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PERSPECTIVE

G Protein-Mediated Inhibition of $Ca_V3.2$ T-Type Channels Revisited

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ABSTRACT

T-type calcium channels are important modulators of both membrane potential and intracellular Ca^{2+} concentration, allowing them to play key roles in such diverse processes as aldosterone production from adrenal glomerulosa cells to boosting pain signals in nociceptors. In both these examples, the $Ca_V3.2$ isoform mediates Ca^{2+} influx. This isoform is also of particular interest because mutations in its gene (*CACNA1H*) that enhance channel activity have been associated with idiopathic generalized epilepsies, whereas mutations that disrupt its activity have been associated with autism spectrum disorders. Block of T-channel activity has been proposed to contribute to the therapeutic usefulness of a wide variety of drugs, such as antihypertensives, antipsychotics, and antidepressants. Recent evidence strongly supports the hypothesis that block of $Ca_V3.2$ channels might be useful in the treatment of neuropathic pain. Therefore, it is of particular interest that $Ca_V3.2$ channels are exquisitely regulated by G protein-coupled receptors and various downstream effectors. This *Perspective* summarizes recent findings (p. 202) on this regulation and the novel pathways specifically activated by either neurokinin I, corticotropin-releasing factor receptor 1, or dopamine D_1 receptors.

Seminal studies on the mechanism of action of antihypertensive agents identified voltage-gated Ca^{2+} channels as key players in smooth muscle contraction (Godfraind et al., 1986). We now call the target of these "calcium channel blockers" L-type Ca^{2+} channels or, specifically, $Ca_V1.2$ channels, which represent just 1 of 10 distinct Ca^{2+} channels as named by their pore-forming $\alpha 1$ subunits. Although many drugs are capable of blocking T-type channels [e.g., mibefradil and efonidipine (Perez-Reyes et al., 2009)], selective antagonists are just being developed (Uebele et al., 2009). It is somewhat surprising that these T-type antagonists were able to prevent weight gain on a high-fat diet, implicating T-channels in the regulation of lipid metabolism. Considerably more evidence suggests that T-type Ca^{2+} channels formed by $Ca_V3.2$ subunits are therapeutic targets for the treatment of idiopathic generalized epilepsies and neuropathic pain (McGivern, 2006). Therefore, inhibition by G protein-coupled receptors (GPCR) provides another therapeutic strategy, much as N-type channel inhibition contributes to morphine analgesia. Early studies of hormonal regulation of native T-currents led to conflicting results, some groups reporting stimulation; others, inhibition; and others, no effect. Studies on recombinant channels have partially resolved this conundrum, revealing that regulation is isoform-specific and pathway-dependent (for review, see Huc et al., 2009; Iftinca and Zamponi, 2009). The most highly regulated T-channel isoform to date is $Ca_V3.2$. In contrast to its brothers, $Ca_V3.1$ and 3.3, it can be regulated by GPCRs via direct action of G protein $\beta_2\gamma_2$ subunits (Wolfe et al., 2003; DePuy et al., 2006) or via unknown mechanisms that do not involve $\beta\gamma$ subunits or canonical protein kinase pathways (Tao et al., 2008) (Fig. 1).

In this issue of *Molecular Pharmacology*, Rangel et al. (2010) describe a new mechanism whereby GPCRs can modulate $Ca_V3.2$ channels. These authors report that activation of neurokinin 1 (NK1) receptors leads to reversible inhibition

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ABBREVIATIONS: GPCR, G protein-coupled receptor; NK1, neurokinin 1; HEK, human embryonic kidney; PLCβ, phospholipase C β; PKC, protein kinase C; CRF-1, corticotropin-releasing factor receptor 1; U73122, 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione.

of recombinant human $Ca_V3.2$ channels transiently expressed in human embryonic kidney (HEK) 293 cells. This finding is of general interest because $Ca_V3.2$ and NK1 receptors, which are activated by the tachykinin peptides substance P and neurokinin A, are both expressed in dorsal root ganglion sensory neurons, and a functional regulation of $Ca_v3.2$ may be an important tool in treating neuropathic pain. Rangel et al. (2010) used a combination of pharmacological and molecular approaches to characterize intracellular signaling involved in this NK1 receptor-mediated inhibition. They were were able to establish that inhibition was mediated by a voltage-independent process via the sequential activation of $G\alpha_{q/11}$ subunits, phospholipase C β (PLCβ), then protein kinase C (PKC). Inhibition was occluded by coexpression with either a dominant-negative form of $G\alpha_q$ or regulators of G protein signaling proteins RGS2 and RGS3T.

