

MENTHOL-INDUCED EXCITATION IN CULTURED COLD-SENSITIVE NEURONS

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When applied to the oral cavity or skin, menthol elicits afferent impulses in cold fibers, and induces coolness or sensation of freshness. However, because it is difficult to access nerve endings, actions of menthol have not been clarified. In this study we analysed effects of menthol on cultured dorsal root ganglion (DRG) cells containing cell bodies of nerve fibers with measurements of cytosolic free Ca^{2+} ion concentration ($[\text{Ca}^{2+}]_i$) and patch-clamp techniques. Wistar rats (2-14 days old) were anesthetized by diethyl ether and decapitated to isolate DRG. After dissociation with collagenase and trypsin, DRG cells were plated on a coverslip and cultured in DMEM containing 10% fetal bovine serum. Menthol (l-menthol) dissolved in Krebs solution was bath-applied. For cold stimulation, temperature was lowered from room temperature (25-29°C) by 10-15°C. Temperature was monitored with a thermocouple close to cells. Before experiments, cells were loaded with Fura-2/AM (5 mM). $[\text{Ca}^{2+}]_i$ was measured with Fura-2 microfluorimetry (ARGUS/HiSCA; Hamamatsu). Patch-clamp recordings were performed with EPC-7 (List). Data were acquired with MacLab (AD Instruments). Cooling induced an increase in $[\text{Ca}^{2+}]_i$ in 10% of DRG neurons. We identified them as cold-sensitive neurons. Menthol induced $[\text{Ca}^{2+}]_i$ response in most (98%) of the cold-sensitive neurons. The number of the menthol-sensitive neurons without cold-sensitivity was small. Menthol induced the $[\text{Ca}^{2+}]_i$ increase in a dose-dependent manner, with an EC_{50} of $37.9 \pm 7.58 \mu\text{M}$ (n=8). When extracellular Ca^{2+} was removed, menthol did not induce the $[\text{Ca}^{2+}]_i$ increase, indicating that menthol induced calcium influx. In whole-cell current-clamp recordings, menthol induced depolarization (receptor potential) leading to impulses. In voltage-clamp recordings (-60 mV), menthol induced inward currents, underlying the receptor potentials. Reversal potentials of the menthol-induced current suggested that menthol activated non-selective cation channels. We conclude that menthol induces depolarization and impulses through activation of non-selective cation channels in most of the cold-sensitive neurons.

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