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New analytical cellular systems: studies of Kv1 channels, their fluorescent ligands, and peptide blockers

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INTRODUCTION & AIM

Voltage-gated potassium channels Kv1.1 and Kv1.2 channels are regulators of neuronal excitability in the CNS, while the Kv1.3 channel mediates the immune response of T lymphocytes and microglial cells in brain. Many peptide toxins from scorpion venoms are high-affinity blockers of Kv1 channels, and some of them (e.g., AgTx2, ChTx, MgTx, and HgTx) are used to study channel structure and functions. The affinities (K_d values) of peptide blockers for the target Kv1 channels are usually measured using electrophysiological, radioligand or Rb+ flux methods. However, new pharmacology-relevant techniques are needed to facilitate the search for the advanced blockers and to systematically characterize their properties for potential drug development.

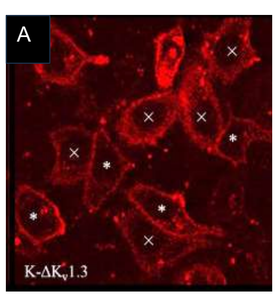
The aim of our study was to develop analytical cellular systems (ACS) for the confocal microscopy study of Kv1 channels, their fluorescent ligands, and peptide blockers. This has been successfully implemented for the bioengineered fluorescently labeled Kv1.1, Kv1.2 and Kv1.3 channels expressed in the plasma membrane of mammalian cells.

METHODS

Plasmids encoding Kv1-subunits fused with red fluorescent protein mKate2, and those encoding hongotoxin 1 (HgTx1) and agitoxin 2 (AgTx2) fused with eGFP were obtained. Fluorescent peptide Atto-HgTx was synthesized. Recombinant fluorescently labeled and unlabeled peptide blockers from scorpion venom were produced. Interactions between blockers and Kv1 channels expressed in mammalian cells were investigated with confocal microscopy and whole-cell patch-clamp technique.

RESULTS & DISCUSSION

1. N-terminal tagging the Kv1 channel with mKate2 fluorescent protein significantly improves membrane expression of the channels compared to the C-terminal tagging



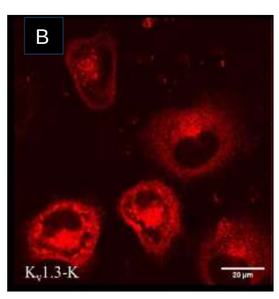
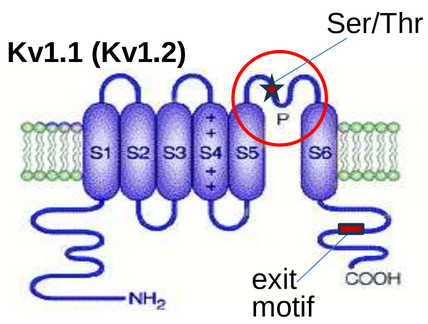


Fig. 1. Expression of mKate2-Kv1.3 (A) and Kv1.3-mKate2 (B) in HEK293 cells

2. Mutation Ser/Thr in the deep pore region of the Kv1.1 and Kv1.2 channels significantly improved their surface expression



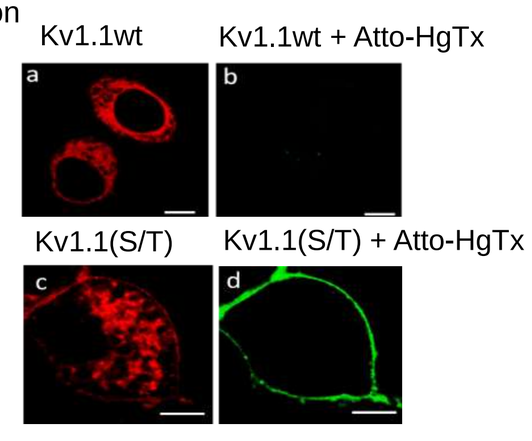


Fig. 2. Alpha-subunit of the Kv1.1(Kv1.2) channel with the mutation in the pore(left panel) and membrane expression of the wt and mutant Kv1.1 channel confirmed by Atto-HgTx binding (right panel)

