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ALTERAÇÕES CARDIOVASCULARES INDUZIDAS POR FENILEFRINA EM ANIMAIS KNOCKOUT PARA O RECEPTOR MAS

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Lista de Abreviaturas e Siglas

A260 = absorbância no comprimento de onda de 260 nanômetros

A-779 = antagonista do receptor Mas

AMPc = monofosfato cíclico de adenosina

Ang I = Angiotensina I

Ang II = Angiotensina II

Ang IV = Angiotensina IV

Ang- (1-7) = Angiotensina 1-7

ANP = peptídeo natriurético atrial

AP = Aminopeptidases

AT1 = subtipo 1 do receptor de angiotensina II

AT2 = subtipo 2 do receptor de angiotensina II

BSA = albumina de soro bovino (do inglês *Bovine Serum Albumin*)

Ca^{2+} = íon cálcio

cDNA = DNA complementar

CT = controle

DMEM = meio de cultura (do inglês *Dulbecco´s Modified Eagle´s Medium)*

DNA= ácido desoxirribonucleico

DNase = deoxirribonuclease

ECA = enzima conversora de angiotensina

ECA 2 = enzima conversora de angiotensina II

EDTA-2Na = Ethylenediaminetetraacetic acid

F/F0 = fluorescência máxima/fluorescência mínima

Fluo-4/AM = Flúor 4 aceto-metil-éster

Gi/0 = proteína G inibitória

GPX = glutationa peroxidase

HRP = do inglês *horseradish peroxidase*

KCl = cloreto de potássio

KO = animal controle com deleção gênica para o receptor Mas

KO-F = animal com deleção gênica para o receptor Mas tratado com fenilefrina

Mas-KO = *knockout* para o receptor Mas

MgCl2 = cloreto de magnésio

M-MLV RT = *Minus Moloney murine leukemia virus-reverse transcriptase*

Na4P2O7 x 10 H20 = pirofosfato de sódio hidratado.

Na3VO4 = ortovanadato de sódio

NaCl = cloreto de sódio

NaF = fluoreto de sódio

NO = óxido nítrico

PBS = tampão fosfato-salino (do inglês *Phosphate buffered saline*)

PBS-T = tampão fostato-salino com tween20 (do inglês *Phosphate buffered saline tween-20*)

NEP = endopeptidase neutra

PCR = reação em cadeia de polimerase (do inglês *polymerase chain reaction*)

PEP = prolil-endopeptidase

PKA = proteína quinase A

PKC = proteína quinase C

PI3K= fosfoinositol 3 quinase

PMSF = Phenylmethylsulphonyl fluoride

PVDF = membrana de Polyvinylidene fluoride

RNA = ácido ribonucleico

RNAm = RNA mensageiro

RNase = ribonuclease

RPM = rotações por minuto

ROS = Espécies reativas de oxigênio

SDS = dodecil sulfato de sódio

SDS-PAGE = gel de acrilamida (do inglês *sodium dodecyl (lauryl)*

SERCA = ATPase-Ca2+ do retículo sarcoplasmático

SOD1 = superóxido dismutase 1

SRA = sistema renina angiotensina

u.a = unidades arbitrárias

WT = Wild Type, camundongos controle

WT-F = Wild type, camundongos tratados com fenilefrina

Resumo

A ativação simpática é modulada pelos receptores adrenérgicos e a estimulação destes receptores pelo excesso de catecolaminas circulantes, causa o remodelamento cardíaco com consequente deterioramento da função miocárdica. É sabido que o sistema Renina-Angiotensina tem importante papel neste processo, sendo o eixo Ang-(1-7)/ receptor Mas a principal via cardioprotetora e contarrregulatória ao eixo Ang II/receptor AT1. A construção de animais com deleção genética do receptor Mas (Mas-Ko), tem trazido grande avanço para o entendimento das funções e mecanismos celulares ativados pelo eixo Ang-(1-7)/Mas. O presente trabalho, teve como principal objetivo, identificar as possíveis alterações cardiovasculares induzidas pelo agente estressor, fenilefrina e comparar a resposta dos animais Mas-Ko com as respostas de camundongos selvagens (WT), buscando desta forma uma maior compreensão do funcionamento do eixo Ang-(1-7)/ Mas. Os animais WT e KO foram tratados com fenilefrina durante 7 e 14 dias e seus respectivos controle com salina. Os resultados mostraram que os animais WT tratados por 7 dias com fenilefrina apresentaram respostas compatíveis ao esperado com relação a este tratamento : (i) hipertrofia do coração e do cardiomiócito, (ii) produção aumentada das espécies reativas de oxigênio (ROS), (ii) alteração na expressão das enzimas anti-oxidantes, (iii) aumento da amplitude do transiente de Ca^{2+} e (iv) aumento da fração de ejeção do coração. O prolongamento do tratamento com fenilefrina levou à (v) diminuição da amplitude do transiente de Ca^{2+} e (vi) diminuição da fração de ejeção. Já o tratamento dos camundongos Mas-KO com fenilefrina durante 7 dias demonstrou um padrão distinto, com repostas menos pronunciadas : (i)ausência de hipertrofia no cardiomiócito (ii) pouca alteração na produção de ROS e enzimas antioxidantes.O prolongamento do tratamento não provocou alterações na amplitude do transiente de Ca^{2+} e na fração de ejeção do coração.Estes dados sugerem que os animais Mas-Ko respondem com menos alterações aparentemente prejudiciais, do que animais selvagens, frente ao agente estressor fenilefrina.

Palavras-chave: fenilefrina, receptor Mas, hipertrofia, estresse oxidativo.

Abstract

Sympathetic activation is modulated by adrenergic receptors and stimulation of these receptors by excessive circulating catecholamines causes cardiac remodeling, with consequent deterioration of myocardial function. It is known that the Renin-Angiotensin system has an important role in this process, being the Ang-(1-7)/Mas receptor axis the main route to cardioprotection and to induce cardio counterregulatory responses to Ang II/AT 1 receptor axis. The development of animals with genetic deletion of the receptor Mas (Mas-Ko), has brought great progress in understanding the functions and cellular mechanisms activated by Ang- (1-7) / Mas axis. The present study aimed to identify the cardiovascular abnormalities induced by the stressor phenylephrine and to compare the response of animals Mas-Ko with the responses of wild-type mice (WT), in order to better understanding the mechanism of the Ang - (1-7) / receptor Mas axis. WT and KO animals were treated with phenylephrine for 7 and 14 days and their respective control with saline solution. Our results showed that WT mice treated for 7 days with phenylephrine presented responses consistent with the expected response to treatment with phenylephrin: (i) hypertrophy of the heart and cardiomyocytes, (ii) increased production of reactive oxygen species (ROS), (ii) change in expression of antioxidant enzymes, (iii) increased amplitude of the Ca2 + transient and (iv) increased heart ejection fraction. Prolonged treatment with phenylephrine led to (v) a decrease in Ca2+ transient amplitude and (vi) a decrease in ejection fraction. On the other hand, treatment of KO mice with phenylephrine for 7 days showed distinct response patterns: (i) cardiomyocytes were not hypertrophied, (ii) ROS production and expression of antioxidant enzymes were not significantly altered. Also, prolonged treatment of KO mice with phenylephrine, was not related to changes in Ca2+ transient amplitude or altered ejection fraction. These data suggest that Mas-Ko mice respond with less harmful alterations when treated with phenylephrine as compared to wild type animals.

Keywords: phenylephrine, Mas receptor, cardiac remodeling, oxidative stress.

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