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Regulation of G-protein Coupled Inwardly Rectifying K^+ Channel Expressed in HEK 293 Cell by Phosphorylation

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Acetylcholine-activated K^+ (K_{ACh}) channels has been introduced as a typical G protein (G_K)-coupled inwardly rectifying K^+ (GIRK) channel, which constructs with four subunit composed of two types of GIRK isoforms, GIRK1 and GIRK4 (or CIR) for the atrial K_{ACh} channel. As a liganded channel, K_{ACh} channel is activated via G_K among the signal pathway including muscarinic (m_2) receptor, G_K and channel proteins for the K_{ACh} channel opening. A recent finding showed that phosphorylation might directly activate K_{ACh} channels via G_K-independent pathway. It remains unclear which site(s) or signal cascade level are responsible for phosphorylation to cause channels to be functional. This study was performed to test whether phosphorylation on the GIRK protein could evoke openings of the K_{ACh} channel. To exclude an involvement of signal cascade unidentified, GIRK channel were expressed by transfection of both plasmid vectors (pcDNA) containing cDNA encoding GIRK1 or CIR simultaneously to human embryo kidney (HEK 293) cells. Although GIRK proteins were successfully expressed on western blotting in HEK 293 cells, single channel activities similar to those in atrial K_{ACh} channels was hard to make record less than 1%. Presence of K^+ channels were screened in HEK cells, prior to this study.

On forming giga-ohm seal, few single channel opening could detect at the cell-attached configuration. Only at inside-out patch configurations, single channels were activated by $G_{\beta\gamma}$ subunit purified from bovine brain, revealing that this channel is liganded by G-protein. Channel conductances were 38.4 pS (2.31 pA at -60 mV) and their mean open time was within 1~2 ms. These properties were similar to those in atrial K_{ACh} channels. This channel activity was also elicited by 1 mM ATP in the absence of $G_{\beta\gamma}$. Channel openings were at a same rate either by 100 nM $G_{\beta\gamma}$ subunit

purified from bovine brain (359 openings/sec) or by 1 mM ATP (319 openings/sec). Intracellular application of mixture ATP and $G_{\beta\gamma}$ increased doubled channel openings (695 openings/sec). Channel activity ($N*P_o$) was also increased from 0.005 to 0.007. This $N*P_o$ was increase according to ATP concentration from 1 mM to 2 mM. However 2 unit of alkaline phosphatase reduced the ATP-induced channel activation to the level of one third (31.3%) of control. These results suggested that the channel could activate by phosphorylation via G_K-independent pathway. Therefore the portion of channel protein out of membrane may be a possible site for phosphorylation responsible to the GIRK channel activation.

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