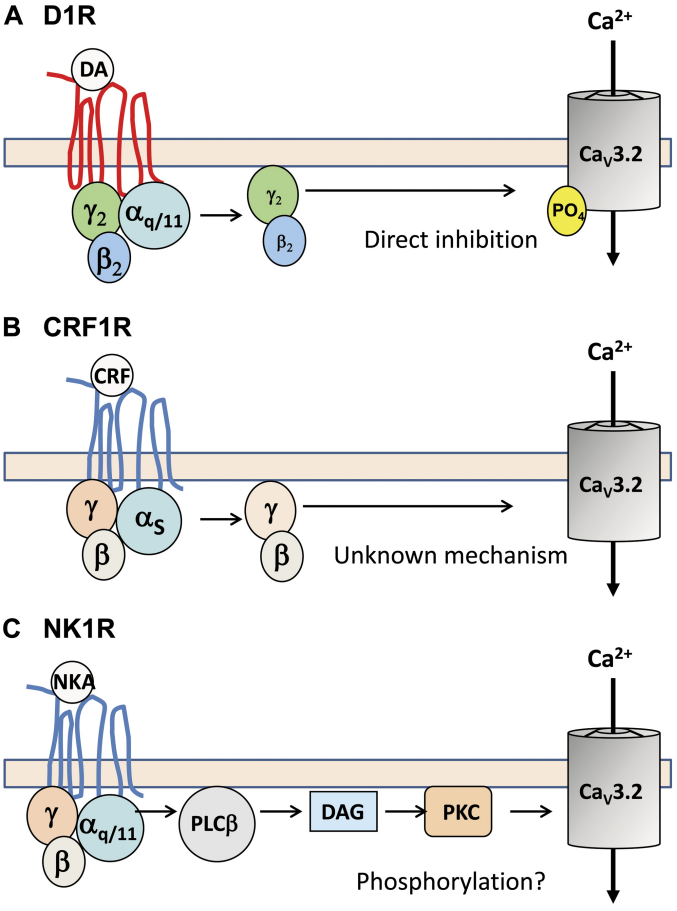

Fig. 1. Three distinct pathways activated by GPCRs that all lead to inhibition of $Ca_v3.2$ channel activity. A, pathway described by the Barrett lab, which indicates that D1 dopamine receptors inhibit channel activity via a direct action of $G\beta_2\gamma_2$ (Wolfe et al., 2003; DePuy et al., 2006; Hu et al., 2009). Inhibition is due to a reduction in the probability of channel opening with no effect on the voltage-dependence of either activation or inactivation. Mutation of serine 1107 to alanine disrupts the ability of $G\beta_2\gamma_2$-mediated inhibition. This pathway may account for the regulation of T-currents observed in adrenal glomerulosa cells (Drolet et al., 1997). B, pathway described by the Soong lab, which indicates CRF-1 receptors act via G_s to inhibit channel activity by a pathway that does not involve any protein kinase (Tao et al., 2008). Inhibition results in a shift in the voltage dependence of inactivation. C, pathway described by the Meza lab, which indicates that neurokinin-1 receptors inhibit channel activity via a pathway that is independent of $G\beta\gamma$, but requires PKC (Rangel et al., 2010). Inhibition occurs with no change in the voltage dependence of channel gating. This pathway may account for the PKC regulation of T-currents observed in DRG neurons (Schroeder et al., 1990).

Inhibition was also occluded by bath application of inhibitors of PLCβ (U73122), and PKC (bisindolylmaleimide I). These results differ with those reported by Wolfe et al. (2003), who demonstrated that inhibition of $Ca_V3.2$ channels by dopamine D_1 receptors (another $G_{q/11}$ protein-coupled receptor) expressed in the adrenocarcinomal cell line H295R was mediated by direct interaction of $G\beta_2\gamma_2$ subunits with the α subunit of $Ca_v3.2$. It is important to note that this form of regulation was specific to the $G\beta_2$ subunit. Thus, a possible explanation for the observed differences in the signaling by these two $G\alpha_{q/11}$ protein-coupled receptors could be attributed to the low endogenous level of $G\beta_2\gamma_2$ dimer expression in HEK-293 cells. Recent studies from the Barrett lab indicate that $Ca_v3.2$ must be phosphorylated by protein kinase A to be responsive to $G\beta_2\gamma_2$ inhibition (Hu et al., 2009). This leads to a scenario in which differences in basal phosphorylation states can alter hormone-mediated inhibition of $Ca_v3.2$ and adds yet another possible explanation for the variability observed in its regulation.

The results of Rangel et al. (2010) also differ from those reported by Tao et al. (2008), who reported that corticotropin-releasing factor receptor 1 (CRF-1) also specifically inhibits recombinant $Ca_v3.2$ channels in HEK-293 cells by a pathway that involves neither $G\beta\gamma$ nor PKCβ. In their study, CRF-1-mediated inhibition was not affected by coexpression with RGS2 and was not affected by blockers of PLC, tyrosine kinases, Ca^{2+}/calmodulin-dependent protein kinase II, or PKC. Surprisingly, pretreatment of HEK-293 cells with cholera toxin abolished regulation, implicating $G\alpha_S$ in the pathway. The role of $G\beta\gamma$ subunits was implicated by the sponge technique, in which overexpression of a protein that binds $G\beta\gamma$ with high affinity [in this case, MAS-GRK, a membrane-associating C-terminal construct of the GPCR receptor kinase 3 (Kammermeier et al., 2000)] is able to outcompete the channel and block inhibition.

These studies highlight the complexity of $Ca_v3.2$ regulation, which partially accounts for the disparate findings of native T-current regulation and, more recently, with recombinant channels. In addition to differences in experimental conditions, these findings suggest that the choice of GPCR activated may also determine the form of regulation observed. Therefore, it will be of great interest when these findings on recombinant channels can be confirmed in native cells where the appropriate combination of GPCR, G protein subunits, and $Ca_v3.2$ isoform are expressed. It will also be important to test the effect of $Ca_v3.2$ splice variation on regulation (Zhong et al., 2006), because mutations that alter $Ca_v3.2$ splicing contribute to the absence of epilepsy phenotypes (Powell et al., 2009).

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