

## TRPV4 in the battle of the sexes

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New JGP paper explains sexual dimorphism and tissue-specific activity of TRPV4.

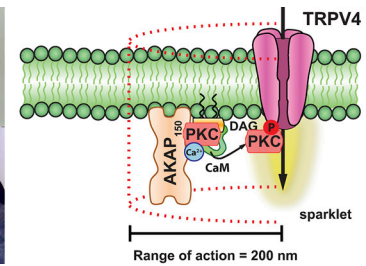
The broadly expressed transient receptor potential vanilloid 4 (TRPV4) calcium channel allows an enormous amount of  $\text{Ca}^{2+}$  into the cell (up to 100-fold greater  $\text{Ca}^{2+}$  flux than those produced by voltage-gated  $\text{Ca}_v1.2$   $\text{Ca}^{2+}$  channels; 1). In vascular smooth muscle, TRPV4 channels help regulate vascular tone (2, 3). In this month's JGP, Tajada et al. provide surprising new insights about how TRPV4 activity is regulated (4).

Prior work has shown that binding of the vasoconstrictor angiotensin II (AngII) to  $\text{G}_q$  protein-coupled receptors stimulates recruitment of protein kinase C  $\alpha$  (PKC $\alpha$ ) to the membrane adaptor protein AKAP150 (1, 5, 6). PKC $\alpha$  then phosphorylates TRPV4, causing the channel to open. Fernando Santana's laboratory, at the University of California Davis, has also conducted super-resolution imaging showing that both AKAP150 and TRPV4 form puncta in the cell membranes of arterial myocytes. These puncta draw closer together when cells are stimulated with AngII, suggesting that AKAP150-TRPV4 interactions may be dynamically regulated (1).

Postdoc Sendoa Tajada led the Santana laboratory's efforts to extend these findings by studying TRPV4 currents in vascular smooth muscle cells isolated from mouse arteries. But, as Santana explains, the endeavor soon hit a snag.

"As do many other labs, we used to work mostly with male mice. But a couple of years ago, we ran out of males and started using female mice, assuming there would be no difference in TRPV4 channel function between male and female myocytes. For a while, we were getting disparate results. Eventually, we figured out what was going on, and when we segregated the data between male and female, things started making sense," recalls Santana.

The extent of sexual dimorphism in TRPV4 activity was striking. Electrophysiological recordings indicated that both basal and AngII-stimulated TRPV4 currents in the cerebral (pial) arteries of male



First author Sendoa Tajada (left), senior author L. Fernando Santana (right), and colleagues show how the distance between AKAP150 and TRPV4 in membranes limits channel activation by PKC $\alpha$ , giving rise to channel behaviors that vary according to anatomical site and sex. PHOTOS COURTESY OF THE AUTHORS.

mice were larger than those in females. Consistent with this, agonist-stimulated TRPV4 activity, gauged using calcium imaging to detect the frequency of localized  $\text{Ca}^{2+}$  increases termed "sparklets," was greater in male than female pial arteries.

Interestingly, the researchers also noticed that, in both sexes, TRPV4 activity varied according to the arterial bed from which a cell was isolated. Thus, currents were greatest in smooth muscle cells from pial arteries, intermediate in parenchymal myocytes, but undetectable in mesenteric myocytes. Yet, each of these tissues expressed all the protein components necessary for TRPV4 activation.

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Because their earlier work indicated that interactions between the pathway's protein components are dynamic, the researchers hypothesized that the distance between AKAP150 puncta and TRPV4 clusters may differ according to sex or tissue. Indeed, super-resolution microscopy demonstrated a clear hierarchy, with myocytes from male pial arteries showing the smallest gaps and female mesenteric myocytes showing the greatest. Correlation of these data with measurements of TRPV4 currents and sparklets indicated that TRPV4 activity is undetectable when the distance between AKAP150 and TRPV4 puncta exceeds 200 nm.

What sets this outer limit? TRPV4 currents can be observed in female mesenteric myocytes infused with activated PKC $\alpha$ , indicating that PKC $\alpha$  activity is the limiting factor in TRPV4 activation. Santana proposes that once activated PKC $\alpha$  detaches from AKAP150, it can only explore a certain distance before it becomes inactivated and unable to phosphorylate TRPV4.

"Knowing the two-dimensional diffusion coefficient for PKC $\alpha$  and that 200 nm is the cutoff for activity, we could back-calculate the time that the protein remains active," notes Santana. Doing this, Tajada et al. estimated PKC $\alpha$  is active for about 8 ms.

Computer modeling incorporating this information, and the AKAP150-TRPV4 distance measurements gleaned from the super-resolution imaging, faithfully reproduced both sexual dimorphic and tissue origin differences in TRPV4 activity. For Santana, this strongly supported the idea that differences in protein positioning drive distinct outcomes in TRPV4 activity. Next, he's interested in probing how these differences translate to larger phenotypic outcomes, such as sexual dimorphism in blood flow regulation or in AngII sensitivity.

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