

## **PGE<sub>2</sub> SELECTIVELY ACTIVATES PERIPHERAL COLD-SENSITIVE NEURONS**

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In response to exogenous pyrogen, immune cells generate endogenous pyrogen, leading to the production of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). PGE<sub>2</sub> may finally evoke heat production (HP) responses and/or heat-seeking (HS) behaviors, resulting in fever. However how PGE<sub>2</sub> acts on neurons is not known. Cooling the skin evokes afferent impulses in cold fibers, which may elicit HP responses and/or HS behaviors. PGE<sub>2</sub> receptors are abundant in dorsal root ganglion (DRG) containing cell bodies of cold fibers. Here we investigated effects of PGE<sub>2</sub> on cultured DRG cold-sensitive neurons with measurements of intracellular Ca<sup>2+</sup> ion concentration ([Ca<sup>2+</sup>]<sub>i</sub>) and patch-clamp techniques. Wistar rats (2-24days old) were anesthetized with diethyl ether and decapitated to isolate DRGs. DRG cells were plated on coverslips (5.5mm), and cultured in DMEM at 37° in a humidified atmosphere containing 5% CO<sub>2</sub> for 1-3 days before recordings. Cultured cells on coverslips were loaded with Fura-2 /AM (Donjindo), and were positioned in a recording chamber mounted on the stage of an upright fluorescence microscope (ECLIPSE E600-FN, Nikon). Cells were perfused with Krebs solution by gravity. Cell temperature was monitored with a thermocouple (0.3mm in diameter) close to cells. Cold stimulation was applied on cells by reducing temperature of perfusing solution from room temperature (26-28°) to 10-12°. [Ca<sup>2+</sup>]<sub>i</sub> in cultured DRG cells was recorded every 10s with a digital image analysis system (AQUACOSMOS, Hamamatsu). Cells which increased [Ca<sup>2+</sup>]<sub>i</sub> in response to cold stimulation were identified as cold-sensitive neurons. PGE<sub>2</sub> (10nM) induced an increase in [Ca<sup>2+</sup>]<sub>i</sub> in most (90%) of the cold-sensitive neurons but not in cold-insensitive neurons. PGE<sub>2</sub>-induced [Ca<sup>2+</sup>]<sub>i</sub> response was dose-dependent (EC<sub>50</sub>=2.8nM). When Ca<sup>2+</sup> was removed from the external solution, PGE<sub>2</sub>-induced [Ca<sup>2+</sup>]<sub>i</sub> response disappeared, indicating that the [Ca<sup>2+</sup>]<sub>i</sub> increase comes from extracellular Ca<sup>2+</sup> ions. In cell-attached patch recordings, PGE<sub>2</sub> directly evoked impulses in neurons showing PGE<sub>2</sub>-induced [Ca<sup>2+</sup>]<sub>i</sub> response. This suggests that PGE<sub>2</sub> receptors leading to cell excitation are present in cold-sensitive neurons. We concluded that immune signal selectively activates peripheral cold-sensitive neurons, even when it is not cold. This might evoke HP responses and/or HS behaviors to induce fever.

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