Development of Fluorescent Dye-based High-throughput System for Screening of T-type Calcium Channel Blockers

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T-type Ca^{2+} channels (T-channels) are enormously important for not only regulation of neuronal excitability[1)], cardiac pacemaker activity, hormone secretion, and gene expression[2)], but also pathophysiological conditions such as absence epilepsy[3)], tremor, tinnitus, neuropsychiatric disorders, and neuropathic pain. Accordingly, T-channels provide important targets for developing novel therapeutical drugs of T-channel-related disorders. Conventional electrophysiological technique has limitation of its slow process for screening T-channel blockers. Thus, high-throughput system is required for faster and efficient screening. In the present study, we developed the high-throughput system for screening T-channel blocker candidates. We firstly developed suitable cell line for activation of T-channels through introduction of inward rectifying K^+ channels (Kir 2.1) in HEK 293 cells stably expressed T-channel isoform (α1H), because of HEK 293 cells have low resting membrane potential (RMP; -26+4 mV, n=6). Thus, we established four clones such as #5, #8, #10, and #30 which had high RMPs of -61±4 (n=3), -63±4 (n=3), -66±5 (n=3), -69±5 (n=3) mV and high T-current density of 25±5 (n=5), 26±4 (n=3), 50±14 (n=4), 19±8 (n=7) pA/pF, respectively. In these cell lines, high K^+-induced depolarization evoked fluorescent Ca^{2+} signals via T-channels, which could be completely blocked by mibefradil, a known T-channel blocker. These Ca^{2+} signals were slightly decreased when the bath temperature was increased to 33°C. Developed high-throughput system could be applicable to screening of T-channel blockers using Flurometric Imaging Plate Reader (FLIPR) system.

References

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