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Molecular Mechanisms of Immune System Involving Thermosensitive TRPM2 Channel

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Summary

Thermoregulation is the ability of organisms to keep their body temperatures within a certain range (~37ºC). Nine thermosensitive transient receptor potential (TRP) channels (thermoTRPs) are known to detect ambient temperature and are believed to be involved in thermoregulation. We investigated the regulatory mechanism and physiological role of TRP melastatin 2 (TRPM2) at the body temperature, which is sensitive to warm temperatures (>35ºC).

TRPM2 is a nonselective, Ca^{2+}-permeable cation channel, and is expressed in various organs such as the brain, pancreas, spleen, kidney and a wide range of immunocytes, such as lymphocytes, neutrophils, and monocytes/macrophages. TRPM2 plays important roles in Ca^{2+} signaling in these tissues and cells, and contributes to cellular functions that include insulin release, cytokine production, cell motility, and cell death. The primary activator of TRPM2 is adenosine diphosphate ribose (ADPR). We found the novel activation mechanism of TRPM2 induced by H_2O_2. The alteration in the temperature sensitivity of TRPM2 by H_2O_2 was mediated by a reduction in the temperature threshold for TRPM2 activation, enabling channel activation and cytosolic Ca^{2+} elevation at the physiological body temperature. Sensitization of TRPM2 by H_2O_2 was found to be via oxidation of methionine residues. Therefore, endogenous TRPM2 channels in vivo could be modulated by redox signals in parallel with adenine-containing second messengers at physiological body temperature.

Sensitization of the heat-evoked response was also observed in wild-type (Wt) but not in TRPM2-deficient macrophages, indicating possible involvement of TRPM2 sensitization in macrophage functions. ROS-mediated elevation of cytosolic Ca^{2+} and Ca^{2+}-dependent ROS production may interact and amplify each other, playing central roles in innate immune responses. Indeed, zymosan-induced cytokine release was affected in TRPM2-deficient macrophages. In addition, elevated temperatures (fever) were found to enhance phagocytic activity of Wt macrophages, but not TRPM2-deficient macrophages, implying that the ROS-TRPM2 activation pathway plays a critical role in macrophage functions. This novel activation mechanism of TRPM2, sensitization to temperature, might provide new approaches to immune research.

We then investigated whether the TRPM2 sensitization by H_2O_2 is a global phenomenon by focusing on the TRPM2 functions in pancreatic β cells. Heat-evoked $[Ca^{2+}]_i$ increases were observed after H_2O_2 treatment in Wt β cells, but not TRPM2-deficient β cells similar to macrophages. In addition, TRPM2 activation downstream from the redox signal plus glucose stimulation enhanced glucose-induced insulin secretion in a temperature-dependent manner. The N-acetyl cysteine (NAC)-sensitive fraction of insulin secretion by Wt islets was increased by temperature elevation and this temperature-dependent enhancement was significantly diminished in TRPM2KO islets. These data suggest that

endogenous redox signals in pancreatic β-cells elevate insulin secretion via TRPM2 sensitization and activity at body temperature. The results could provide new therapeutic approaches for the regulation of diabetic conditions by focusing on the physiological function of TRPM2 and redox signals.

We also investigated the physiological role of TRP vanilloid 4 (TRV4) at the body temperature, which is sensitive to warm temperatures (>30ºC). TRPV4, a calcium-permeable channel, is highly expressed in the apical membrane of choroid plexus epithelial cells (CPECs) in the brain. The function of TRPV4 is unknown. We show physical and functional interaction between TRPV4 and anoctamin (ANO) 1, one of the Ca^{2+}-activated chloride channels, in HEK293T cells and CPECs. Chloride currents induced by a TRPV4 activator (GSK1016790A) were markedly increased in an extracellular calcium-dependent manner in HEK293T cells expressing TRPV4 with ANO1, but not with ANO4, ANO6 or ANO10, the mRNAs of which were expressed in the choroid plexus. GSK-induced chloride currents were observed in wild-type CPECs but not in TRPV4-deficient CPECs. We also found physical interaction between TRPV4 and ANO1 in both HEK293T cells and choroid plexus. We observed that ANO1 was activated at a warm temperature (37˚C) in HEK293T cells and that the heat-evoked chloride currents were markedly enhanced after GSK1016790A application in CPECs. Simultaneous stimulation by warmth and hyposmosis induced chloride current activation in wild-type, but not in TRPV4-deficient CPECs. Cell volume changes were induced by ANO1-mediated chloride currents in parallel with membrane potential changes, and the cell volume was significantly decreased at negative membrane potentials by TRPV4-induced ANO1 activation. Thus, physical and functional interactions between TRPV4 and ANO1 can modulate water transport in the choroid plexus, and it could be one of the mechanisms for cerebrospinal fluid production in choroid plexus.

To find another example the functional interaction between Ca^{2+}-permeable TRP channels and ANO1, we focused on mouse sensory neurons. Because it is known that cytosolic chloride concentrations are high in the sensory neurons, opening of ANO1 is supposed to lead to chloride efflux, resulting in the membrane depolarization. Capsaicin receptor TRPV1 is activated by various noxious stimuli, and the stimuli are converted into electrical signals in primary sensory neurons. It is believed that cation influx through TRPV1 causes depolarization, leading to the activation of voltage-gated sodium channels, followed by action potential generation. We found that the capsaicin-evoked action potential could be induced by two components: a cation influx-mediated depolarization due to TRPV1 activation and a subsequent anion efflux-mediated depolarization via activation of anoctamin 1 (ANO1), a calcium-activated chloride channel, due to the entry of Ca^{2+} through TRPV1. The interaction between TRPV1 and ANO1 is based on their physical binding. Capsaicin activated the chloride currents in an extracellular calcium-dependent manner in HEK293T cells expressing TRPV1 and ANO1. Similarly, in mouse DRG neurons, capsaicin-activated inward currents were significantly inhibited by a specific ANO1 antagonist, T16Ainh-A01 (A01) in the presence of a high concentration of EGTA, but not BAPTA. Furthermore, pain-related behaviors in mice treated with capsaicin, but not with αβmethylene ATP, were significantly reduced by the concomitant administration of A01. These results indicate that TRPV1-ANO1 interaction is a significant pain-enhancing mechanism in the peripheral nervous system.