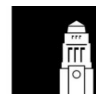

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Peers, C, Elies, J and Gamper, N (2015) Novel ways to regulate T-type Ca2+ channels. Channels, 9 (2). pp. 68-69. ISSN 1933-6950

https://doi.org/10.1080/19336950.2015.1017995

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Novel ways to regulate T-type Ca^{2+} channels

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Key words: T-type Ca^{2+} channel; H_2S; carbon monoxide; gasotransmitter; Zn^{2+} affinity; nociception

Main text:

T-type (Ca_V3) Ca^{2+} channels are distinguished from other voltage-gated Ca^{2+} channels by their rapid activation and inactivation, slow deactivation, smaller single channel conductances and very negative activation threshold (as low as -60mV). Ca_V3 are encoded by *CACNA1G, CACNA1H* and *CACNA1I* genes which give rise to pore-forming α subunits ($Ca_V3.1$-3.3 respectively). These channels serve surprisingly diverse roles; in central neurons they are responsible for pacemaker activity and low threshold spikes, contributing to "rebound" bursts of spikes following a hyperpolarizing postsynaptic potential. They also display a significant window current (i.e. are tonically active) at potentials close to resting membrane potential (RMP), so can contribute to setting the RMP. In the peripheral nervous system, Ca_V3 are expressed in several types of sensory neurons, including a subpopulation of small, capsaicin-sensitive (presumed nociceptive) DRG neurons where they influence excitability and so play an important role in nociception[6,8]. Also due to their window currents, $Ca_V3.1$ and 3.2 strongly influence cell proliferation, as has been studied in vascular smooth muscle and different types of cancers (see[2]).

In sensory neurons CaV3.2 is the dominant T-type channel form and may even be the exclusive form in some mechanosensitive neurons. Their control of burst firing in DRG neurons indicates they are of central importance to nociception since stimulus intensity correlates with burst frequency[8]. Native nociceptive and recombinant CaV3 currents are enhanced by reducing agents (which induce hyperalgesia) and inhibited by the oxidising agents. This sensitivity to redox modulation of Cav3.2 is specific amongst the Cav3 isoforms, and arises because of the presence of an extracellular, high affinity binding site for trace metals (Zn^{2+}, Ni^{2+}) formed by interacting regions of the S1-S2 and S3-S4 loops within domain I of the channel protein. Mutation of the histidine residue H191 (in the S3-S4 region; Fig. 1) abolishes high affinity current inhibition by these metals and markedly reduces redox sensitivity[7]. Since this site is clearly important in nociception, it represents an attractive site for therapeutic development.

The increasing awareness of the biological importance of endogenous gases (firstly NO, but more recently CO and H_2S – these are now labelled as "gasotransmitters") has led to a wealth of studies indicating that they are important in diverse physiological functions, and can exert important influences on disease progression. These gases modulate a number of intracellular signalling pathways, and ion channels were amongst the first type of target proteins recognised as mediating some of their effects[4,5]. More recently, we have shown that T-type channels are sensitive to

gasotransmitters: CO blocks all three isoforms of T-type Ca^{2+} channels with similar affinity, although the underlying mechanisms vary significantly[1]. Such modulation appears to contribute to the inhibitory effects of this gas on cell proliferation[2]. By contrast, H_2S is selective in its effects: at low (presumed physiological) levels it selectively inhibits Cav3.2, whilst Cav3.1 and 3.3 are unaffected[3]. Similarly to redox modulation, the effect of H_2S depended on the presence of the metal binding site. Thus, mutation of H191 to glutamine (H191Q) abolished H_2S sensitivity. Furthermore, the analogous reciprocal mutation (Q172H) conferred H_2S sensitivity on Cav3.1.

Since reducing agents augment currents through $Ca_V3.2$ channels by relieving H191-dependent tonic Zn^{2+} inhibition[8], we next explored a potential role for Zn^{2+} in the inhibitory effects of H_2S. Consistent with previous studies[7,8], we found that Zn^{2+} chelation using N,N,N',N'-tetra-2-picolylethylenediamine (TPEN) augmented $Ca_V3.2$ currents, an effect which could be reversed by sub-micromolar levels of Zn^{2+}, importantly suggesting that ambient levels of Zn^{2+} influences current amplitudes. Furthermore, Zn^{2+} chelation with TPEN fully reversed the H_2S inhibition and led to a similar augmentation as was observed following TPEN application in the absence of H_2S. Finally, H_2S was ineffective in the continued presence of TPEN (i.e. in the absence of free extracellular Zn^{2+}). Clearly, these findings point to a fundamental involvement of Zn^{2+} in the inhibitory actions of H_2S and have led us to propose that H_2S inhibition may result from enhancement of the channel sensitivity to ambient Zn^{2+}. Total plasma concentrations of Zn^{2+} in humans range within 5-20 μM with free Zn^{2+} likely to be at sub-micromolar levels; thus enhancing the Cav3.2 sensitivity to Zn^{2+} should result in increase of channel inhibition by physiological levels of this transition metal.

The mechanism by which H_2S may alter the sensitivity or apparent affinity of $Ca_V3.2$ for Zn^{2+} remains to be elucidated. H_2S might interact directly with the extracellular loop residues (including H191) which confer high affinity, or may act intracellularly to modify the channel at a distant but influential site, perhaps via sulfhydration or alternative modifications (see[4]). It is also conceivable that metal binding site of Cav3.2 may serve as a convergence point for several types of signals, thus it was hypothesized that His191 may also bind other transition metals such as coper or iron which, in turn, may promote the metal-catalyzed oxidation (MCO) reaction at this site (see[8]) providing the mechanism for redox modulation. Therefore, H_2S and redox modulation of the channel may be mediated by different metals yet require a common site within the channel protein. Given the important role of CaV3.2 particularly in nociception, it would seem worthwhile to clarify signaling processes mediated by this 'modulatory hub' of Cav3.2.

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Figure Legend

Cartoon of the structure of the T-type Ca^{2+} channel Cav3.2, highlighting the location in the extracellular S3-S4 linker of domain I which confers high redox sensitivity and susceptibility to block by trace metals such as zinc. Our recent work suggests H_2S may inhibit this channel by increasing the apparent affinity of this channel for zinc.

Figure 1