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RESEARCH NEWS

A TREK inhibitor takes multiple tracks

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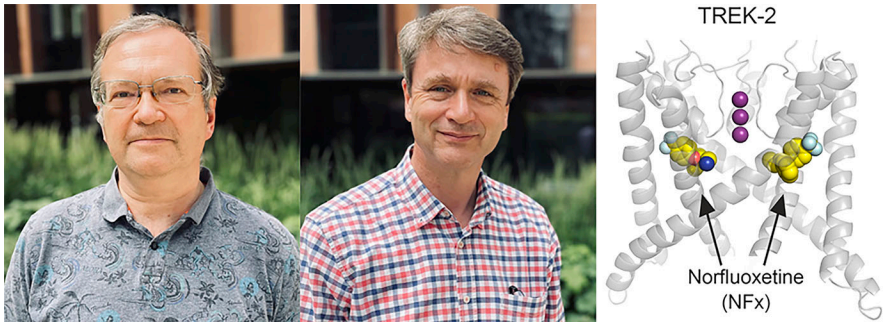
Single-channel recordings reveal that norfluoxetine inhibits the two-pore domain K^+ channel TREK-2 by a complex array of mechanisms.

The TREK subfamily of two-pore domain K^+ channels are expressed throughout the central and peripheral nervous systems and are involved in a diverse range of processes such as mechanosensation, thermosensation, and nociception. Accordingly, channel gating—which is thought to involve changes in the selectivity filter of TREKs—can be regulated by a wide variety of factors, including pressure, temperature, and multiple endogenous ligands (1). In this issue of *JGP*, Proks et al. reveal that this regulatory complexity is reflected in the fact that the TREK inhibitor norfluoxetine impairs channel activity via several different mechanisms (2).



Peter Proks (left), Stephen J. Tucker (center), and colleagues use single-channel recordings to investigate how norfluoxetine inhibits the two-pore domain K^+ channel TREK-2. Norfluoxetine binds exclusively to the "down" conformation of TREK-2 (right) and prevents the channel's transmembrane domains from transitioning to the "up" configuration. But Proks et al. find that TREK-2 can be fully active in the down conformation and that norfluoxetine works via multiple mechanisms to inhibit both the open and closed states of the channel.

Norfluoxetine is a metabolite of fluoxetine (Prozac), and both compounds are among the few known inhibitors of TREK activity (3). "TREK channels are not the principal targets of fluoxetine, which is mainly a selective serotonin reuptake inhibitor," explains Stephen J. Tucker from the University of Oxford. "But fluoxetine and norfluoxetine are useful tools to study the mechanisms of TREK channel gating."

Tucker and colleagues previously helped solve the crystal structures of TREK-2 in the presence and absence of norfluoxetine (4). The channel can adopt two distinct conformations, named "up" or "down" depending on the orientation of its transmembrane helices, and norfluoxetine was found to bind within the inner cavity of TREK-2 in a gap that is only formed when the transmembrane helices are in the down configuration. Norfluoxetine can therefore block the transition from the down to up conformation, and it was originally suggested that this might inhibit channel activity by locking the selectivity filter in its closed state. But the mechanism of filter gating appears to be more complex. Tucker's group, for example, has previously shown using macroscopic recordings that TREK-2 can adopt several open states, some of which may occur in the down conformation (5).

To learn more about the mechanisms underlying filter gating and norfluoxetine inhibition, Tucker and colleagues, including first author Peter Proks, turned to single-channel recordings of purified TREK-2 channels embedded in lipid bilayers (2). "We found that norfluoxetine affects both the open and closed states of the channel and is therefore a state-independent inhibitor of TREK-2," Tucker says. "That information is lost in macroscopic recordings."

Moreover, the fact that highly active channels are sensitive to norfluoxetine inhibition confirms that TREK channels can be fully open in the down conformation. It also indicates that, in addition to blocking changes in transmembrane conformation, norfluoxetine must inhibit TREK channels by other mechanisms as well.

"We found that there are several mechanisms involved, all of which converge on the selectivity filter gate," Tucker says. The researchers also observed a mild voltage dependence of norfluoxetine inhibition, suggesting that it can influence voltage-dependent gating as well.

"The complexity with which the drug works reflects the many different ways in which the selectivity filter can gate the channel," Tucker says. "This, in turn, reflects the polymodal regulation of TREK channels and their ability to integrate a wide variety of signals."

References

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