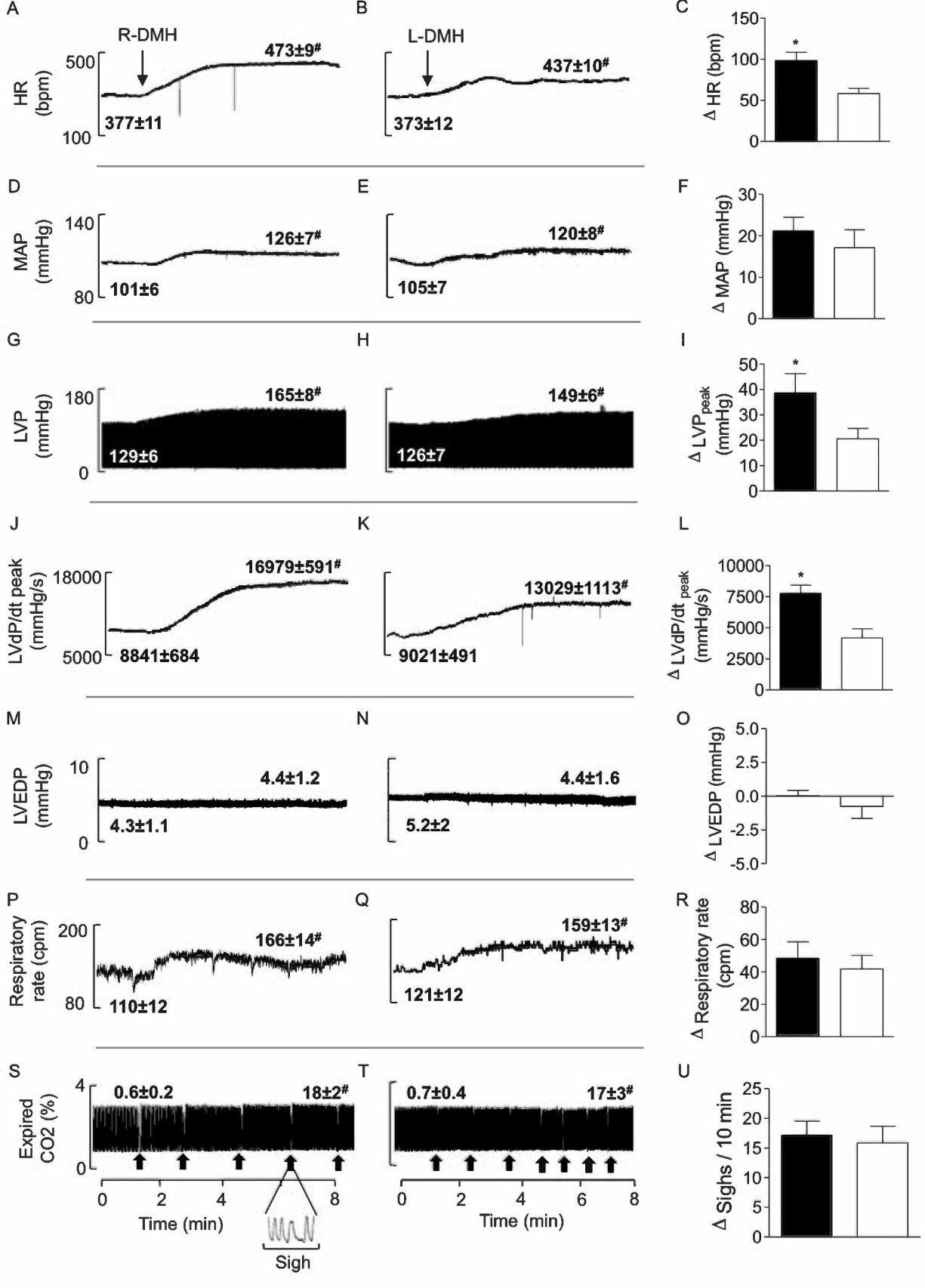
45. **Xavier C.H., Beig M. I., Ianzer D., Fontes MA, Nalivaiko E.** Cardiac chronotropic and inotropic responses evoked from right or left sides of dorsomedial hypothalamus. *FASEB Journal* 24: 1019.1020, 2010.

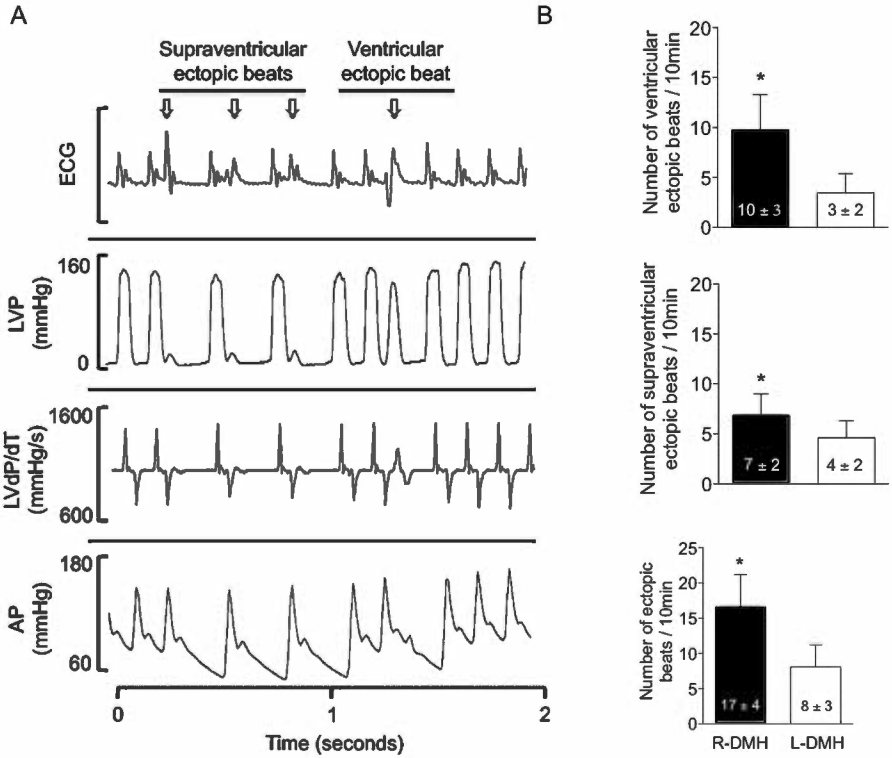
46. **Xavier CH, Campagnole-Santos MJ, Fontes MAP.** Comparison of the cardiovascular responses evoked by chemical activation of the left and right sides of dorsomedial hypothalamus. *Experimental Biology meeting abstracts FASEB J* 22, 1943, 2008.

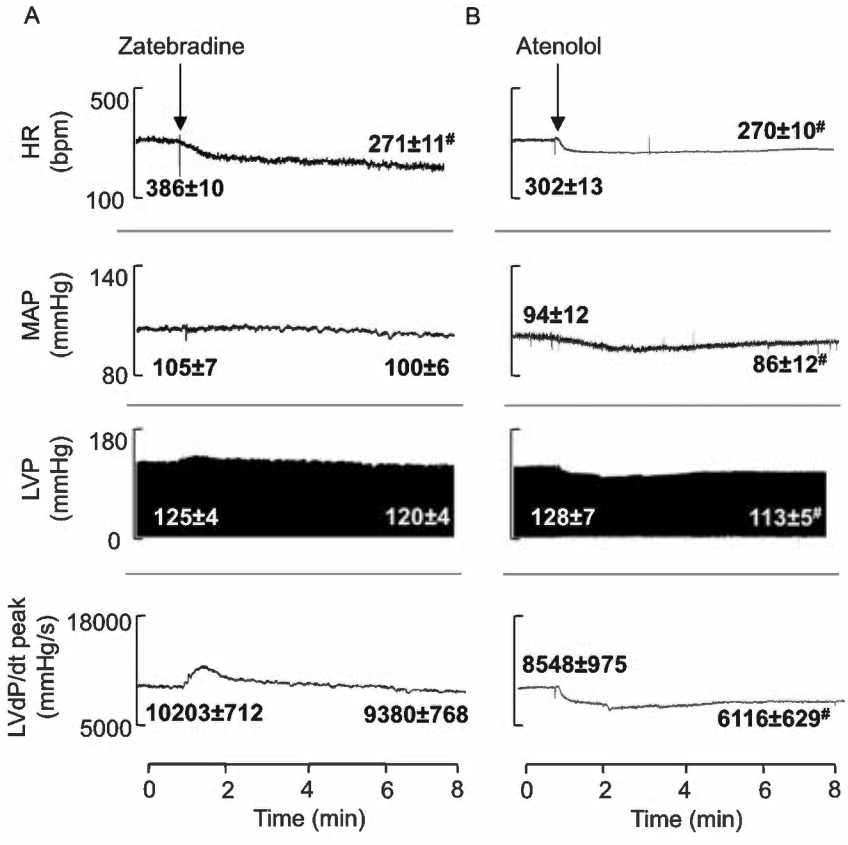
47. **Xavier CH, Nalivaiko E, Beig MI, Menezes GB, Cara DC, Campagnole-Santos MJ, and Fontes MA.** Functional asymmetry in the descending cardiovascular pathways from dorsomedial hypothalamic nucleus. *Neuroscience* 164: 1360-1368, 2009.

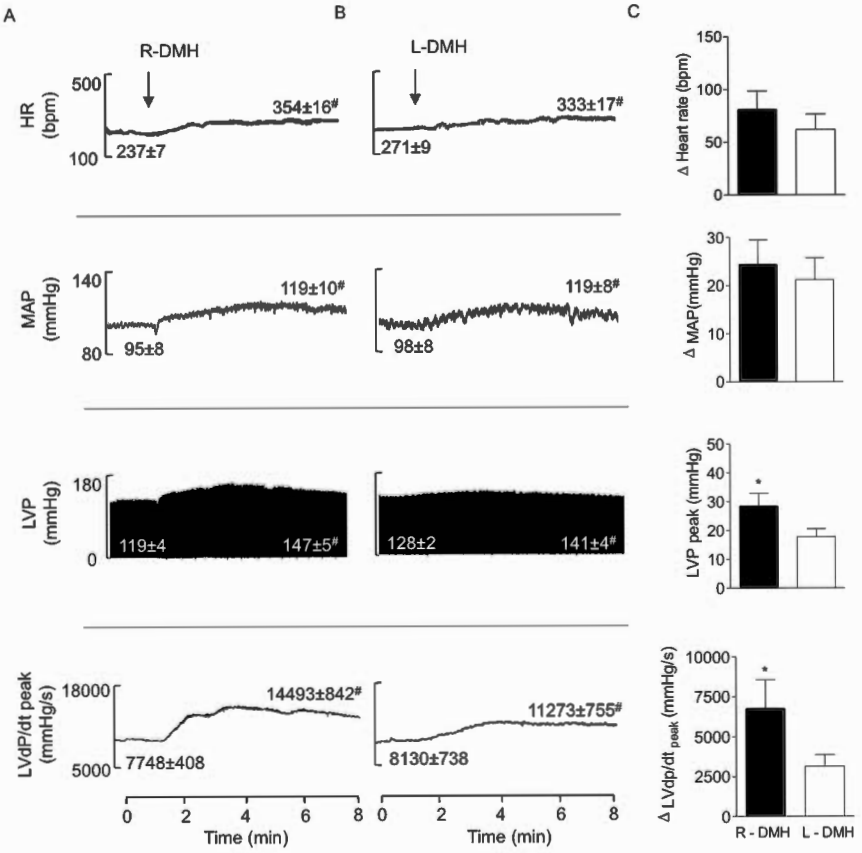
48. **Zagon A and Smith AD.** Monosynaptic projections from the rostral ventrolateral medulla oblongata to identified sympathetic preganglionic neurons. *Neuroscience* 54: 729-743, 1993.

