Ligand-gated ion channels of sensory neurons: from purines to peppers

O. Krylova, C.-C. Chen, A. Akopian, V. Souslova, K. Okuse, N. Abson, S. Ravenall and J. N. Wood*

Department of Biology, Medawar Building, University College, London WC1 E 6BT, U.K.

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The activation of peripheral sensory neurons by tissue-damaging stimuli is known as nociception; this effect may lead to a sensation of pain. A variety of chemical mediators have been implicated in the activation of nociceptive sensory neurons, including ATP which is present at millimolar levels in all cells [1,2]. More recently, ATP has also been suggested to play a role in both mechanoreception and the detection of noxious mechanical, rather than chemical, stimulation [3]. In this review we describe recent progress in the identification of sensory neuron-specific receptors, and discuss their properties with respect to sensory neuron activation.

The sensory neurons of the peripheral nervous system arise from migrating cells of the neural crest in the trunk, as well as from ectodermal placodes in some cranial ganglia; their survival is influenced by competition for target-derived trophic factors. Sensory neurons are activated by a variety of external stimuli (mechanical, thermal and chemical) and release a range of neuropeptides as well as glutamate as their principal excitatory neurotransmitter, at synapses within the spinal cord [4]. Sensory neurons have been subdivided into two broad populations: neurons with large-diameter cell bodies and fast conducting myelinated axons (A-fibres), and neurons with small-diameter cell bodies and unmyelinated slowly conducting axons (C-fibres). Most of the small-diameter neurons are activated by tissue-damaging stimuli, whereas the large-diameter neurons seem to be principally involved in mechanoreception or proprio-reception. However, there are clearly many subpopulations of sensory neurons and it has proved difficult to correlate sensory modality with particular markers. Fortunately, primary cultures of sensory neurons retain many of the properties of their in vivo counterparts and it is possible to investigate chemical activation of these cells in vitro [5].

Extracellular ATP acts via P2 purinoceptors which have been subdivided into two families: P2X purinoceptors are a novel type of ligand-gated non-selective cation channels, whereas P2Y purinoceptors are G-protein-coupled receptors [6]. The cDNAs that are encoded by distinct P2X purinoceptor genes have been cloned and characterized from the rat vas deferens P2X, [7], P2X from PC12 cells [8], P2X, from rat sensory neurons [9,10], P2X from rat epithelia and brain [11,12], rat brain P2X, and P2X, [13] and rat superior cervical ganglia P2X, [14].

P2X, purinoceptors are expressed by cells within dorsal root ganglia including sensory neurons as judged by reverse transcriptase PCR [13]. Some purinoceptors, however, show a restricted distribution. The G-protein-coupled receptor P2Y, is selectively expressed by large-diameter sensory neurons, and was expression (re)cloned using an oocyte-based assay for mechanosensitive channels [3]. Mechanical stimulation of the oocyte resulted in the release of enough ATP from the cell to activate the expressed receptor. It is possible that specialized cells such as Pacinian corpuscles and Merkel cells may release ATP on mechanical distortion, resulting in the depolarization of mechanoreceptors. Similarly, more intense mechanical stimula-
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The rat P<sub>2X</sub> receptor is a 397-amino acid polypeptide that shows substantial identity (40–47%) with the other five receptors, and with the first 400 amino acids of P<sub>2X</sub>. As with all members of the P<sub>2X</sub> family, P<sub>2X</sub> appears to have two transmembrane domains, intracellular N- and C-termini, and the greater part of the polypeptide (about 300 amino acids) lies between the two putative transmembrane domains and is thus predicted to be extracellular. The position of ten cysteine residues in the extracellular domain is conserved in all P<sub>2X</sub> receptors, suggesting that the secondary structure of this class of receptors is conserved. The molecular architecture of P<sub>2X</sub>, but not the nucleotide sequence, is similar to subunits of the mechanosensitive channel of Escherichia coli [15], inward-rectifier K<sup>+</sup> channels [16], amiloride-sensitive epithelial Na<sup>+</sup>-channels [17], the FMRFamide-gated Na<sup>+</sup> channel [18] and Caenorhabditis elegans degenerins (deg-1, mec-4 and mec-10) [19,20].

