Development of Stable Cell Lines for Fluorescent Dye-based High-throughput Screening of T-type Calcium Channel Blockers

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T-type Ca^{2+} channels (T-channels) play a critical role in various physiological functions such as neuronal excitability, olfaction, vision, pain reception, fertilization, cardiac pacemaking, and hormone secretion[1]. Furthermore, T-channels are also associated with pathogenesis of absence epilepsy, neuropathic pain, cardiac arrythmia, cancer, diabetes, etc. Although three isoforms (α 1G, α 1H, and α 1I) of T-channels have been molecularly defined, isoform-selective blockers have not been developed yet. Accordingly, T-channels became significant targets of the drug discovery process. For high-throughput screening (HTS) of drugs, development of an efficient T-channel assay system is critical. In the present study, thus, we were generated stable cell lines co-expressing α 1G, α 1H or α 1I and Kir2.1, an inwardly rectifying K^+ channel (IRK), and evaluated whether they fit fluorescent dye-based T-channel assay. In three cell lines of α 1G-Kir2.1, α 1H-Kir2.1, and α 1I-Kir2.1, heterologous expression of Kir2.1 conferred high resting membrane potential to the HEK 293 cells expressing T-channel isoforms, which might increase the number of T-channels availability at resting state. Application of Ba^{2+} (100 μM), an IRK antagonist, evoked Ca^{2+} spikes by depolarizing the stable cell lines and selectively negated by pretreatment of mibefradil (10 μM). Likewise, bath perfusion of a high K^+ (60 mM)-containing external solution evoked fluorescent Ca^{2+} signals when measured using fura-2/AM in the stable cell lines. The high K^+-induced Ca^{2+} signals were negated in the presence of mibefradil, as known to be a specific T-channel blocker. And also,

biophysical properties and pharmacological properties of T-channel isoforms were little affected when expressed alone or co-expressed with Kir2.1 in HEK 293 cells. Taken together, these data suggest that the stable cell lines co-expressing T-channel isoform and Kir2.1 may be suitable for the non-electrophysiological HTS of T-channel blocker candidates.

References

1. Perez-Reyes E. Molecular physiology of low-voltage-activated T-type calcium channels. 2003. Physiol Rev, 83, 117-161.