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Two Distinct Modes of Action of TRPM8 Agonists
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TRPM8, a transient receptor potential (TRP) cation channel expressed in sensory neurons, functions both as a cold sensor and as an ionotropic receptor for various natural and synthetic ligands, including menthol, eucalyptol, icilin and mustard oil (AITC). The mechanisms whereby TRPM8 agonists act on the channel are incompletely understood. Here we analyzed in detail the changes in kinetics of TRPM8 gating induced by different ligands, and identified two clearly distinct modes of agonist action. The majority of agonists (type I), including menthol, cause a prominent slowing of the channel relaxation kinetics in response to voltage steps, whereas AITC (type II) causes a clear acceleration. These results can be reproduced using a Monod-Wyman-Changeux model, where each subunit can bind a single ligand. In this model, type I agonists cause an stabilization of the open state, while type II ligands destabilize the closed state. These results indicate that agonists can exert energetically distinct effects on the TRPM8 channel protein, and suggest that ''equipotent'' concentrations of type I and type II agonists may differentially affect electrical activity in sensory neurons.

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Evidence for the Role of Camkinase II and Synapsin I in the Restoration of Neurotransmission in Botulinum Neurotoxin a Intoxicated Nerve Terminals
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At the active zones in the nerve terminals, presynaptic Ca^{2+} is recognized as an important second messenger that regulates neurotransmission. However, the precise role of Ca^{2+} in the regulation of exocytosis still remains to be elusive. Synapsin I (SYN1) is a member of the synapsin family (SYN1/2/3) of synaptic vesicle phosphoproteins that is phosphorylated by Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) at the Serine 603. At the motor nerve terminals (MNT), SYN I regulates synaptic transmission and the depolarization signal mediated rise in Ca^{2+} influx stimulates CaMKII dependent phosphorylation of SYN1. Botulinum neurotoxin A (BoNT/A) selectively proteolyzes SNAP-25 to inhibit acetylcholine (ACh) release and causes life-threatening neuroparalysis. In our efforts to develop therapeutic strategies to counteract BoNT/A at its primary site of action, the MNT, we discovered that mouse MNT express transient receptor potential vanilloid 1 (TRPV1) channel protein and that capsaicin (TRPV1 agonist) antagonized the neuroparalytic effects of BoNT/A. Capsaicin, when injected post BoNT/A exposure, significantly accelerated recovery from neuroparalysis by restoring ACh release and muscle functions. Capsaicin treatment also decreased the total duration of paralysis by 50% and synergistically increased the effects of 3,4 diaminopyridine (blocks K^+ channels and prolongs action potential) on ACh release measured by stimulus evoked twitch tension in BoNT/A poisoned nerve muscle preparations. Further, BoNT/A treatment, in vitro, decreased the expression of CaMKII and SYN1 and the phosphorylation of SYN1 in cholinergic Neuro 2a cells. Capsaicin treatment, post BoNT/A, reversed this. We hypothesize that at the MNT, capsaicin stimulated Ca^{2+} influx via TRPV1 triggers exocytosis by facilitating synaptic transmission via CaMKII mediated phosphorylation of SYN1.

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Species-Dependent Effects of Mustard Oil on TRPM8
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Allyl isothiocyanate (AITC; mustard oil) confers the typical pungency to condiments such as wasabi and mustard, and has been widely used as a pharmacological tool to study pain and neurogenic inflammation. The somatosensory effects of AITC have been largely attributed to direct activation of TRPA1 and TRPV1, two excitatory Transient Receptor Potential (TRP) ion channels expressed in nociceptor neurons. Nevertheless, a subset of sensory neurons responds to AITC in a TRPA1- and TRPV1-independent manner. We show that AITC activates TRPA1/TRPV1-deficient sensory neurons through activation of TRPM8, a TRP channel involved in cool/cold sensation. Whole-cell and inside-out patch-clamp recordings revealed that AITC rapidly and reversibly activates heterologously expressed TRPM8 in a membrane-delimited manner. Moreover, we found that, at higher doses, AITC induced channel activation is followed by channel block, a process that is more pronounced in the human TRPM8 than in the mouse orthologue. Analysis of chimeric channels combining human and mouse TRPM8 orthologues cytosolic revealed domains that determine this AITC-induced channel blockade. TRPM8 may contribute to the complex psychophysical effects of AITC.

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Activation and Sensitization of the Capsaicin Receptor TRPV1 by Allyl Isothiocyanate
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Allyl isothiocyanate (AITC, or oil) is a powerful plant-derived irritant that functions as a defensive trait against herbivores and confers pungency to mustard and wasabi. AITC is extensively used experimentally to induce acute pain and neurogenic inflammation, which are largely mediated by the activation of nociceptive cation channels TRPA1 and TRPV1. We have recently shown that AITC activates TRPV1 through a mechanism that is similar to that underlying the activation induced by capsaicin. In the present study, we tested whether AITC sensitizes TRPV1 for activation by extracellular acidosis and heat. Patch-clamp experiments in TRPV1-expressing HEK293T cells revealed that AITC enhances the responses to low pH and heat. These results were confirmed with intracellular calcium imaging experiments in the same cells and in dorsal root ganglion (DRG) neurons isolated from Trpa1 knockout (KO) mice. The responses to low pH and heat in the presence of AITC were strongly reduced by the TRPV1 inhibitor capsazepine and nearly absent in DRG neurons isolated from Trpa1/Trpv1 KO mice. The mechanism of cross sensitization between AITC, low pH and heat seem to occur via the induction of additive shifts of the voltage dependence of channel activation. These findings indicate that TRPV1 is a locus for cross sensitization between AITC, acidosis and heat in nociceptive neurons and help understanding the molecular bases underlying the role of this channel as mediator of the algesic properties of AITC.

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Molecular Adaptations to Extreme Thermogenesis in Mammalian Hibernators
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Thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) are obligate mammalian hibernators that make the transition from deep torpor- where body temperatures can be as low as 2°C- to the fully active state within as little as two hours. During arousal from hibernation, squirrels undergo an extreme thermogenesis phase, where core body temperatures can increase above normal (37°C) and reach 45°C, without causing distress or discomfort to the animal. To elucidate protective mechanisms that enable tolerance to hyperthermia, we examined the functional properties of TRPV1, the capsaicin- and heat-activated channel that senses noxious heat (>42°C). Sequence alignment showed a high level of identity between rat and squirrel homologues. Histological examination showed no changes in the neuronal profile of squirrel sensory ganglia or decreases in the number of neurons expressing TRPV1. Electrophysiological recordings revealed that both channels are activated by acidic pH and capsaicin. However, whereas rat TRPV1 is also activated by heat at ~43°C, we detected no thermal activation of the squirrel homologue even at temperatures as high as 48°C. Furthermore, in accord with the electrophysiological data, we found that squirrels fail to clearly discriminate between innocuous (25°C) and noxious (50°C) temperatures in behavioral tests. These experiments provide a molecular mechanism for hyperthermia tolerance in mammals at the level of the somatosensory system, and present a novel TRPV1 homologue, which can be used to delineate protein region(s) important for thermal activation.