

## Thimerosal decreases TRPV1 activity by oxidation of extracellular sulfhydryl residues

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### Abstract

TRPV1, a receptor for capsaicin, plays a key role in mediating thermal and inflammatory pain. Because the modulation of ion channels by the cellular redox state is a significant determinant of channel function, we investigated the effects of sulfhydryl modification on the activity of TRPV1. Thimerosal, which oxidizes sulfhydryls, blocked the capsaicin-activated inward current ( $I_{\text{cap}}$ ) in cultured sensory neurons, in a reversible and dose-dependent manner, which was prevented by the co-application of the reducing agent, dithiothreitol. Among the three cysteine residues of TRPV1 that are exposed to the extracellular space, the oxidation-induced effect of thimerosal on  $I_{\text{cap}}$  was blocked only by a point mutation at Cys621. These results suggest that the modification of an extracellular thiol group can alter the activity of TRPV1. Consequently, we propose that such a modulation of the redox state might regulate the physiological activity of TRPV1.

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Capsaicin, the main pungent ingredient of hot peppers, produces burning pain and neurogenic inflammation through the excitation of small sensory neurons [3,25]. In cultured dorsal root ganglion (DRG) neurons, capsaicin activates a ligand-gated, non-selective cation channel [19]. A cDNA that encodes a channel that is activated by capsaicin was cloned recently, and was classified as TRPV1 [4,18]. The TRPV1 channel has properties that resemble those of the capsaicin-activated channel that is present in sensory neurons. In addition, TRPV1 is activated by heat and extracellular acid [4,27], and the lipid metabolic products of lipoxygenases and anandamide activate TRPV1 [13,23,31]. TRPV1-deficient mice exhibit reduced inflammatory thermal hyperalgesia, so TRPV1 appears to be essential for mediating thermal hyperalgesia that is induced by inflammation [5,6].

During inflammation or reperfusion injury, reactive oxygen species are generated, which affects the redox state of tissues [11]. Among the many amino acids that are present

within biologically active proteins, cysteine residues are reactive to the cellular redox state. Thus, redox modification of cysteinyl sulfhydryl (SH) groups due to a change in the redox state constitute an important mechanism for regulating cellular function. Oxidation of cysteine residues modulates the activity of the channels in *N*-methyl-D-aspartate receptors [2], GABA<sub>A</sub> receptors [1], ATP-sensitive K<sup>+</sup> channels [20], large conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels [7], and voltage-dependent Ca<sup>2+</sup> [17,26] and Na<sup>+</sup> channels [24].

Recently, intradermal injection of a reducing agent, dithiothreitol (DTT), was reported to induce thermal hyperalgesia, an effect that could be blocked by co-injection of an oxidizing agent, 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) [26]. These results suggested that changes in the redox state in peripheral tissues may influence the activity of ion channels that are expressed in sensory neurons. Because TRPV1 has been implicated in mediating inflammatory pain [5,6], it is possible that changes in the redox state in peripheral tissues modulate the activity of TRPV1. In the present study, we examined the effects of a sulfhydryl-oxidizing agent, thimerosal, on TRPV1-dependent currents in cultured DRG neurons. In ad-

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