

4 α -Phorbol 12,13-didecanoate activates cultured murine dorsal root ganglia neurons independently of TRPV4

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The Ca²⁺ permeable cation channel TRPV4 is a member of the Transient Receptor Potential Vanilloid family of channel proteins. Data from immunohistochemical and functional studies suggest that it is expressed in sensory neurons originating from the dorsal root ganglia (DRG), in addition to a wide-range of non-neuronal tissues (e.g. pulmonary and renal epithelium, vascular endothelium). It is activated by mechanical disturbance of the cell membrane and decreased extracellular osmolarity, and is implicated in mechanical hyperalgesia and osmosensation. The phorbol ester 4 α -phorbol 12,13-didecanoate (4 α PDD) is a chemical activator of TRPV4 (EC₅₀ at mTRPV4 of ~3 μ M), and has been described as a selective agonist. More recently, GSK1016790A (GSK101), a small molecule synthetic agonist of TRPV4 (EC₅₀ at mTRPV4 of 18nM), was described. We investigated the ability of exposure to 4 α PDD or GSK101 to stimulate increases in [Ca²⁺]_i in primary cultures of murine DRG sensory neurons.

Male and female C57BL/6 wild type (+/+) and TRPV4, TRPV1 and TRPA1 knockout (-/-) mice (20-30g) were used in this study. Mice were euthanased with an overdose of pentobarbital (1mg/g) and the spinal column removed. DRG were collected from all spinal levels and individual neurons dissociated by incubation in collagenase (1.25%) and trituration. Changes in intracellular Ca²⁺ concentration in response to perfusion of TRPV4 agonists were studied after 18 – 24 hours in culture, by ratiometric imaging using the Ca²⁺-sensitive dye Fura-2. All changes were recorded as a % of the maximum response to depolarisation with 50mM KCl.

Exposure to 4 α PDD (1-10 μ M) stimulated a pronounced, dose-dependent increase in [Ca²⁺]_i in neurons from both wild type and knockout mice. The proportion of responding neurons (10 μ M: TRPV4 +/+ = 85.8%; TRPV4 -/- = 82.9% of cells) and the magnitude of the increase (10 μ M: TRPV4 +/+ = 63.4 \pm 7.3%; TRPV4 -/- = 63.8 \pm 6.4% of KCl response) were unaffected by the genotype. In contrast, 1 μ M GSK101 failed to stimulate an increase in intracellular Ca²⁺ in a greater proportion of cells than vehicle (DMSO = 5.1%; GSK101 = 3.7% of cells). The percentage of responding neurons and magnitude of response to 4 α PDD (3 μ M) in cultures from TRPV1 and TRPA1 -/- mice was the same as that in wild type animals. Responses to 4 α PDD were abolished in the absence of extracellular Ca²⁺.

We conclude that 4 α PDD activates neurons independently of TRPV4, TRPV1 or TRPA1. It does not seem appropriate to refer to 4 α PDD as a selective TRPV4 agonist when studying neuronal responses. Additionally, the lack of difference in response to 4 α PDD between TRPV4 +/+ and -/- neurons, and the failure of GSK101 to stimulate a measurable increase in intracellular Ca²⁺ in these cells suggests that there is little, if any, expression of functional TRPV4 in DRG sensory neurons.