A novel long chain toxin variant from Androctonus Australis scorpion venom activates currents carried by K_v7.4 channels

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The K_v7 subfamily of voltage-gated potassium channels (K_v7.1-K_v7.5), plays a key pathophysiological role in controlling excitability of cardiac, neuronal, and sensory cells. Mutations in four of five K_v7 genes are responsible for human diseases like cardiac arrhythmia, epilepsy or deafness (Soldovieri et al., 2011). In particular, K_v7.4 channels are expressed in central auditory pathways and in sensory hair cells of the inner ear; mutations in K_v7.4 underlie a rare form of slowly progressive deafness. K_v7.4 channels also regulate vascular (Yeung et al., 2007) and non-vascular smooth muscle contraction, and skeletal muscle differentiation (Iannotti et al., 2013). Drugs acting as openers of K_v7 channels might be effective against neuropsychiatric disorders characterized by neuronal hyperexcitability (including migraine, epilepsy, and neurophatic pain), as well as genitourinary, vascular, and skeletal muscle diseases.
Since scorpion's venoms contain peptides active on sodium and potassium channels, the aim of the present work has been to investigate the Androctonus australis (Aa) scorpion venom as a potential source for novel molecular entities active on K_v7 channels.
To this aim, the whole venom from the Aa scorpion was separated by gel filtration chromatography to obtain five partially-resolved fractions; among these, four correspond to non-toxic fractions (M1, M2, M3, and M4), and one (G50) was toxic *in-vivo*. The M2 nontoxic fractions dose-dependently increased K_v7.4 currents in Xenopus oocytes and caused a 7-8 mV negative voltage shift in current activation threshold. To identify the active molecule(s) in the M2 fraction, we performed different steps of HPLC purification, obtaining 9 fractions; among these, only the P8 fraction showed K_v7.4-activating properties. Within this fraction, by means of mass spectral techniques, we identified a novel variant of AaTXKß(AaTXKß$_{(2-64)}$), a toxin belonging to long chain peptides active on K^+ channels (ß-KTX), whose sequence had been previously predicted by screening of a venom glands cDNA library from Aa (Legros et al., 1998). This variant differs form the canonical AaTXKß$_{(1-64)}$ for the lack of the N-terminal lysine.
To investigate whether AaTXKß$_{(2-64)}$ was selective for K_v7.4 subunits, we performed electrophysiological experiment using pach-clamp technique in whole-cell configuration in CHO mammalian cells expressing homomeric K_v7.1, K_v7.2, K_v7.3, and K_v1.1 channels, as well as heteromeric K_v7.2/3 channels. Similarly to Kv7.4 channels, aTXKß$_{(2-64)}$ significantly increased K_v7.3, K_v7.2/3, and K_v7.4 currents by about 70% (at -40 mV) and caused a 5-10 mV hyperpolarization shift in the activation threshold. By contrast, AaTXKß$_{(2-64)}$ failed to modify current amplitudes at depolarized potentials or activation threshold in K_v7.1, K_v7.2 and K_v1.1 channels.
In conclusion, in the present study, we have identified and characterized a novel variant of AaTXKß from the Aa scorpion acting as a the first natural activator of K_v7 channels. This study may set the basis for developing novel pharmacological tool acting on K_v7 channels.

Bibliography

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