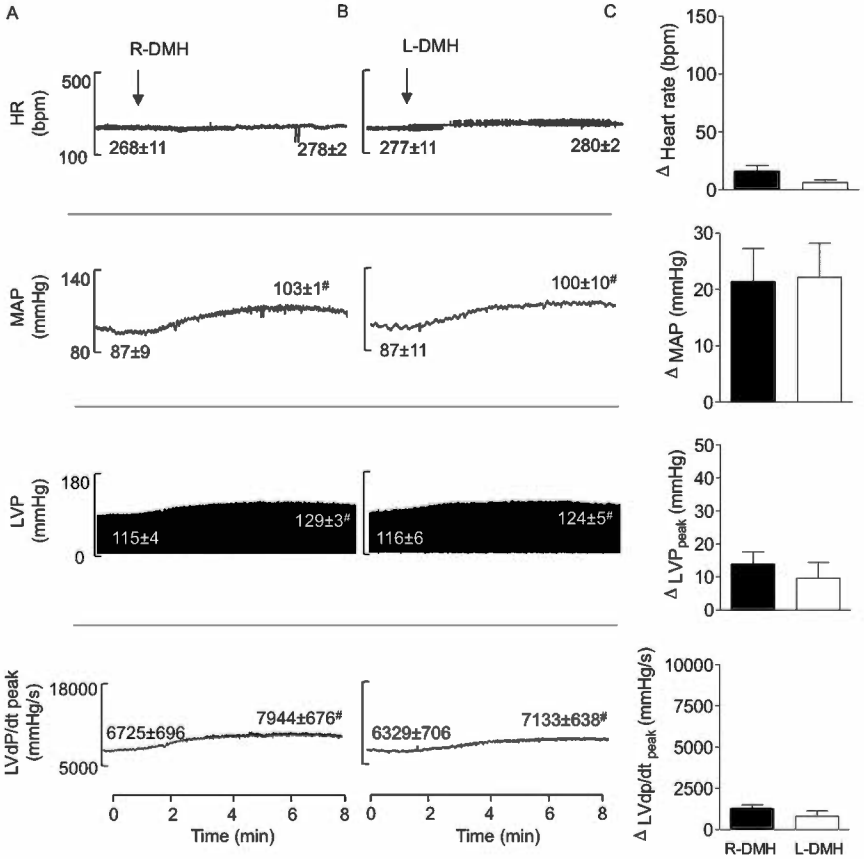

ot
mt
DMH
VMH
f
Bregma -3.1mm
1 mm

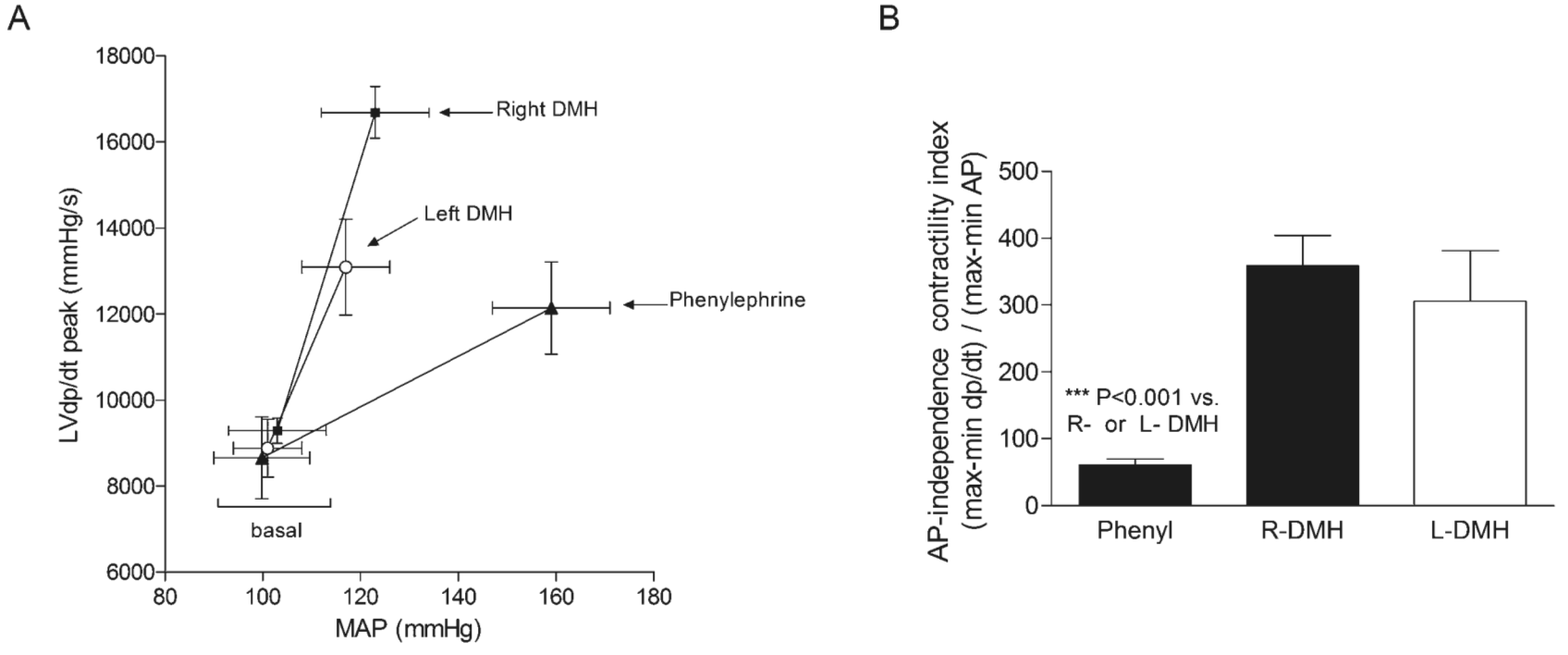

A
R-DMH
473±9#
500
100
HR (bpm)
377±11
B
L-DMH
437±10#
373±12
C
150
100
50
0
Δ HR (bpm)
*
D
140
80
MAP (mmHg)
126±7#
101±6
E
120±8#
105±7
F
30
20
10
0
Δ MAP (mmHg)
G
180
0
LVP (mmHg)
165±8#
129±6
H
149±6#
126±7
I
50
40
30
20
10
0
Δ LVP peak (mmHg)
*
J
18000
5000
LVdP/dt peak (mmHg/s)
16979±591#
8841±684
K
13029±1113#
9021±491
L
10000
7500
5000
2500
0
Δ LVdP/dt peak (mmHg/s)
*
M
10
0
LVEDP (mmHg)
4.4±1.2
4.3±1.1
N
4.4±1.6
5.2±2
O
5.0
2.5
0.0
-2.5
-5.0
Δ LVEDP (mmHg)
P
200
80
Respiratory rate (cpm)
166±14#
110±12
Q
159±13#
121±12
R
80
60
40
20
0
Δ Respiratory rate (cpm)
S
4
0
Expired CO2 (%)
0.6±0.2
18±2#
0
2
4
8
Time (min)
Sigh
T
0.7±0.4
17±3#
0
2
4
6
8
Time (min)
U
25
20
15
10
5
0
Δ Sighs / 10 min

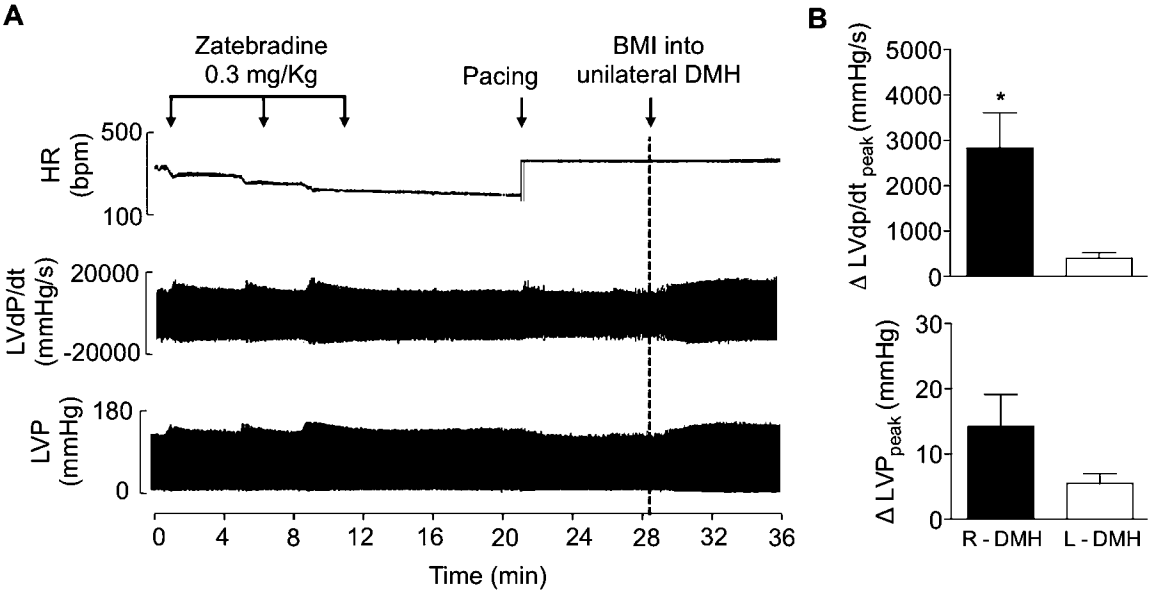

A
Supraventricular ectopic beats
Ventricular ectopic beat
ECG
LVP (mmHg)
160
0
LVdP/dT (mmHg/s)
1600
600
AP (mmHg)
180
60
0
1
2
Time (seconds)
B
Number of ventricular ectopic beats / 10min
20
15
10
5
0
*
10 ± 3
3 ± 2
Number of supraventricular ectopic beats / 10min
20
15
10
5
0
*
7 ± 2
4 ± 2
Number of ectopic beats / 10min
25
20
15
10
5
0
*
17 ± 4
8 ± 3
R-DMH
L-DMH


A
B
Zatebradine
Atenolol
HR (bpm)
500
100
386±10
271±11#
270±10#
302±13
MAP (mmHg)
140
80
105±7
100±6
94±12
86±12#
LVP (mmHg)
180
0
125±4
120±4
128±7
113±5#
LVdP/dt peak (mmHg/s)
18000
5000
10203±712
9380±768
8548±975
6116±629#
0
2
4
6
8
Time (min)
Time (min)


A
B
C
R-DMH
L-DMH
HR (bpm)
500
100
354±16#
237±7
333±17#
271±9
MAP (mmHg)
140
80
119±10#
95±8
119±8#
98±8
LVP (mmHg)
180
0
119±4
147±5#
128±2
141±4#
LVdP/dt peak (mmHg/s)
18000
5000
14493±842#
7748±408
11273±755#
8130±738
0 2 4 6 8
Time (min)
0 2 4 6 8
Time (min)
Δ Heart rate (bpm)
150
100
50
0
Δ MAP(mmHg)
30
20
10
0
LVP peak (mmHg)
50
40
30
20
10
0
*
Δ LVdp/dt peak (mmHg/s)
10000
7500
5000
2500
0
*
R - DMH
L - DMH


A
B
C
R-DMH
L-DMH
HR (bpm)
500
100
268±11
278±2
277±11
280±2
MAP (mmHg)
140
80
87±9
103±1#
87±11
100±10#
LVP (mmHg)
180
0
115±4
129±3#
116±6
124±5#
LVdP/dt peak (mmHg/s)
18000
5000
6725±696
7944±676#
6329±706
7133±638#
0 2 4 6 8
Time (min)
0 2 4 6 8
Time (min)
Δ Heart rate (bpm)
150
100
50
0
Δ MAP (mmHg)
30
20
10
0
Δ LVP peak (mmHg)
50
40
30
20
10
0
Δ LVdp/dt peak (mmHg/s)
10000
7500
5000
2500
0
R-DMH
L-DMH


A
18000
16000
14000
12000
10000
8000
6000
LVdp/dt peak (mmHg/s)
Right DMH
Left DMH
Phenylephrine
basal
80
100
120
140
160
180
MAP (mmHg)
B
AP-independence contractility index
(max-min dp/dt) / (max-min AP)
500
400
300
200
100
0
*** P<0.001 vs.
R- or L- DMH
Phenyl
R-DMH
L-DMH


A
Zatebradine
0.3 mg/Kg
Pacing
BMI into
unilateral DMH
HR
(bpm)
500
100
LVdP/dt
(mmHg/s)
20000
-20000
LVP
(mmHg)
180
0
0
4
8
12
16
20
24
28
32
36
Time (min)
B
Δ LVdp/dt peak (mmHg/s)
5000
4000
3000
2000
1000
0
*
Δ LVP peak (mmHg)
30
20
10
0
R - DMH
L - DMH

Anexo 4

Elsevier Editorial System(tm) for Brain Research

Manuscript Draft

Manuscript Number: BRES-D-12-01168

Title: Excitatory amino acid receptors contribute to the functional asymmetry in the descending cardiovascular pathways from the dorsomedial hypothalamus

Article Type: Research Report

Section/Category: Systems Neuroscience and Behavior

Keywords: Asymmetry, lateralization, heart rate, stress, dorsomedial hypothalamus, periaqueductal gray, excitatory amino acid.

