SENSORY NEURONS

A new target for G protein signaling

G protein-coupled receptor stimulation inhibits TRPM3 channel activity through direct binding of the Gβγ subunit to the channel.

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Related research article
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Many of the cells in our body communicate by releasing small molecules that bind to receptors on the surface of target cells. These molecules include hormones and, in the case of nerve cells, neurotransmitters. Signal transduction pathways then relay the information from the receptor to inside the cell and either activate or inhibit ‘effector’ proteins that cause the cells to respond appropriately.

Heterotrimeric G proteins – protein complexes that consist of three different subunits named α, β and γ – provide one such pathway, and work with cell surface receptors called G protein-coupled receptors (GPCRs; Figure 1A).

The structure and mechanism of heterotrimeric G proteins has been studied at atomic resolution (Oldham and Hamm, 2008). In the resting state, the α subunit (Gα) binds to a molecule called GDP and is tightly associated with the β and γ subunits, forming a heterotrimer. When the complex interacts with an activated GPCR, the molecule of GDP is exchanged for GTP, and the G protein complex dissociates into two parts: Gα-GTP and a stable Gβγ dimer. Both Gα and Gβγ contain ‘anchors’ that keep them attached to the cell membrane, but allow them to diffuse laterally along the membrane to find their target effector proteins. Eventually, Gα breaks down the GTP to form GDP, and Gα-GDP associates with Gβγ to reform the heterotrimer.

In the ‘conventional’ mode of signal transduction (Gilman, 1987) Gα-GTP activates or inhibits a target enzyme, depending on which class of α subunit is involved (Figure 1B). For example, an inhibitory α subunit (Gαi) inhibits the enzyme that produces a chemical messenger called cyclic AMP (or cAMP). Heterotrimeric G proteins may also regulate ion channels within the membrane via a different pathway. For instance, the GIRK channels (which are responsible for slowing the heart rate) are activated by Gβγ directly binding to them (Figure 1C; Logothetis et al., 1987).

Now, in eLife, three groups featuring researchers based at institutes in Germany, the
UK, the US and Canada independently present evidence of a new target for GPCR signaling: an ion channel called Transient Receptor Potential Melastatin 3 (TRPM3; Badheka et al., 2017; Quallo et al., 2017; Dembla et al., 2017). Expressed abundantly in sensory neurons, TRPM3 channels help organisms to sense heat and make them more sensitive to pain during inflammation (Vriens et al., 2011). The new reports suggest that the activation of TRPM3 channels is greatly reduced if the channels are also stimulated by any of a variety of GPCRs. The three studies systematically probed individual steps of the G protein regulatory pathway to dissect its mechanism. First, each of the groups independently show that the inhibition of TRPM3 can be overcome by pre-treatment with pertussis toxin. This toxin prevents the inhibitory G\textsubscript{ai} subunit from interacting with the activated GPCR, locking the complex in the resting trimeric state (Figure 1A). Thus, TRPM3 inhibition requires the heterotrimeric G protein to dissociate. But is the inhibiting signal carried through G\textsubscript{ai}-G\textsubscript{TTP} or through G\textsubscript{bg}?

The studies accumulate strong evidence showing that the G\textsubscript{ai} subunit is not involved. Johannes Oberwinkler of Philipps-Universität Marburg and colleagues (including Sandeep Dembla and Marc Behrendt as joint first authors) did not detect any interaction between G\textsubscript{ai} and TRPM3. Moreover, they and Tibor Rohacs of New Jersey Medical School and co-workers – who include Doreen Badheka and Yevgen Yudin as joint first authors – show that wild-type inhibitory G\textsubscript{ai} subunits do not decrease the activity of TRPM3, and neither can mutant subunits that...
cannot break down GTP and are therefore permanently active. Furthermore, Talissa Quallo and colleagues at King’s College London show that GPCR-mediated TRPM3 inhibition is unaffected by an inhibitor that selectively acts upon the inhibitory G$i$ subunit. Dembla et al. and Badheka et al. also show that altering the concentration of the chemical messenger cAMP (which is decreased by the activity of inhibitory G$i$ subunits) has no effect on either the activity or inhibition of TRPM3.

On the other hand, all evidence points to a role for G$a$ in signaling to TRPM3. Badheka et al. and Dembla et al. both show that TRPM3 activity is strongly inhibited by the overexpression of G$a$, whereas the overexpression of engineered proteins that bind to G$a$ (and so prevent it from interacting with TRPM3) eliminates GPCR-mediated TRPM3 inhibition. Co-immunoprecipitation experiments demonstrate a direct interaction between G$a$ and TRPM3. Finally, Badheka et al. show that the flow of ions through TRPM3 channels is strongly and reversibly inhibited when the cell membrane is flushed with purified G$a$, but not with G$i$ or boiled G$a$. These elegant studies thus reveal that G$a$ inhibits TRPM3 by directly binding to the channel (Figure 1D).

Interesting mechanistic questions remain. Not all G$a$ subunits are inhibitory and it is unclear whether G$a$-mediated TRPM3 regulation also occurs with heterotrimers containing other G$a$ subunits. Like GIRK channels, TRPM3 channels require a molecule called PIP$_2$ in the membrane in order to open (Badheka et al., 2015; Tóth et al., 2015). Because some other G$a$ subunits (arbitrarily named G$_a$ subunits) lead to the localized depletion of PIP$_2$ (Figure 1B), heterotrimers containing these subunits do not activate GIRK (Wang et al., 2014; Figure 1C). However, they should enhance inhibition of TRPM3 through GPCRs. Indeed, artificially expressing TRPM3 in human embryonic kidney cells enabled Badheka et al. to show that TRPM3 is readily inhibited through co-expressed G$_{a}$-linked receptors even when the concentration of PIP$_2$ inside cells is buffered. This demonstrates that G$a$ released from G$_{a}$-containing heterotrimers can also inhibit TRPM3. However it remains to be established whether G$_{a}$-linked GPCRs (or any other GPCRs for that matter) contribute to this process in any native cell.

Finally, in vivo experiments by all three groups highlight the practical relevance of the uncovered pathway for pain signaling in the peripheral nervous system: molecules that bind to and activate two types of GPCRs – the GABA-B and $\mu$ opioid receptors – significantly reduce TRPM3-dependent pain. Of note, the strongest peripheral painkillers currently available activate the $\mu$ opioid receptor, but cause severe adverse effects in the brain such as addiction, tolerance or respiratory depression. The new findings suggest that peripheral pain might be better treated by drugs that inhibit TRPM3 directly.

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