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Novel Probes for G-Protein-Coupled Receptor Signaling

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Review of Robbins et al. (<http://www.jneurosci.org/cgi/content/full/26/30/7950>)

G-protein-coupled receptors (GPCRs) constitute the largest known protein family. They are activated by a wide range of ligands to transduce an extracellular signal into an intracellular one that evokes a variety of physiological responses, including regulation of ion channels by neurotransmitters and hormones. One such example is modulation of M-type potassium current by $G_{q/11}$ G-proteins. This plays an important role in regulating neuronal excitability. M current is encoded by Kv7.2 (KCNQ2) and Kv7.3 (KCNQ3) channel subunits. Multiple mutations in these subunits cause neuronal hyperexcitability and underlie a mild epileptic syndrome, benign familial neonatal convulsions. The membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) is the principal determinant of M current activity, and its cleavage on activation of $G_{q/11}$ G-proteins underlies channel closure and slow membrane depolarization (Fig. 1). Many ion channels are modulated by PIP_2 , including voltage-gated calcium channels, inward rectifier potassium channels, and some transient receptor potential ion channels (Suh and Hille, 2005). Given our increasing understanding of the role of G-proteins and PIP_2 in cellular signaling, it is important to develop tools to examine GPCR–G-protein and PIP_2 –channel interactions.

Several approaches have been used to study GPCR-mediated pathways. The more successful of these involve selectively attenuating the function of candidate signaling molecules and determining how this affects the end result of GPCR activation. One such technique is the use of anti-G-protein antibodies. The antibodies inhibit GPCR signaling (Caulfield et al., 1994) but can lack specificity, are slow acting and irreversible, and are difficult to use in intact cells. Another approach has been to use dominant-negative proteins or RNA interference to disrupt protein function or expression. The irreversibility of these methods and the time required for expression precludes their use in many experiments. The ideal technique will be highly specific, fast, and reversible, and act in an intact cell. Membrane-targeted molecular interference tools, such as specially designed peptides, come closer to meeting these criteria than several existing techniques. Covic et al. (2002) designed palmitoylated peptides, termed “pepducins,” that were directed against the third intracellular loop (one putative G-protein binding region) of GPCRs. These pepducins penetrated the cell membrane and activated G-proteins in the manner of the GPCRs they were based on.

The recent *Journal of Neuroscience* paper by Robbins et al. (2006) introduced novel applications of this palmitoylated peptide approach. Their technique, modeled after the pepducins designed by Covic et al. (2002), used palmitoyl-tagged peptides (10-mer “palpeptides”) that

mimicked the binding site for the target molecule and were cell permeant. This method allowed the researchers to prevent activation of specific G-proteins or to sequester PIP_2 .

The $G_{q/11}$ palpeptide, which contained the receptor-binding region from the C terminus of $G\alpha_q$, effectively blocked coupling between the M_1 muscarinic acetylcholine receptor (M_1R) and downstream inhibition of both M current [Robbins et al., their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F1>)] and calcium current. $G_{q/11}$ palpeptide action was dose dependent, fast, and G-protein subtype specific, because it did not interfere with calcium channel inhibition via G_o -coupled adrenergic receptors [Robbins et al., their Fig. 2 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F2>)]. Likewise, the G_o palpeptide selectively blocked norepinephrine (NE)-induced calcium current inhibition, but not inhibition by the muscarinic agonist oxotremorine-M (Oxo-M) [Robbins et al., their Fig. 3 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F3>)] acting via M_1R . However, both G-protein palpeptides appear to have enhanced calcium channel inhibition nonspecifically. A scrambled version of the G_o palpeptide substantially augmented NE-evoked inhibition relative to palpeptide-free controls [Robbins et al., their Figs. 2B (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F2>) and 3A (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F3>)]. The authors showed that palmitoyl tethering prevented a *Shaker* inactivation ball from blocking the pore of a

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Kv1.2 channel [Robbins et al., their Fig. 11 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F11>)] but did not rule out the possibility of $G\alpha$ palpeptide occlusion of calcium channels.

The second set of palpeptides was directed against PIP₂, using a region of the Kv7.2 C terminus shown to be important in regulating channel affinity for PIP₂. PIP₂ palpeptide inhibition of M current was dose dependent, rapid, M₁R independent, and specific because it only inhibited PIP₂-independent potassium currents to a small extent. It was only slightly reversible on washout [Robbins et al., their Fig. 5 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F5>)]. The PIP₂ palpeptide acted in a manner consistent with a reduction in the available pool of PIP₂, because its inhibitory action on M current was rescued by addition of the PIP₂ analog, diC₈PIP₂ [Robbins et al., their Fig. 8 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F8>)]. Furthermore, the PIP₂ palpeptide enhanced M₁R suppression of M current [Robbins et al., their Fig. 9 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F9>)]. Importantly, the authors found a loose relationship between the structure and function of PIP₂ palpeptides; both a scrambled palpeptide and a palpeptide formed exclusively of lysine residues had higher affinities for PIP₂ than the potential PIP₂ binding sequence from Kv7.2 [Robbins et al., their Table 1 (<http://www.jneurosci.org/cgi/content/full/26/30/7950/T1>)]. This suggests either the absence of a highly conserved PIP₂ binding domain, or the inability of the palpeptides to recreate the binding motif of Kv7.2. Sequence and functional analysis from many channels supports the former; electrostatic attraction to basic residues may constitute the mechanism of PIP₂ binding. Palpeptides

Figure 1. Schematic illustration of muscarinic modulation of M current. Activation of the M₁ muscarinic receptor couples through $G\alpha_q$ and phospholipase C β (PLC β) to catalyze the degradation of the membrane phospholipid PIP₂ with concomitant inhibition of M-type potassium current. ACh, Acetylcholine; IP₃, inositol 1,4,5-trisphosphate; DAG, diacylglycerol.

based on other channel domains could help to further characterize this mechanism.

Robbins and colleagues motivated their experiments by suggesting that membrane-targeted peptide probes could be used to delineate multiple contributions to regulation of M current. Their results suggest that PIP₂ depletion accounts for >95% of M current inhibition [Robbins et al., their Fig. 5B,D (<http://www.jneurosci.org/cgi/content/full/26/30/7950/F5>)], making the maximal effect of PIP₂ palpeptides virtually indistinguishable from that of saturating concentrations of Oxo-M. However, because PIP₂ palpeptides may interfere with hydrolysis of bound PIP₂, both release of calcium stores and activation of protein kinase C could be attenuated in their presence. Consequently, it remains difficult to rule out additional roles of other $G_{q/11}$ effectors such as calcium/calmodulin or protein kinase C.

Despite these caveats, Robbins et al. (2006) have succeeded in developing a

useful and exciting new tool to add to the molecular repertoire for studying GPCR signaling and its many manifestations. This technique may also prove to be useful in the design of new therapeutics that can specifically and rapidly target components of GPCR signaling pathways.

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