Corresponding Author: Dr. Carlos Henrique Xavier, MSc., PhD.

Corresponding Author's Institution: Universidade Federal de Ouro Preto

First Author: Carlos Henrique Xavier, MSc., PhD.

Order of Authors: Carlos Henrique Xavier, MSc., PhD.; Danielle Ianzer, PhD; Augusto M Lima, MSc. PhD st.; Fernanda R Marins, MSc. st.; Gisele C Vaz, MSc. PhD st.; Gustavo B Menezes, PhD; Eugene Nalivaiko, PhD; Marco A Fontes, PhD

Manuscript Region of Origin: BRAZIL

Abstract: The dorsomedial hypothalamus (DMH) and lateral / dorsolateral periaqueductal gray (PAG) are anatomically and functionally connected. Both DMH and PAG depend on glutamatergic inputs to be activated. We recently reported that removal of gabaergic tone in unilateral DMH produces: asymmetry - right-sided predominance in cardiac chronotropism; lateralization - greater increases in ipsilateral renal sympathetic activity. We next investigated whether excitatory amino acids (EAA) receptors in the DMH and PAG contribute to the functional interhemispheric difference reported previously. In urethane (1.4g/kg) anesthetized rats we found that: i) injections of N-methyl D-aspartate (NMDA 100pmol/100nl) into unilateral DMH revealed the same right (R-) sided asymmetric predominance for the cardiac chronotropism; ii) ipsilateral injections of NMDA into unilateral DMH or PAG showed lateralization in the control renal sympathetic activity; iii) blockade of EAA receptors in the left (L-) DMH attenuated cardiovascular responses evoked by injection of NMDA into unilateral PAG. In awake animals, injection of kynurenic acid (1nmol/100nl) into L- DMH or R- and L- PAG attenuated tachycardia evoked by air stress. However, the magnitude of the stress-evoked tachycardia was smallest only when blocking EAA receptors in the R-DMH. We conclude that activation of EAA receptors contribute to the right-sided predominance in the cardiac chronotropism. This interhemispheric difference / asymmetry involving EAA receptors is observed in the DMH, but not in PAG.

Excitatory amino acid receptors contribute to the functional asymmetry in the descending cardiovascular pathways from the dorsomedial hypothalamus

*Carlos Henrique Xavier[1,4], Danielle Ianzer[2], Augusto Martins Lima[1], Fernanda Ribeiro Marins[1], Gisele Cristiane Vaz[1], Gustavo Batista Menezes[5], Eugene Nalivaiko[3] and Marco Antônio Peliky Fontes[1]

[1] Instituto Nacional de Ciência e Tecnologia (INCT-NanoBioFar). Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

[2] Laboratório Especial de Toxinologia Aplicada, CAT-CEPID, Instituto Butantan, São Paulo, SP, Brazil.

[3] Neurocardiology Laboratory, School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia.

[4] Departamento de Ciências Biológicas, Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil.

[5] Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Corresponding author: Dr. Carlos Henrique Xavier. Departamento Ciências Biológicas. Universidade Federal de Ouro Preto. Ouro Preto, MG. Brazil. carloshxc@live.com

Running title: excitatory amino acid in the cardiovascular pathways from dorsomedial hypothalamus and periaqueductal gray.

ABSTRACT

The dorsomedial hypothalamus (DMH) and lateral / dorsolateral periaqueductal gray (PAG) are anatomically and functionally connected. Both DMH and PAG depend on glutamatergic inputs to be activated. We recently reported that removal of gabaergic tone in unilateral DMH produces: *asymmetry* – right-sided predominance in cardiac chronotropism; *lateralization* – greater increases in ipsilateral renal sympathetic activity. We next investigated whether excitatory amino acids (EAA) receptors in the DMH - PAG pathway contribute to the functional interhemispheric difference reported previously. In urethane (1.4g/kg) anesthetized rats we found that: i) injections of N-methyl D-aspartate (NMDA 100pmol/100nl) into unilateral DMH revealed the same right (R-) side predominance in the control of cardiac chronotropy; ii) injections of NMDA into ipsilateral DMH or PAG showed lateralization in the control renal sympathetic activity; iii) blockade of EAA receptors in the left (L-) DMH attenuated cardiovascular responses evoked by injection of NMDA into either R- or L-PAG. In awake animals, injection of kynurenic acid (1nmol/100nl) into L- DMH or R- and L- PAG attenuated tachycardia evoked by air stress. However, the magnitude of the stress-evoked tachycardia was smallest only when blocking EAA receptors in the R-DMH. We conclude that activation of EAA receptors contribute to the right-sided predominance in the cardiac chronotropism. This interhemispheric difference / asymmetry involving EAA receptors is observed in the DMH, but not in PAG.

KEY WORDS: Asymmetry, lateralization, heart rate, stress, dorsomedial hypothalamus, periaqueductal gray, excitatory amino acid.

1 - INTRODUCTION

Asymmetries are known as the left-right differences in the properties of the central nervous system, found at different levels of the neuraxis (Harris et al., 1996; Schwartz et al., 1976; Toga and Thompson, 2003). We have previously reported functional asymmetry and lateralization in the cardiovascular responses evoked by nanoinjection of $GABA_A$ antagonist into unilateral DMH; right DMH predominantly drives cardiac performance and tail vasoconstriction, whereas unilateral DMH seems to exert prominent autonomic control over the ipsilateral hemibody (Xavier et al., 2008; Xavier et al., 2009; Xavier et al., 2010a).

Studies have highlighted the involvement of diencephalic and mesencephalic regions in the organization of physiological responses to stress [for review, see (Carrive, 2009)]. Anatomic tracings revealed dense connections between these two encephalic levels (Carrive and Gorissen, 2008; Farkas et al., 1998; ter Horst and Luiten, 1986). In addition, specific functional assessments have been done in the connections between hypothalamus and lateral / dorsolateral regions of periaqueductal gray (PAG) (da Silva et al., 2003; da Silva et al., 2006; de Menezes et al., 2009; Vianna and Brandao, 2003; Villela et al., 2009).

Aversive reactions are mediated by periaqueductal gray (Carrive, 1993; Carrive, 2009), known to be responsive to efferent excitatory inputs (Albin et al., 1990) from upper regions, as hypothalamus (Beart et al., 1990; da Silva et al., 2006). PAG is considered an exit synaptic relay for defensive responses (Bandler and Carrive, 1988; Carrive, 1993). Activation of the PAG with the excitatory amino acids (EAA) agonist N-methyl D-aspartate (NMDA) evokes sympathetically mediated increases in body temperature, heart rate and arterial pressure (da Silva et al., 2006; de Menezes et al., 2006; de Menezes et al., 2008; de Menezes et al., 2009).

Dorsomedial hypothalamus (DMH) is responsive to nanoinjections of both $GABA_A$ antagonists and EAA agonists. Inhibition of DMH neurons attenuates the responses provoked

by stress exposure (Rusyniak et al., 2008; Stotz-Potter et al., 1996a; Stotz-Potter et al., 1996b). Inversely, DMH disinhibition by blocking the $GABA_A$ receptors in this nucleus, produce a series of effects that mimics stress-related physiological responses, such as behavioral changes, thermogenesis, tachycardia and increases in blood pressure [for review, see (DiMicco et al., 2002; Fontes et al., 2011)]. Previous studies demonstrated that this selective gabaergic blockade activates DMH neurons allows a predominant local excitatory input (Soltis and DiMicco, 1991a; Soltis and DiMicco, 1991b; Soltis and DiMicco, 1992), which suggest that both phenomena should happen together to achieve the responses described.

Recent studies have revealed a functional projection between DMH and PAG (da Silva et al., 2003; da Silva et al., 2006; de Menezes et al., 2008; de Menezes et al., 2009; Horiuchi et al., 2009). Interestingly, attenuation of the tachycardia evoked by unilateral DMH activation was detected only following ipsilateral inhibition of the lateral/dorsolateral columns of PAG (da Silva et al., 2003). Indeed, it was also described that this connection depends of EAA receptors, particularly NMDA receptors (da Silva et al., 2006).

In this study, we evaluated the contribution of the excitatory input to the asymmetry and lateralization in the descending cardiovascular pathways to emotional stress at the level of DMH and PAG. For that, we compared the cardiovascular responses evoked by activation and blockade of EAA receptors between right and left sides of DMH and PAG. Part of our results was presented in abstract form (Xavier et al., 2010b; Xavier et al., 2011).

2 - RESULTS

Figure 1 presents a microphotograph of coronal section exemplifying nuclei targeted on experimental groups. DMH and PAG are showed in panels A and B, respectively. Baseline values sampled before starting experimental procedures are showed in table 1. There were no differences between basal values found before central injections within each experimental series.

Experiment 1. Effects of unilateral activation of DMH and PAG on heart rate, blood pressure and renal sympathetic activity in anesthetized rats.

Results and chart records obtained in this experimental series are grouped in figures 2 and 3. Nanoinjection of NMDA into unilateral DMH (Fig. 2) and PAG (Fig. 3) positively changed cardiovascular parameters. These effects were evident by one minute following nanoinjections and lasted for about 5 minutes. We observed marked and equipotent increases in arterial pressure after unilateral injections of NMDA into DMH or PAG, (ΔMAP DMH: R = 13±2 vs. L = 14±3; PAG: R = 16±3 vs. L = 18±4 mmHg; $P<0.05$ vs. baseline), but no differences were found in the range of the responses when comparing right and left sided nanoinjections within same experimental group.

Unilateral nanoinjections of NMDA into the unilateral DMH or PAG increased RSNA sampled in the left renal nerve ($P<0.05$ vs. baseline). However, compared to the changes found after injecting into R- side, the greater responses were from activation of left DMH and PAG, i.e. ipsilateral to the side where renal sympathetic activity was sampled (ΔRSNA DMH: R = 30±7 vs. L = 66±13; PAG: R = 26±3 vs. L = 92±14 %; $P<0.05$). Nanoinjections into DMH and PAG also increased heart rate ($P<0.05$ vs. baseline), but those reaching R-DMH provoked greater positive chronotropy compared to L-DMH (ΔHR DMH: R = 59±5 vs. L = 28±4 bpm; $P<0.05$) (Fig. 2). Tachycardia evoked by nanoinjection of

NMDA into unilateral PAG was similar to that found in the contralateral side (ΔHR PAG: R = 39±6 vs. L = 45±6 bpm) (Fig. 3).

Intriguingly, a difference in the pattern of the time course responses evoked from unilateral PAG was detected during our recordings (see "peak change" highlights in the Fig.3). Peak changes in MAP and RSNA were likely close, about 30-60s after stimulation of both R- and L- PAG (Fig. 3A and B). However, the maximal HR responses followed a different time course, delayed about 90-120s past injection (Fig. 3C). No differential pattern in the time course of the responses evoked from unilateral DMH was detected (Fig. 2).

Experiment 2. Contribution of unilateral EAA receptors of the DMH to the heart rate, blood pressure and renal sympathetic activity responses evoked by stimulation of PAG in anesthetized rats.

