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Estudos estruturais e funcionais da toxina Gi-Tx1 da peçonha da aranha caranguejeira brasileira *Grammostola iheringi* (MYGALOMORPHAE: Theraphosidae)

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Fisiologia e Farmacologia, Depto. de Fisiologia e Biofísica e Depto. de Farmacologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, como requerimento parcial, para obtenção do título de mestre em Fisiologia e Farmacologia.

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"O valor de praticar com rigor, por algum tempo, uma ciência rigorosa, não está propriamente em seus resultados: eles sempre serão uma gota ínfima, ante o mar das coisas dignas de saber.

Mas isso produz um aumento de energia, de capacidade dedutiva, de tenacidade; aprende-se a alcançar um fim de modo pertinente. Neste sentido é valioso, em vista de tudo o que se fará depois de ter sido um homem de ciência"

Nietzsche

RESUMO

A maioria dos trabalhos com venenos de aranhas é dedicada ao estudo de peçonhas de espécies de importância médica. Venenos de baixa toxicicidade, antes negligenciados, hoje tem mostrado grande interesse científico porque são importantes fontes de sondas bioquímicas para dissecação molecular de processos farmacológicos. Peçonhas de caranguejeiras são ricas misturas de sais, nucleotídeos, aminoácidos livres, neurotransmissores, poliaminas, peptídeos, proteínas e enzimas. Este trabalho, iniciou-se com o veneno bruto de *Grammostola iheringi,* uma caranguejeira brasileira encontrada principalmente no sul do país, que foi purificado gerando várias frações e toxinas. O foco de nosso estudo foi uma nova toxina pura, com massa molecular de 3.585 Da, cuja sequência foi resolvida e chamada de Gi-Tx1. As atividades biológicas desta toxina foram caracterizadas via bioensaios de toxicidade, triagem eletrofisiológica realizada em neurônios obtidos de gânglio da raiz dorsal (DRG) de ratos e em subtipos de canais para sódio e potássio dependentes de voltagem, transfectados em ovócitos de *Xenopus laevis*. Estes estudos mostraram que Gi-Tx1 é capaz de bloquear parcialmente as correntes de entrada (40%) e de saída (20%) dos neurônios DRG, a 2 μM e é capaz de bloquear totalmente os canais Nav1.2, Nav1.3, Nav1.4 e Nav1.6 e parcialmente, os canais Nav1.5, DmNav, além de bloquear totalmente, os canais Kv4.3 e hERG nesta mesma concentração. Em células tumorais de fígado e mama, a toxina foi capaz de reduzir a proliferação (células SKHep-1 e MGSO3, respectivamente) mas não afetou as taxas de proliferação de células mononucleares de sangue periférico humano (PBMC's, não tumorais). Estes resultados em conjunto, revelam a ação promíscua de Gi-Tx1 em canais iônicos para sódio e para potássio, bem como seu efeito antitumoral. Será interessante verificar se os efeitos bloqueadores em canais iônicos pela toxina, estariam relacionados ao seu efeito antitumoral e se esta toxina poderia concorrer para indicações de possíveis modelos de fármacos para o combate a determinados tipos de câncer.

ABSTRACT

Most of the initial work on spider venoms has been devoted to the study of medically important species. Venoms of apparent lower toxicity, before neglected, have shown increased interest for scientific studies, because they are sources of important biochemical probes for the pharmacological dissection of molecular processes. Tarantula venoms are rich mixtures of salts, nucleotides, free amino acids, neurotransmitters, polyamines, peptides, proteins and enzymes. *Grammostola iheringi* is a Brazilian tarantula spider found mainly in southern part of Brazil. Here, we studied the poison fractions and mainly a new toxin of 3.585 Da, named Gi-Tx1**.** The activities of this toxin were characterized by bioassays, electrophysiological screening performed on dorsal root ganglia neurons (DRG) and in voltage-gated sodium and potassium channels subtypes that have been transfected in ovocites of *Xenopus leavis*. Our results shows that Gi-Tx1 causes a partial block of inward (40%) and outward (20%) currents in DRG cells at 2 μM and it is able to block Nav 1.2-.4, 1.6 subtypes, partially blocks Nav1.5 andDmNav, and fully blocks currents from Kv 4.3 subtypes and hERG channels at 2 μM. In tumor cells, the toxin was able to reduce the proliferation (specifically on SKHep-1 and MGSO3 cells) but did not affect proliferation rates of normal cells (human PBMC's). These results reveal the promiscuous action of Gi-Tx1 on ion channels and indicate its possible antiproliferative effects. It would be interesting to verify if exist a correlation of the effects of this toxin in ionic channels and its antitumoral activity.

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