The contribution of different P<sub>2X</sub> receptor subunits to the formation of functional ATP-gated channels in sensory neurons is uncertain. Even the stoichiometry of functional channels remains to be clarified. Expression of the P<sub>2X</sub> subunit alone may not account for the functional responses of nodose ganglion sensory neurons to ATP, but does seem to reproduce the P<sub>2X</sub> receptor activity defined in sensory neurons of dorsal root ganglia [21]. However, Lewis and colleagues [10] have suggested that heteromultimerization of P<sub>2X</sub> and P<sub>2X</sub> may better account for P<sub>2X</sub> activity in sensory neurons derived from the nodose ganglion. The situation is further complicated by the demonstrations of splice variants of the P<sub>2X</sub> receptor [22].

Both P<sub>2X</sub> and P<sub>2X</sub> genes consist of 12 exons, the P<sub>2X</sub> gene spanning approx. 40 kb of genomic DNA [22,23]. The chromosomal localization of the mouse P<sub>2X</sub> gene has been determined by fluorescence in situ hybridization (FISH)-mapping to be on the proximal part of mouse chromosome 2, immediately adjacent to the recombination activation gene (RAG) locus. A hybridization signal only on chromosome 2 supports the view that a single copy of the P<sub>2X</sub> gene is present in the mouse genome.

Many nociceptive neurons that express the P<sub>2X</sub> receptor are also activated by the sensory neurotoxin capsaicin (8-methyl-N-vanillyl-6-non-ename) [2,24]. Because of this selectivity of action, capsaicin has been a useful tool in elucidating the function of these neurons. At low doses, capsaicin excites and desensitizes a variety of peripheral sensory systems, whereas high doses of capsaicin, particularly in neonatal animals, can lead to the loss of sensitive cells through Ca<sup>2+</sup>-mediated damage.

Evidence for a specific capsaicin receptor comes from structure–function studies of capsaicin congeners, the well-defined species and tissue specificity of capsaicin action, and the development of a functionally defined competitive antagonist of capsaicin, capsazepine [2]. Capsaicin has been shown to induce cation fluxes in sensitive cells through a direct mechanism not involving second-messenger systems. Using isolated membrane patches from sensory neurons, capsaicin has also been found to activate a non-selective cation flux [25]. Evidence from whole-cell studies that the capsaicin-gated channel may also be activated by protons has been obtained [26]: pH values lower than 6.5 are found in inflamed tissue, suggesting that the capsaicin-activated channel may play a physiological role in the response to tissue-damaging stimuli. This idea is strengthened by the analgesic actions of a number of capsaicin analogues that are likely to act at the same site as capsaicin [27]. However, some reports do not support the view that protons gate the capsaicin channel [2,28].

There are some intriguing parallels in the properties of capsaicin-gated channels and P<sub>2X</sub> receptors, including the mechanism of desensitization through calcineurin action [29,30], the pronounced pH-dependence of channel gating, the distribution of expression of capsaicin sensitivity and the P<sub>2X</sub> receptor, and the ion selectivity of the channel. The size of the capsaicin receptor channel complex has been estimated by radio-inactivation studies to be approx. 270 kDa, using the ultrapotent capsaicin analogue resiniferatoxin, but the channel remains to be characterized by molecular cloning [31]. By analogy with the structure of other ligand-gated ion channels, it seems reasonable to assume that the capsaicin...
receptor channel complex will be multimeric, perhaps comprising known ligand-gated channel subunits such as the P2X receptors or other subunits [2] that show a relatively selective distribution within sensory neurons, in association with a capsaisin-binding site.

As well as receptors for the exogenous activators, capsaisin and resiniferatoxin, and the P2X receptors described above, a number of other glutamate- and serotonin-activated channels. However, only the P2X receptor is selectively expressed by small-diameter sensory neurons, and only the response to capsaisin is selectively associated with these same cells. The molecular cloning of the capsaisin receptor, and the generation of null mutant animals for both P2X and capsaisin receptors will define whether this remarkable selectivity of expression is functionally associated with nociception, or has a wider significance.

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