The pharmacological blockade of EAA receptors in the left DMH caused slight reduction in cardiovascular parameters. After this blockade, injections of NMDA reaching either ipsilateral or contralateral PAG did not cause responses with similar amplitudes observed in Experiment 1. MAP (ΔMAP: R = 1±2 vs. L = 2±1 mmHg), HR (ΔHR: R = 3±5 vs. L = 4±1 bpm) and RSNA (ΔRSNA: R = -1±2 vs. L = 3±5 %) responses were dramatically attenuated. Asymmetry and lateralization in the control of HR and RSNA, respectively, were not found. Also, the differential timing previously observed was not evident when blocking EAA in the unilateral DMH prior to the injections of NMDA into unilateral PAG (Fig. 4).

Experiment 3. Contribution of EAA receptors of the unilateral DMH and PAG to the stress-induced tachycardic and pressor responses.

Figure 5 (panels A and C) shows the time course of the responses during entire experiments. Panels B and D show the peak changes in HR and MAP sampled during stress exposure. Compared to the amplitude of the control responses (vehicle), nanoinjections of kynurenic acid into either R- or L- sides of DMH and PAG attenuated tachycardia evoked from airjet stress. However, the range of the stress-evoked tachycardia was dramatically reduced when inhibiting EAA receptors in the R- DMH (ΔHR DMH: R = 12±18 vs. L = 87±17; PAG: R = 73±15 vs. L = 74±12; bilateral vehicle: 132±9 bpm; $P<0.05$; Fig. 5A and B). Blockade of EAA receptors into either R- or L- sides of DMH and PAG did not change the amplitude of the stress-induced pressor responses (ΔMAP DMH: R = 12±4 vs. L = 17±3; PAG: R = 18±4 vs. L = 18±5; bilateral vehicle: 19±3 mmHg) (Fig. 5C and D).

Experiment 4. Anatomic pathways between DMH and PAG

Photomicrographs depicting injection sites of retrobeads into right DMH and left PAG and labeled neurons are showed in Figure 1 A and B. As proposed, nanoinjections of green and red beads reached DMH and the lateral/dorsolateral columns of PAG, respectively. In the left DMH, contralaterally to the side where green beads were injected, there were neurons labeled with red beads from left PAG. Similarly, in the right PAG there were green-labeled neurons with beads injected into right DMH. The PAG of the animal that only received green beads into right DMH showed bilateral labeling, which included sparse cells in ventral portions of PAG. However, the ipsilateral dl/PAG appeared to show a little denser labeling. This evidences a prominently lateralized pathway. Altogether, these data reinforce the collateral two-way connection between DMH and PAG.

3 - DISCUSSION

The major findings of this study are: i) the nanoinjection of NMDA into the right DMH evoked greater cardiac chronotropic changes compared to those observed in the left DMH; ii) blockade of EAA receptors in the right DMH almost abolished the stress-evoked tachycardia, whereas same blockade at L-DMH only showed an attenuation of the tachycardic response.

Current data supports previous findings showing that stimulation of the right hypothalamus evokes more prominent chronotropic changes (Fang and Wang, 1962; Xavier et al., 2009). We recently demonstrated that disinhibition of R-DMH produces greater tachycardia when compared to the L-DMH. In addition, compared to L-DMH, inhibition of the neuronal activity in R-DMH greatly attenuates the stress-evoked tachycardia (Xavier et al., 2009). The removal of the gabaergic tone implicates in predominant excitatory input to the DMH (Soltis and DiMicco, 1991a; Soltis and DiMicco, 1991b; Soltis and DiMicco, 1992), that changes the sympathetic output (Cao et al., 2004; Fontes et al., 2011; Horiuchi et al., 2004) and regional blood flow to different target organs (Dimicco et al., 1992; Xavier et al., 2009). In this regard, it is important to highlight the amygdala as one of the sources of excitatory input to the DMH (Soltis et al., 1998). Therefore, the current data demonstrates that the functional asymmetry observed for the control of cardiac chronotropism by DMH is also present after stimulation of EAA receptors in this region.

Altogether, these data allow us to conclude that the grater chronotropic response evoked by removal of GABA tone in the R-DMH (Xavier et al., 2009) may involve a differential response from right side DMH neurons to the glutamatergic input. Several mechanisms could account for this difference. One possibility is that this functional asymmetry could be due to particular characteristics of right or left DMH neurons such as

electrophysiological properties or density of glutamatergic receptors, but this remains to be investigated. It is also important to consider other possible functional and neuroanatomical differences between right and left descending pathways from DMH, including synaptic relays other than the PAG, and the organization of sympathetic projections to the heart. The DMH projects to other nuclei involved in the control cardiovascular responses such as RVLM (Fontes et al., 2001) and RP (Cao et al., 2004; Horiuchi et al., 2004). In addition, right and left cardiac sympathetic neurons and nerves differ in terms of function (Campos and McAllen, 1999; Furukawa et al., 1990; Kamosinska et al., 1989; Randall and Rohse, 1956; Randall et al., 1968; Randall et al., 1972; Schwartz et al., 1976; Ter Horst et al., 1993; Yanowitz et al., 1966), where, in general, stimulation of right sided components is more likely to evoke positive chronotropic responses (Campos and McAllen, 1999; Furnival et al., 1968; Furukawa et al., 1990; Rogers et al., 1978; Winter et al.). Undoubtedly, further studies are necessary to unravel the areas and mechanisms underlying this functional interhemispheric difference.

We also showed that stimulation of unilateral DMH and PAG with NMDA evokes greater RSNA responses in the ipsilateral renal nerve. Due to technical reasons, we recorded only one renal nerve (left) in all experiments. Although we recognize that future experiments recording both nerves simultaneously would be extremely important for confirming this data, previous evidence allows concluding that there is a certain degree of hypothalamic lateralization for controlling RSNA. At least for the DMH, a similar pattern was recently reported after injections of bicuculline (Xavier et al., 2009). Previous anatomical studies shall support these functional data as if the lateralization and asymmetry result from a lack of communication between the two sides of these descending pathways, likely organized as ipsilateral mirrors (ter Horst and Luiten, 1986; Thompson et al., 1996). In addition, other anatomical and functional studies further support the lateralization for the control of the

RSNA (Huang et al., 2002; Moon et al., 2002; Shafton et al., 1998; Taylor and Weaver, 1992). Using Fos-immunoreactivity, Zaretskaia and colleagues clearly demonstrated that activation of unilateral DMH neurons with bicuculline recruits mainly ipsilateral synaptic relays (Zaretskaia et al., 2008), including nuclei involved in the cardiovascular control. Therefore, not only the descending pathways from the DMH provide evidences for lateralization. Unilateral nanoinjection of a nitric oxide donor into PAG was reported to recruit ipsilateral neurons in the DMH, suggesting that the specific PAG-DMH ascending pathway underlies organized responses that may not be consequence of the generalized arousal (de Menezes et al., 2009; de Oliveira et al., 2000). Together with these data, our anatomic findings extend the previous idea of a lateralized DMH – PAG bidirectional connection (Cameron et al., 1995a; Cameron et al., 1995b; ter Horst and Luiten, 1986; Thompson et al., 1996). Because the PAG is a synaptic relay in the descending cardiovascular pathways from the DMH (Fontes et al., 2011) and the activity of DMH neurons is required for PAG-evoked responses (de Menezes et al., 2009; Horiuchi et al., 2009), all these evidences support the hypothesis of functional lateralization from and to both DMH and PAG, which comprise a collateral background facilitation system for controlling RSNA.

We found a different timing for peak changes for the tachycardia during the course of the cardiovascular response observed after PAG stimulation. This pattern was different from that observed after DMH stimulation (current data) or from that observed after the exteroceptive stimulus in conscious rats (Bandler and Carrive, 1988). Despite not having an exact explanation that might help us understanding these findings, the response evoked from PAG is recruiting the ascending functional projection via DMH, recently reported (de Menezes et al., 2009; Horiuchi et al., 2009) and confirmed by current data. This would then cause a delayed HR response when compared to that evoked by direct stimulation of the

DMH, likely governed by cardiac premotor neurons of the ventromedial medulla (Cao and Morrison, 2003; Cao et al., 2004; Samuels et al., 2002; Samuels et al., 2004). However, inhibition of left DMH attenuated the lateralized responses in RSNA and abolished the delayed tachycardia evoked by unilateral PAG stimulation. Given that right DMH would be working after inhibition of its contralateral side, it is worth suggesting that the PAG-DMH ascending pathway may be involved in the organization of lateralized but not asymmetric responses. We also confirmed that PAG indeed acts via DMH, as R-PAG stimulation failed in revealing any cardiac dominance. Then, two important questions still require further addressing: i) the reason for the delayed tachycardia and; ii) whether or not the descending pathways from DMH are the same governing those PAG-evoked responses.

Conclusion

A growing body of research has described a functional interaction between DMH and PAG in the descending autonomic pathways mediating the cardiovascular response to emotional stress. Our recent results add the asymmetry and lateralization as a novelty to this idea and this could strength the neural substrate for understanding cardiovascular autonomic control during emotions (Fontes et al., 2011). In this regard, there is substantial evidence for interhemispheric specialization for autonomic control in humans (Lane et al., in press; Oppenheimer, 2006; Oppenheimer, 2007). During stress and emotional arousal, most central components may be influenced asymmetrically, unbalancing sympathetic outflow to the heart improving the chances of cardiac arrhythmic events (Critchley et al., 2005; Lane and Schwartz, 1987; Lane et al., 2005; Wittling, 1997) and even so, causing sudden death (Lane and Schwartz, 1987; Ozdemir and Hachinski, 2008). Together with these studies, current data reinforces the necessity for further experiments addressing autonomic interhemispheric differences and their functional implications for the cardiovascular control. Studying the

differential autonomic responses organized by each brain side helps to pave the way to the understanding of the interindividual variability in the stress-induced cardiovascular responses.

4 - EXPERIMENTAL PROCEDURES

General procedures

All experiments were performed on male Wistar rats (250-320 g) bred at the animal facilities of the Biological Sciences Institute (CEBIO, UFMG, Belo Horizonte, MG, Brazil) and conducted in accordance with the guidelines established by our local institutional animal welfare committee (CETEA/UFMG protocol number 137/2006), and in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used. Animals were housed in individual home cages (47 cm x 31 cm x 16 cm) and had free access to food and water.

In this study we recorded the following cardiovascular parameters in two different experimental conditions*: i) anesthetized* - heart rate (HR), mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA); ii) *non-anesthetized* – HR and MAP.

4.1 - Experiments in anesthetized animals

General procedures

Rats were anesthetized with urethane (1.2 to 1.4 g/kg i.p.), and the trachea was cannulated to maintain the airways opened. The adequacy of anesthesia was verified by the absence of a withdrawal response to nociceptive stimulation of a hindpaw. Supplemental doses of urethane (0.1 g/kg i.v.) were given when necessary. Body temperature was kept in the range of 36.5 to 37.0° C with a heating lamp. The head was positioned in a stereotaxic frame (Stoelting, IL, USA) with the tooth bar fixed at –3.3 mm below the interaural line. Small craniotomies were made to allow for later insertion of thin tip graduated glass pipettes into: DMH (3.2 mm posterior, 0.6 mm lateral, 8.5 mm ventral); PAG (6.8mm posterior, 0.6mm lateral, 4.8mm ventral).

Catheters were placed into a femoral artery and vein to record AP and HR and for supplementary anesthesia, respectively. Using a retroperitoneal approach, the left renal nerve and left renal artery were isolated and covered with mineral oil. Following, the renal nerve was put on a silver bipolar electrode. RSNA signal was amplified by 10K, filtered (100-1000 Hz), displayed on an oscilloscope and monitored by means of an audio amplifier. The filtered nerve activity signal was rectified; integrated (resetting every second), displayed online and acquired using Powerlab 4/20 - LabChart 7.1 (ADInstruments, Sydney, Australia). All data were digitized at 1 kHz. The noise level of the RSNA recording system was determined *post mortem* and subtracted from RSNA values obtained during the experiment. After 20 minutes of stabilization period, experiments were performed as described ahead.

Experiment 1. Effects of unilateral activation of DMH and PAG on heart rate, blood pressure and renal sympathetic activity in anesthetized rats.