3. Functional properties of mKate2-tagged Kv1 channels were confirmed by electrophysiological methods

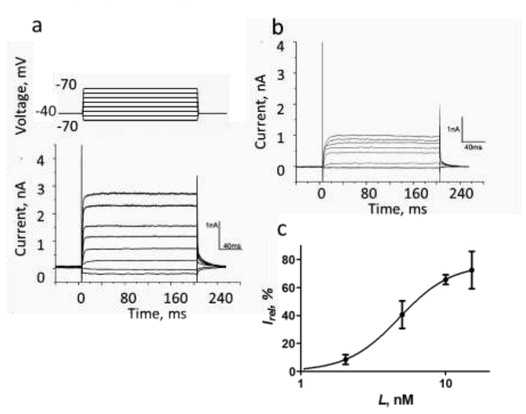


Fig. 3. The whole-cell recording of currents in Neuro2a cells expressing K-Kv1.1 in the absence (a) or after the addition of 5 nM A-HgTx (b). Dependence of inhibition of K-Kv1.1 current on the concentration of A-HgTx at +50 mV (c).

4. Fluorescently labeled peptide toxins were used for Kv1 channel binding

5. Analytical binding procedure was developed to measure K_d values for peptide blockers

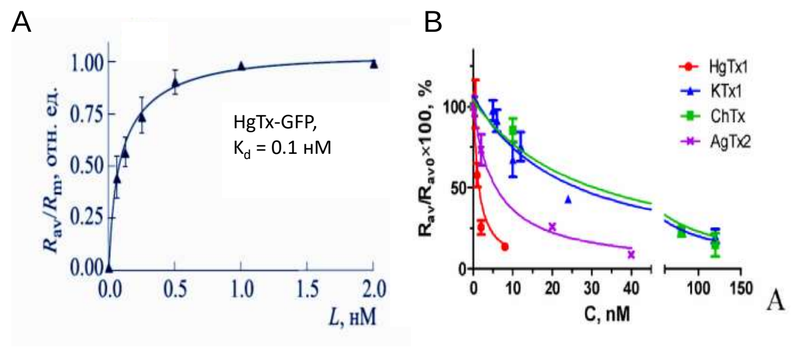


Fig. 3. Measuring of the K_d values for HgTx-GFP (A) and unlabeled peptides (B) using HgTx-GFP as a probe

6. Using ACS, it was found that Ce1 and Ce4 peptides from *Centruroides sp.* scorpion are high-affinity (K_d of 10 and 30 pM) and selective blockers of the Kv1.2 channel

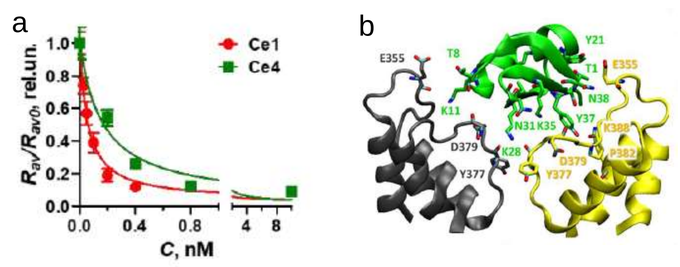


Fig. 4. Measuring of the K_d values of Ce1 and Ce4 for the Kv1.2 channel (a) and structural model of Kv1.2-Ce1 complex

Using confocal microscopy and electrophysiological studies, it was shown that fluorescently labeled Kv1 channels and peptide toxins retained the functional activity of the native counterparts. The values of K_d obtained for the known peptide toxins correlated well with the published data. The developed ACS helped to detect high affinity and selective for the Kv1.2 channel peptides Ce1 and Ce4 that are perspective tools to study functional activity of the target Kv1.2 channel.

CONCLUSION/FUTURE WORK

The proposed concept of ACS is universal and can be applied to various potassium channels. Peptides Ce1 and Ce4 can be useful in the studies of Kv1.2-mediated currents in neurons, and can also be used to construct fluorescent ligands selective for Kv1.2.

FUNDING/REFERENCES

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