In the first series of experiments, we compared the changes in HR, MAP and RSNA evoked by nanoinjection of NMDA (100 pmol/100 nl) into the left (L-) and right (R-) DMH and PAG. It has been described that activation of DMH and PAG require integrity in their neuronal activity due to a two-way connection between these nuclei (da Silva et al., 2006; de Menezes et al., 2009; Vianna and Brandao, 2003). Thus, these experiments were performed in two separated groups of animals (n=5 each), one with nanoinjections into DMH and other into PAG. The side for the first injection into unilateral DMH or PAG was chosen randomly. For example, if nanoinjection of NMDA was made first into R-DMH, the second one was into L-DMH. The same rationale was adopted for the group microinjected into unilateral PAG. Between nanoinjections, we waited minimum intervals of 15 minutes or until the variables returned to the baseline.

Experiment 2. Contribution of unilateral EAA receptors of the DMH to the heart rate, blood pressure and renal sympathetic activity responses evoked by stimulation of PAG in anesthetized rats.

Based on previous reports that tracked the influence of DMH neurons in the responses evoked from PAG (de Menezes et al., 2009; Horiuchi et al., 2009), in this series we aimed to reveal whether inhibition of EAA receptors of unilateral DMH is able to modify the range of the responses evoked by stimulation of either unilateral or contralateral PAG (n=5). Following 20 minutes of stabilization period, we injected Kyn (1nmol/100nl) into left DMH. After 15 minutes, we performed the first injection of NMDA (100pmol/100nl) into unilateral PAG. 15 minutes later or until the variables returned to baseline, the second nanoinjection of NMDA was done into contralateral PAG. The side for the first injection into unilateral PAG was chosen randomly, and the second injection was always contralateral to that previously performed.

4.2 - Experiments in non-anesthetized animals

General procedures

Under tribromoethanol anesthesia (250 mg/kg i.p.), rats were placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA) and instrumented with bilateral stainless steel guide cannulas (22 gauge, 16 mm length) targeted to DMH or PAG. The guide cannulas were fixed to the skull by dental acrylic cement anchored with stainless steel screws. After surgical procedures, animals were allowed to recover in their home cages for at least three days. Subsequently, they were anesthetized again with tribromoethanol (250 mg/kg i.p.), and polyethylene catheter (0.011 ID, Clay Adams, Parsippany, NJ, USA) filled with heparinized saline and sealed with a stylet was inserted in the abdominal aorta through the femoral artery for recording of blood pressure and HR. The catheter was routed subcutaneously to the nape

of the neck where it was exteriorized and secured. Rats were then allowed to recover in their home cages for at least 24 h before experiments began. All animals remained in good health conditions throughout surgical procedures and experimental protocol as assessed by appearance, behavior, and maintenance of body weight.

Experiment 3. Contribution of EAA receptors of the unilateral DMH and PAG to the stress-induced tachycardic and pressor responses.

In this experimental series our aim was to know the contribution of EAA receptors in the unilateral DMH or PAG to the cardiovascular responses evoked by acute stress exposure. In one group (n=5), bilateral guide cannulas were targeted only to DMH. In another group (n=5), guide cannulas were targeted only to bilateral PAG, following coordinates mentioned below. Both DMH and PAG groups were submitted to the same experimental procedures, as following. After 20 min of baseline heart rate and blood pressure monitoring, the EAA antagonist kynurenic acid (Kyn, 1 nmol/100 nl) was injected unilaterally into DMH or PAG. Rats were then placed into a plastic restrainer (60-mm inner diameter) and subjected to a 10-min air jet stress – a stream of air (10 l/min) directed to the head. After the air jet, the animals remained in the restrainer for additional 10 min. Air jet stress was repeated on 3 consecutive days. In the day one, the side for first nanoinjection was chosen randomly. In the second day, nanoinjection was contralateral to that targeted in the first day. In the third day, air jet stress was repeated after bilateral nanoinjection of vehicle (NaCl 0.9%, 100nl) in all animals, aiming to evaluate the control responses provoked by stress.

4.3 - Anatomic tracings

Experiment 4. Anatomic pathways between DMH and PAG

Animals were anesthetized as described in Experiment 2. Craniotomy procedures to reach DMH and PAG were the same used in Experiment 1. Small craniotomies were made to allow for later insertion of thin tip graduated glass pipettes into: right DMH (3.2 mm posterior, +0.6 mm lateral, 8.5 mm ventral); left PAG (6.8mm posterior, -0.6mm lateral, 4.8mm ventral). Procedures for neuronal tracings were as previously described (Apps and Ruigrok, 2007). In one animal, undiluted green and red Retrobeads^{TM} (Luma Fluor) were nanoinjected (100nl) into right DMH and left PAG, respectively. Because the descending DMH-PAG pathway is extensively described, in other animal, green retrobeads were injected only into right DMH to double-check the ascending PAG-DMH anatomic pathway. After surgeries, animals received injections (i.m.) of antibiotics (Pentabiotic – 0.2ml) and analgesic (Banamine Pet – 1.1 mg/kg). Seven days later, animals were again anesthetized and underwent transcardial perfusion with 250ml of heparinized (5000 U.I.) saline followed by 250ml of PFA 4%. Brains were removed, kept in PFA 4% for 24h and then transferred to 30% sucrose solution for 48h. Before cutting the brains, left-sided fiducial mark was performed in regions far from those surrounding DMH and PAG, as superior thalamus. Brain slices (40μm) of DMH and PAG regions were taken and mounted in silanized glass slides. Coverslip used was ProLong Gold® antifade (Invitrogen). Microscopic analyses of the fluorescence were performed using excitation max of 460 and 530 nm / emission max of 505 and 590 nm for green and red beads, (fluorescein and rhodamine) respectively.

4.4 - Injections and drugs

The drugs employed in physiology experiments were: i) NMDA (100 pmol/100 nl); ii) the antagonist of EAA receptors, kynurenic acid (1 nmol/100 nl); iii) vehicle (NaCl 0.9%, 100nl). Drugs were purchased from Sigma. Nanoinjections in awake animals were made with a 30-gauge injection needle connected to a Hamilton syringe (5 μl) as described before (da

Silva et al., 2003), using a dual manipulator stereotaxic instrument. The coordinates for injections into DMH were: -3.2 mm posterior and ± 0.6 mm lateral to the bregma and at a depth of -8.5 mm. For PAG, coordinates were: -6.8mm posterior, ±0.6mm lateral and -4.8mm ventral (Paxinos and Watson, 1986).

4.5 - Histology

At the end of experiments (1-3), rats were euthanized by an overdose of anesthetic, and a nanoinjection of alcian blue dye 2% (100 nl) was made into injection sites for subsequent histological confirmation according to the methodology described previously (da Silva et al., 2003; Menezes and Fontes, 2007). The atlas of Paxinos & Watson (Paxinos and Watson, 1986) was used as a reference for confirmation of the injection sites in the DMH and PAG.

4.6 - Statistical Analysis

We used student *t test* and ANOVA, when appropriated (see figure legends for details). Significance was taken at $P<0.05$. Data are expressed as mean ± SEM.

ACKNOWLEDGMENTS

Xavier CH was recipient of CNPq PhD fellowship (Postgraduation program in Biological Sciences - Physiology and Pharmacology, ICB, UFMG). Ianzer D was recipient of FAPESP postdoctoral fellowship. Financial support: INCT NanoBiofar, FAPEMIG and CNPq.

CONFLICT OF INTEREST

None.

SUBMISSION DECLARATION

The present article has not been published previously, except part of the results in the abstract form.

CONTRIBUTORS

Xavier CH, Ianzer D, Marins FR, Lima AM, Menezes GB and Vaz GC performed experimental procedures. Analysis, conception and design of the whole study were performed by Xavier CH, Nalivaiko E and Fontes MAP.

REFERENCES

Albin, R.L., Makowiec, R.L., Hollingsworth, Z., Dure, L.S.t., Penney, J.B., Young, A.B., 1990. Excitatory amino acid binding sites in the periaqueductal gray of the rat. Neurosci Lett. 118**,** 112-5.

Apps, R., Ruigrok, T.J., 2007. A fluorescence-based double retrograde tracer strategy for charting central neuronal connections. Nat Protoc. 2**,** 1862-8.

Bandler, R., Carrive, P., 1988. Integrated defence reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal grey region of the unrestrained cat. Brain Res. 439**,** 95-106.

Beart, P.M., Summers, R.J., Stephenson, J.A., Cook, C.J., Christie, M.J., 1990. Excitatory amino acid projections to the periaqueductal gray in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. Neuroscience. 34**,** 163-76.

Cameron, A.A., Khan, I.A., Westlund, K.N., Cliffer, K.D., Willis, W.D., 1995a. The efferent projections of the periaqueductal gray in the rat: a Phaseolus vulgaris-leucoagglutinin study. I. Ascending projections. J Comp Neurol. 351**,** 568-84.

Cameron, A.A., Khan, I.A., Westlund, K.N., Willis, W.D., 1995b. The efferent projections of the periaqueductal gray in the rat: a Phaseolus vulgaris-leucoagglutinin study. II. Descending projections. J Comp Neurol. 351**,** 585-601.

Campos, R.R., McAllen, R.M., 1999. Cardiac inotropic, chronotropic, and dromotropic actions of subretrofacial neurons of cat RVLM. Am J Physiol. 276**,** R1102-11.

Cao, W.H., Morrison, S.F., 2003. Disinhibition of rostral raphe pallidus neurons increases cardiac sympathetic nerve activity and heart rate. Brain Res. 980**,** 1-10.

Cao, W.H., Fan, W., Morrison, S.F., 2004. Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. Neuroscience. 126**,** 229-40.

Carrive, P., 1993. The periaqueductal gray and defensive behavior: functional representation and neuronal organization. Behav Brain Res. 58**,** 27-47.

Carrive, P., Gorissen, M., 2008. Premotor sympathetic neurons of conditioned fear in the rat. Eur J Neurosci. 28**,** 428-46.

Carrive, P., 2009. Emotional Control of the Autonomic Nervous System. In Encyclopedia of Neuroscience. Vol., R.S. Larry, ed.^eds. Academic Press, Oxford, pp. 923-928.

Critchley, H.D., Taggart, P., Sutton, P.M., Holdright, D.R., Batchvarov, V., Hnatkova, K., Malik, M., Dolan, R.J., 2005. Mental stress and sudden cardiac death: asymmetric midbrain activity as a linking mechanism. Brain. 128**,** 75-85.

da Silva, L.G., de Menezes, R.C., dos Santos, R.A., Campagnole-Santos, M.J., Fontes, M.A., 2003. Role of periaqueductal gray on the cardiovascular response evoked by disinhibition of the dorsomedial hypothalamus. Brain Res. 984**,** 206-14.

da Silva, L.G., Jr., Menezes, R.C., Villela, D.C., Fontes, M.A., 2006. Excitatory amino acid receptors in the periaqueductal gray mediate the cardiovascular response evoked by activation of dorsomedial hypothalamic neurons. Neuroscience. 139**,** 1129-39.

de Menezes, R.C., Zaretsky, D.V., Fontes, M.A., DiMicco, J.A., 2006. Microinjection of muscimol into caudal periaqueductal gray lowers body temperature and attenuates increases in temperature and activity evoked from the dorsomedial hypothalamus. Brain Res. 1092**,** 129-37.

de Menezes, R.C., Zaretsky, D.V., Sarkar, S., Fontes, M.A., Dimicco, J.A., 2008. Microinjection of muscimol into the periaqueductal gray suppresses cardiovascular and neuroendocrine response to air jet stress in conscious rats. Am J Physiol Regul Integr Comp Physiol. 295**,** R881-90.

de Menezes, R.C., Zaretsky, D.V., Fontes, M.A., DiMicco, J.A., 2009. Cardiovascular and thermal responses evoked from the periaqueductal grey require neuronal activity in the hypothalamus. J Physiol. 587**,** 1201-15.

de Oliveira, R.W., Del Bel, E.A., Guimaraes, F.S., 2000. Behavioral and c-fos expression changes induced by nitric oxide donors microinjected into the dorsal periaqueductal gray. Brain Res Bull. 51**,** 457-64.

Dimicco, J.A., Soltis, R.P., Anderson, J.J., Wible, J.H., Jr., 1992. Hypothalamic Mechanisms and the Cardiovascular Response to Stress. . In:: Central Neural Mechanisms in Cardiovascular Regulation. Vol. II, Ed. by G. Kunos and J. Ciriello, Birkhauser, Boston.

DiMicco, J.A., Samuels, B.C., Zaretskaia, M.V., Zaretsky, D.V., 2002. The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. Pharmacol Biochem Behav. 71**,** 469-80.

Fang, H.S., Wang, S.C., 1962. Cardioaccelerator and cardioaugmentor points in hypothalamus of the dog. Am J Physiol. 203**,** 147-50.

Farkas, E., Jansen, A.S., Loewy, A.D., 1998. Periaqueductal gray matter input to cardiac-related sympathetic premotor neurons. Brain Res. 792**,** 179-92.

Fontes, M.A., Tagawa, T., Polson, J.W., Cavanagh, S.J., Dampney, R.A., 2001. Descending pathways mediating cardiovascular response from dorsomedial hypothalamic nucleus. Am J Physiol Heart Circ Physiol. 280**,** H2891-901.

Fontes, M.A., Xavier, C.H., de Menezes, R.C., Dimicco, J.A., 2011. The dorsomedial hypothalamus and the central pathways involved in the cardiovascular response to emotional stress. Neuroscience. 184**,** 64-74.

Furnival, C.M., Linden, R.J., Snow, H.M., 1968. Response to stimulation of the cardiac sympathetic nerves. J Physiol. 197**,** 74P-75P.

Furukawa, Y., Wallick, D.W., Martin, P.J., Levy, M.N., 1990. Chronotropic and dromotropic responses to stimulation of intracardiac sympathetic nerves to sinoatrial or atrioventricular nodal region in anesthetized dogs. Circ Res. 66**,** 1391-9.

Harris, J.A., Guglielmotti, V., Bentivoglio, M., 1996. Diencephalic asymmetries. Neurosci Biobehav Rev. 20**,** 637-43.

Horiuchi, J., McAllen, R.M., Allen, A.M., Killinger, S., Fontes, M.A., Dampney, R.A., 2004. Descending vasomotor pathways from the dorsomedial hypothalamic nucleus: role of medullary raphe and RVLM. Am J Physiol Regul Integr Comp Physiol. 287**,** R824-32.

Horiuchi, J., McDowall, L.M., Dampney, R.A., 2009. Vasomotor and respiratory responses evoked from the dorsolateral periaqueductal grey are mediated by the dorsomedial hypothalamus. J Physiol. 587**,** 5149-62.

Huang, J., Chowhdury, S.I., Weiss, M.L., 2002. Distribution of sympathetic preganglionic neurons innervating the kidney in the rat: PRV transneuronal tracing and serial reconstruction. Auton Neurosci. 95**,** 57-70.

Kamosinska, B., Nowicki, D., Szulczyk, P., 1989. Control of the heart rate by sympathetic nerves in cats. J Auton Nerv Syst. 26**,** 241-9.

Lane, R.D., Schwartz, G.E., 1987. Induction of lateralized sympathetic input to the heart by the CNS during emotional arousal: a possible neurophysiologic trigger of sudden cardiac death. Psychosom Med. 49**,** 274-84.

Lane, R.D., Laukes, C., Marcus, F.I., Chesney, M.A., Sechrest, L., Gear, K., Fort, C.L., Priori, S.G., Schwartz, P.J., Steptoe, A., 2005. Psychological stress preceding idiopathic ventricular fibrillation. Psychosom Med. 67**,** 359-65.

Lane, R.D., Critchley, H.D., Taggart, P., in press. Asymmetric autonomic innervation. In Handbook of Cardiovascular Behavioral Medicine. Edited by Waldstein S, Kop W and Katzel L. New York, Springer

Menezes, R.C., Fontes, M.A., 2007. Cardiovascular effects produced by activation of GABA receptors in the rostral ventrolateral medulla of conscious rats. Neuroscience. 144**,** 336-43.

Moon, E.A., Goodchild, A.K., Pilowsky, P.M., 2002. Lateralisation of projections from the rostral ventrolateral medulla to sympathetic preganglionic neurons in the rat. Brain Res. 929**,** 181-90.

Oppenheimer, S., 2006. Cerebrogenic cardiac arrhythmias: cortical lateralization and clinical significance. Clin Auton Res. 16**,** 6-11.

Oppenheimer, S., 2007. Cortical control of the heart. Cleve Clin J Med. 74 Suppl 1**,** S27-9.

Ozdemir, O., Hachinski, V., 2008. Brain lateralization and sudden death: its role in the neurogenic heart syndrome. J Neurol Sci. 268**,** 6-11.

Paxinos, G., Watson, C., 1986. The rat brain in stereotaxic coordinates. Vol., New York.

Randall, W.C., Rohse, W.G., 1956. The augmentor action of the sympathetic cardiac nerves. Circ Res. 4**,** 470-5.

Randall, W.C., Szentivanyi, M., Pace, J.B., Wechsler, J.S., Kaye, M.P., 1968. Patterns of sympathetic nerve projections onto the canine heart. Circ Res. 22**,** 315-23.

Randall, W.C., Armour, J.A., Geis, W.P., Lippincott, D.B., 1972. Regional cardiac distribution of the sympathetic nerves. Fed Proc. 31**,** 1199-208.

Rogers, M.C., Battit, G., McPeek, B., Todd, D., 1978. Lateralization of sympathetic control of the human sinus node: ECG changes of stellate ganglion block. Anesthesiology. 48**,** 139-41.

Rusyniak, D.E., Zaretskaia, M.V., Zaretsky, D.V., DiMicco, J.A., 2008. Microinjection of muscimol into the dorsomedial hypothalamus suppresses MDMA-evoked sympathetic and behavioral responses. Brain Res. 1226**,** 116-23.

Samuels, B.C., Zaretsky, D.V., DiMicco, J.A., 2002. Tachycardia evoked by disinhibition of the dorsomedial hypothalamus in rats is mediated through medullary raphe. J Physiol. 538**,** 941-6.

Samuels, B.C., Zaretsky, D.V., DiMicco, J.A., 2004. Dorsomedial hypothalamic sites where disinhibition evokes tachycardia correlate with location of raphe-projecting neurons. Am J Physiol Regul Integr Comp Physiol. 287**,** R472-8.

Schwartz, P.J., Stone, H.L., Brown, A.M., 1976. Effects of unilateral stellate ganglion blockade on the arrhythmias associated with coronary occlusion. Am Heart J. 92**,** 589-99.

Shafton, A.D., Ryan, A., Badoer, E., 1998. Neurons in the hypothalamic paraventricular nucleus send collaterals to the spinal cord and to the rostral ventrolateral medulla in the rat. Brain Res. 801**,** 239-43.

Soltis, R.P., DiMicco, J.A., 1991a. Interaction of hypothalamic GABAA and excitatory amino acid receptors controlling heart rate in rats. Am J Physiol. 261**,** R427-33.

Soltis, R.P., DiMicco, J.A., 1991b. GABAA and excitatory amino acid receptors in dorsomedial hypothalamus and heart rate in rats. Am J Physiol. 260**,** R13-20.

Soltis, R.P., DiMicco, J.A., 1992. Hypothalamic excitatory amino acid receptors mediate stress-induced tachycardia in rats. Am J Physiol. 262**,** R689-97.

Soltis, R.P., Cook, J.C., Gregg, A.E., Stratton, J.M., Flickinger, K.A., 1998. EAA receptors in the dorsomedial hypothalamic area mediate the cardiovascular response to activation of the amygdala. Am J Physiol. 275**,** R624-31.

Stotz-Potter, E.H., Morin, S.M., DiMicco, J.A., 1996a. Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air stress-induced neuroendocrine and cardiovascular changes in rats. Brain Res. 742**,** 219-24.

Stotz-Potter, E.H., Willis, L.R., DiMicco, J.A., 1996b. Muscimol acts in dorsomedial but not paraventricular hypothalamic nucleus to suppress cardiovascular effects of stress. J Neurosci. 16**,** 1173-9.

Taylor, R.B., Weaver, L.C., 1992. Spinal stimulation to locate preganglionic neurons controlling the kidney, spleen, or intestine. Am J Physiol. 263**,** H1026-33.

ter Horst, G.J., Luiten, P.G., 1986. The projections of the dorsomedial hypothalamic nucleus in the rat. Brain Res Bull. 16**,** 231-48.

Ter Horst, G.J., Van den Brink, A., Homminga, S.A., Hautvast, R.W., Rakhorst, G., Mettenleiter, T.C., De Jongste, M.J., Lie, K.I., Korf, J., 1993. Transneuronal viral labelling of rat heart left ventricle controlling pathways. Neuroreport. 4**,** 1307-10.

Thompson, R.H., Canteras, N.S., Swanson, L.W., 1996. Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. J Comp Neurol. 376**,** 143-73.

Toga, A.W., Thompson, P.M., 2003. Mapping brain asymmetry. Nat Rev Neurosci. 4**,** 37-48.

Vianna, D.M., Brandao, M.L., 2003. Anatomical connections of the periaqueductal gray: specific neural substrates for different kinds of fear. Braz J Med Biol Res. 36**,** 557-66.

Villela, D.C., da Silva, L.G., Jr., Fontes, M.A., 2009. Activation of 5-HT receptors in the periaqueductal gray attenuates the tachycardia evoked from dorsomedial hypothalamus. Auton Neurosci. 148**,** 36-43.

Winter, J., Tanko, A.S., Brack, K.E., Coote, J.H., Ng, G.A., Differential cardiac responses to unilateral sympathetic nerve stimulation in the isolated innervated rabbit heart. Auton Neurosci.

Wittling, W., 1997. The right hemisphere and the human stress response. Acta Physiol Scand Suppl. 640**,** 55-9.

Xavier, C.H., Campagnole-Santos, M.J., Fontes, M.A.P., 2008. Comparison of the cardiovascular responses evoked by chemical activation of the left and right sides of dorsomedial hypothalamus. Experimental Biology meeting abstracts. FASEB Journal. 22.

Xavier, C.H., Nalivaiko, E., Beig, M.I., Menezes, G.B., Cara, D.C., Campagnole-Santos, M.J., Fontes, M.A., 2009. Functional asymmetry in the descending cardiovascular pathways from dorsomedial hypothalamic nucleus. Neuroscience. 164**,** 1360-8.

Xavier, C.H., Beig, M.I., Ianzer, D., Fontes, M.A., Nalivaiko, E., 2010a. Cardiac chronotropic and inotropic responses evoked from right or left sides of dorsomedial hypothalamus. FASEB Journal. 24**,** 1019.20.

Xavier, C.H., Ianzer, D., Nalivaiko, E., Fontes, M.A., 2010b. Lateralized changes in renal sympathetic activity evoked by unilateral stimulation of lateral/dorsolateral periaqueductal gray. FASEB Journal. 24**,** 1050.6.

Xavier, C.H., Vaz, G.C., Lima, A.M., Ianzer, D., Nalivaiko, E., Fontes, M.A., 2011. Contribution of excitatory amino acid receptors in the dorsomedial hypothalamus and periaqueductal gray to the asymmetry and lateralization in the cardiovascular responses to stress in rats. Autonomic Neuroscience. 163**,** 57.

Yanowitz, F., Preston, J.B., Abildskov, J.A., 1966. Functional distribution of right and left stellate innervation to the ventricles. Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. Circ Res. 18**,** 416-28.

Zaretskaia, M.V., Zaretsky, D.V., Sarkar, S., Shekhar, A., DiMicco, J.A., 2008. Induction of Fos-immunoreactivity in the rat brain following disinhibition of the dorsomedial hypothalamus. Brain Res. 1200**,** 39-50.

	Anesthetized				Non-Anesthetized			
	NMDA DMH		NMDA PAG		Kyn DMH		Kyn PAG	
	Left	Right	Left	Right	Left	Right	Left	Right
MAP (mmHg)	93±3	94±2	96±6	98±7	102±3	105±5	106±4	108±8
HR (bpm)	319±13	325±4	353±9	357±10	321±8	334±9	346±13	333±9

Table 1. Baseline values of mean arterial pressure (MAP) and heart rate (HR) collected immediately before microinjections into unilateral DMH or PAG in the following experimental series where we used anesthetized and non-anesthetized animals. There were no differences between basal values found before microinjections between L- and R- within each experimental series (see experimental protocols for details).

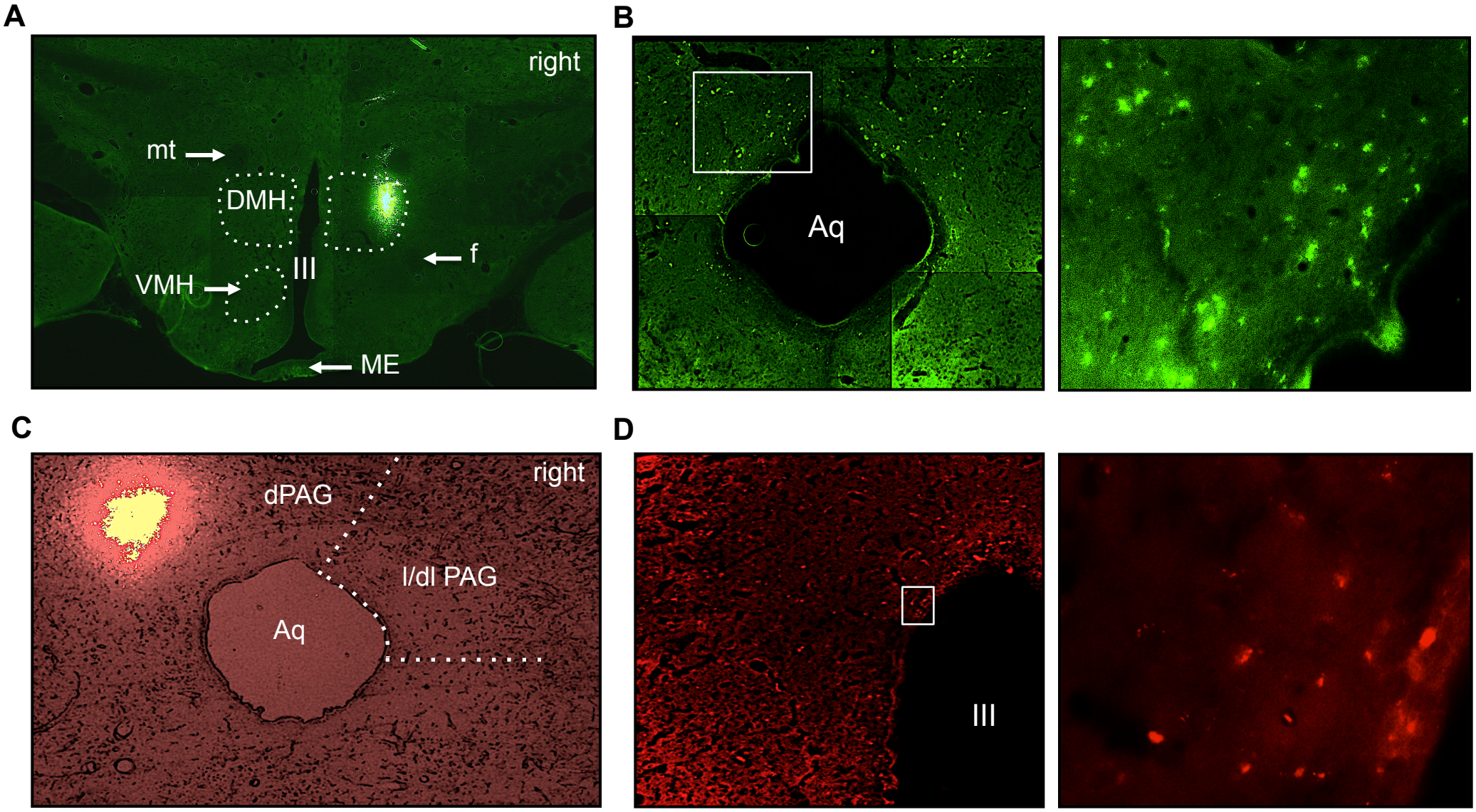
Figure 1. A and C: Photomicrographs of rat brain slices depicting injection sites into the nuclei we targeted in all experimental series. Spots are from animals injected with green and red retrobeads at level of (A) DMH and (C) PAG, respectively. C and D: Zoom view in (B) PAG and (D) DMH neurons retrogradely labeled with green and red retrobeads, respectively. DMH – dorsomedial hypothalamus; ME – median eminence; mt – mammillothalamic tract; f – fornix; VMH – ventromedial hypothalamus; III – third ventricle; l/dlPAG – lateral/dorsolateral periaqueductal gray; Aq – aqueduct.

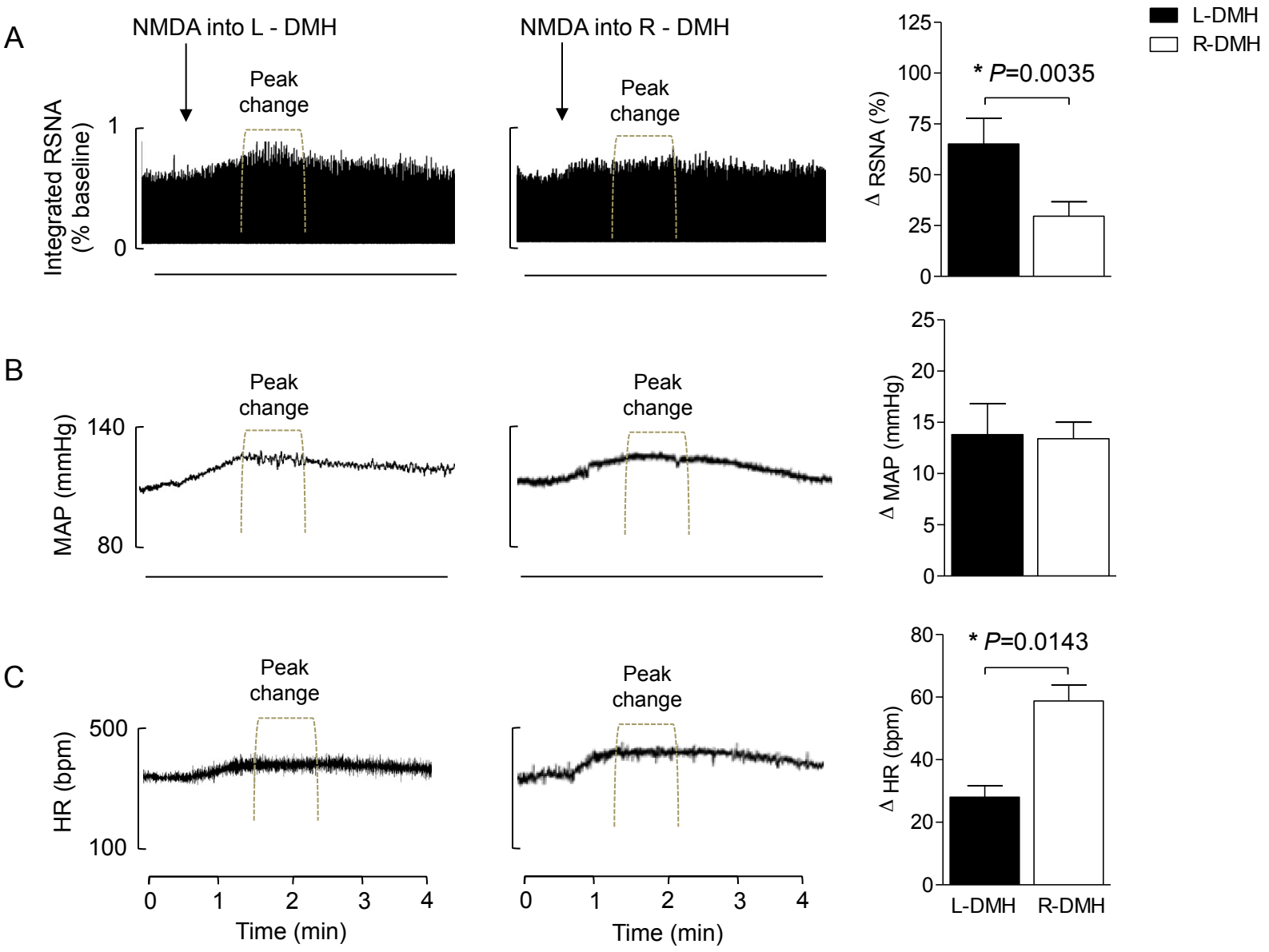
Figure 2. Representative charts records and peak changes in RSNA (A) recorded in the left renal nerve, MAP (B) and HR (C) evoked by microinjections of NMDA (100 pmol/100 nl) into right (white bars) and left (black bars) DMH in the first experimental series. **P*<0.05 R- vs. L-DMH (paired Student *t* test). Results are shown as mean ± SEM.

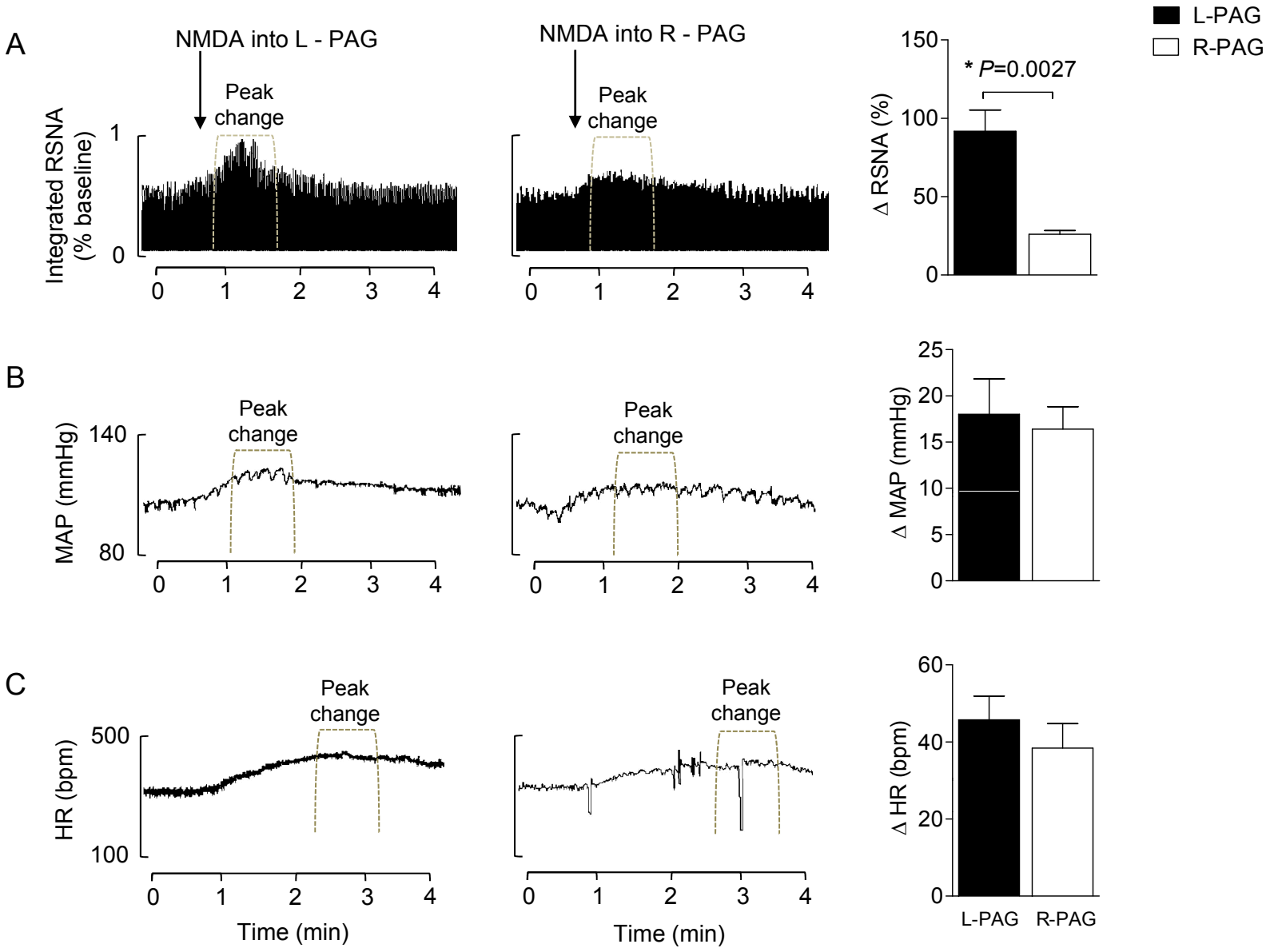
Figure 3. Representative charts records and peak changes in RSNA (A) recorded in the left renal nerve, MAP (B) and HR (C) evoked by microinjections of NMDA (100 pmol/100 nl) into right (white bars) and left (black bars) PAG in the first experimental series. **P*<0.05 R- vs. L- PAG (paired Student *t* test). Results are shown as mean ± SEM.

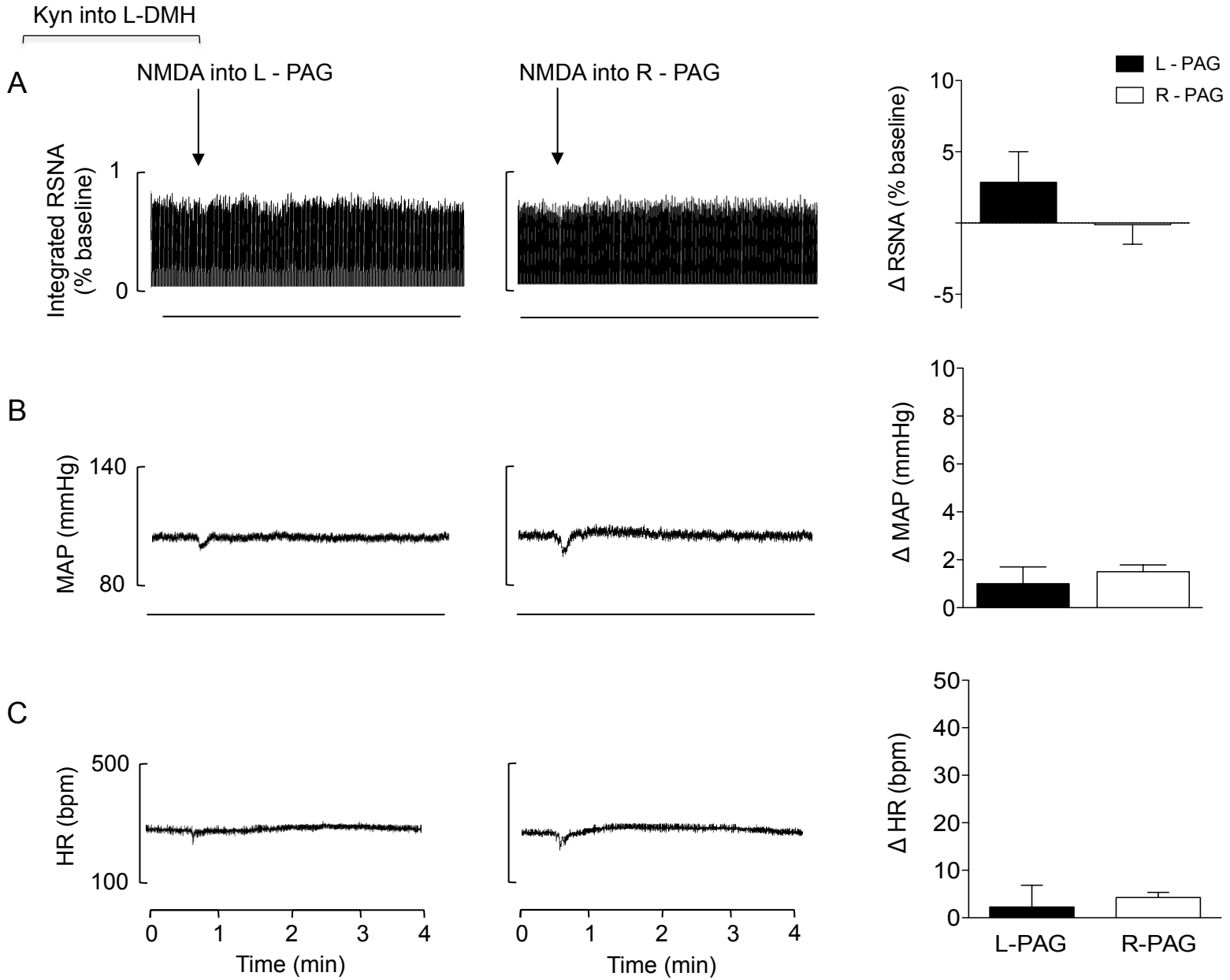
Figure 4. Representative charts records and peak changes in RSNA (A) recorded in the left renal nerve, MAP (B) and HR (C) evoked by microinjections of NMDA (100 pmol/100 nl) into right (white bars) and left (black bars) PAG after inhibition of EAA receptors of left DMH in the second experimental series. Results are shown as mean ± SEM.

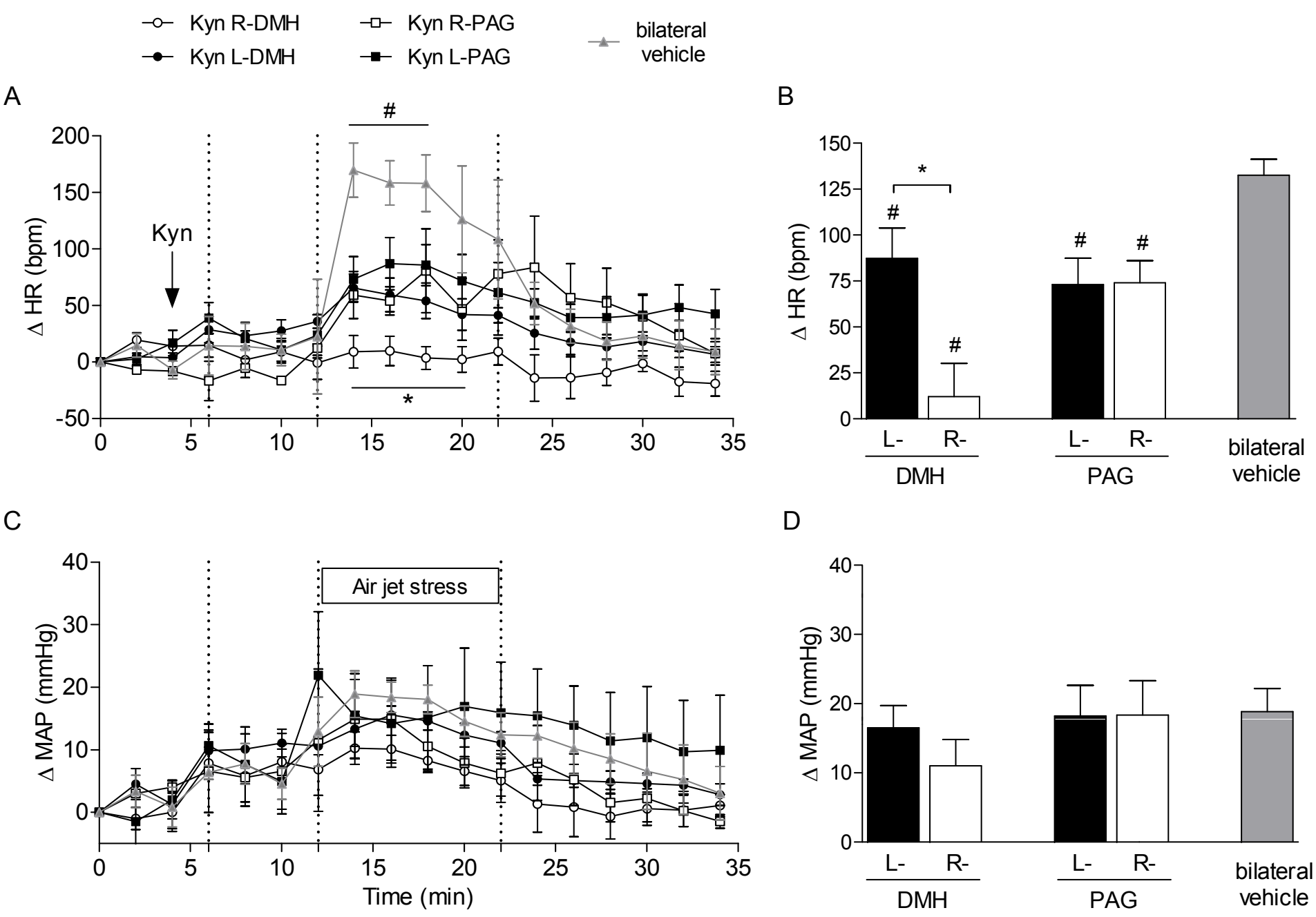
Figure 5. Results of non-anesthetized rats submitted to air jet stress at the second experimental series. **Panels A and C:** time course of HR and MAP before and after injection of Kynurenic acid (1 nmol/100 nl) into right (open symbols) and left (black symbols) DMH (circles) or l/dl PAG (squares); or bilateral vehicle (gray triangles). **P*<0.05 R- vs. L-DMH; $^{\#}P$<0.05 vehicle vs. R- or L-DMH and R- or L-PAG (Two-way ANOVA followed by Bonferroni post hoc test). **Panels B and D**: peak changes in HR and MAP during stress trial in animals previously microinjected with Kynurenic acid (1 nmol/100 nl) into right (open bars) and left (black bars) DMH or l/dl PAG; or bilateral vehicle (gray bars). **P*<0.05 R vs. L and $^{\#}P$<0.05 vs. bilateral vehicle (One-way ANOVA followed by Newman-Keuls post hoc test). Results are shown as mean ± SEM.


A
right
mt
DMH
III
f
VMH
ME
B
Aq
C
right
dPAG
l/dl PAG
Aq
D
III


A
NMDA into L - DMH
NMDA into R - DMH
Peak change
Peak change
Integrated RSNA (% baseline)
1
0
L-DMH
R-DMH
125
100
75
50
25
0
* P=0.0035
Δ RSNA (%)
B
Peak change
Peak change
MAP (mmHg)
140
80
25
20
15
10
5
0
Δ MAP (mmHg)
C
Peak change
Peak change
HR (bpm)
500
100
80
60
40
20
0
* P=0.0143
Δ HR (bpm)
0
1
2
3
4
Time (min)
L-DMH
R-DMH


A
NMDA into L - PAG
Peak change
NMDA into R - PAG
Peak change
Integrated RSNA (% baseline)
1
0
0 1 2 3 4
L-PAG
R-PAG
150
100
50
0
* P=0.0027
Δ RSNA (%)
B
MAP (mmHg)
140
80
Peak change
Peak change
25
20
15
10
5
0
Δ MAP (mmHg)
C
HR (bpm)
500
100
Peak change
Peak change
Time (min)
Time (min)
60
40
20
0
Δ HR (bpm)
L-PAG R-PAG


Kyn into L-DMH
A
NMDA into L - PAG
NMDA into R - PAG
Integrated RSNA (% baseline)
1
0
L - PAG
R - PAG
Δ RSNA (% baseline)
10
5
-5
B
MAP (mmHg)
140
80
Δ MAP (mmHg)
10
8
6
4
2
0
C
HR (bpm)
500
100
0
1
2
3
4
Time (min)
Time (min)
Δ HR (bpm)
50
40
30
20
10
0
L-PAG
R-PAG


Kyn R-DMH
Kyn L-DMH
Kyn R-PAG
Kyn L-PAG
bilateral vehicle
A
B
C
D
Kyn
Δ HR (bpm)
Δ MAP (mmHg)
Air jet stress
Time (min)
L-
R-
DMH
PAG
bilateral vehicle