

Inflammatory Pain: The Cellular Basis of Heat Hyperalgesia

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Abstract: Injury or inflammation release a range of inflammatory mediators that increase the sensitivity of sensory neurons to noxious thermal or mechanical stimuli. The heat- and capsaicin-gated channel TRPV1, which is an important detector of multiple noxious stimuli, plays a critical role in the development of thermal hyperalgesia induced by a wide range of inflammatory mediators. We review here recent findings on the molecular mechanisms of sensitisation of TRPV1 by inflammatory mediators, including bradykinin, ATP, NGF and prostaglandins. We describe the signalling pathways believed to be involved in the potentiation of TRPV1, and our current understanding of how inflammatory mediators couple to these pathways.

Key Words: Pain, sensory transduction, inflammation, protein kinase, intracellular signalling, capsaicin, heat, TRPV1.

INTRODUCTION

Pain-producing stimuli are detected *in vivo* by the nerve terminals of primary sensory neurons, whose cell bodies are found in sensory ganglia such as the dorsal root ganglia (DRG). The signal, in the form of action potentials, is then transmitted along primary sensory nerve fibres to the dorsal horn of the spinal cord, and from there on to higher brain centres, where it is interpreted as pain [67]. The specialised primary sensory neurons involved in the transduction of painful stimuli into action potentials are called nociceptors, a term coined by Sherrington 100 years ago [104]. Nociceptors can be activated by a wide range of thermal, mechanical and chemical stimuli (reviewed in ref. [30]). A unique feature of nociceptors is that the gain of signal transduction can be enhanced, or sensitised, by inflammatory mediators, such as prostaglandins, bradykinin, ATP, protons and nerve growth factor (NGF), which are released during tissue injury, metabolic stress and inflammation [13,21,106,120,126]. The effects of these inflammatory mediators on nociceptors are commonly produced by activation or sensitisation of membrane ion channels at nociceptor terminals, through the action of receptor-initiated second messenger cascades (reviewed in ref. [68]).

The heat and capsaicin receptor TRPV1 (formerly known as VR1), an important ion channel involved in pain sensation, plays a critical role in the development of inflammatory hyperalgesia. Knockout studies have shown that mice lacking the TRPV1 receptor fail to develop thermal hyperalgesia during inflammation [18,25]. In addition, pharmacological studies using capsazepine and other more potent TRPV1 antagonists have found that mechanical hyperalgesia was also attenuated in a variety of pain models, supporting the hypothesis that activation of TRPV1 causes mechanical hyperalgesia even though TRPV1 is not directly involved in the detection of noxious mechanical stimuli [48,55,89,129]. Thus, the central implication of TRPV1 in hyperalgesia makes it a potential target for novel analgesic and anti-inflammatory

drugs. Since the channel was first cloned, intensive work has been carried out to understand the pharmacological properties of TRPV1 and its regulation by inflammatory mediators.

THE TRPV1 RECEPTOR

TRPV1 is a member of the transient receptor potential (TRP) channel family, and was first isolated from a rodent DRG cDNA library by using a calcium-imaging based expression assay [19]. In agreement with an essential role in nociception, the expression of TRPV1 is mainly detected in small to medium size neurons in the trigeminal ganglia (TG) and dorsal root ganglia (DRG) [38]. Within DRG neurons the level of TRPV1 mRNA is highly variable, being high in a group of neurons expressing the TrkA receptor for NGF but low in somatostatin containing cells [70]. TRPV1 is localised both with the peptidergic marker substance P, and with the lectin IB4 which labels non-peptidergic neurons in the DRG [29,37,43]. TRPV1 is also found in most cells of the nodose ganglia [38,70]. TRPV1 immunopositive fibers are detected in a variety of tissues such as skin [27,37,45], urinary bladder [7,134], gastrointestinal tract [130], tooth pulp [95], lung [53] and prostate [124]. However, the expression of TRPV1 is not restricted to peripheral neuronal tissues. Functional TRPV1 channels are also found in many areas of the central nervous system [69, 97] and in some non-neuronal tissues, such as epidermal keratinocytes of human skin [112], gastric epithelial cells [51], and epithelial cells of the urothelium and smooth muscle [11]. While our knowledge of the *in vivo* distribution of TRPV1 is expanding, the function of TRPV1 in cells other than primary sensory neurons remains poorly understood.

In common with other TRP channels, functional TRPV1 channels are homo- or heterotetramers of four single subunits [52]. Each single subunit of TRPV1 contains six transmembrane domains and a pore loop between domain five and six (see Fig. 1) [19]. When expressed together with other TRPV members in HEK 293 cells, the TRPV1 subunits preferentially assemble into homomeric complexes [39]. In addition, expression of TRPV1 alone in heterologous systems [19,36, 90] can fulfill the major electrophysiological properties of native receptors in DRG neurons [21]. These observations suggest that TRPV1 is most likely to form a homotetramer *in*

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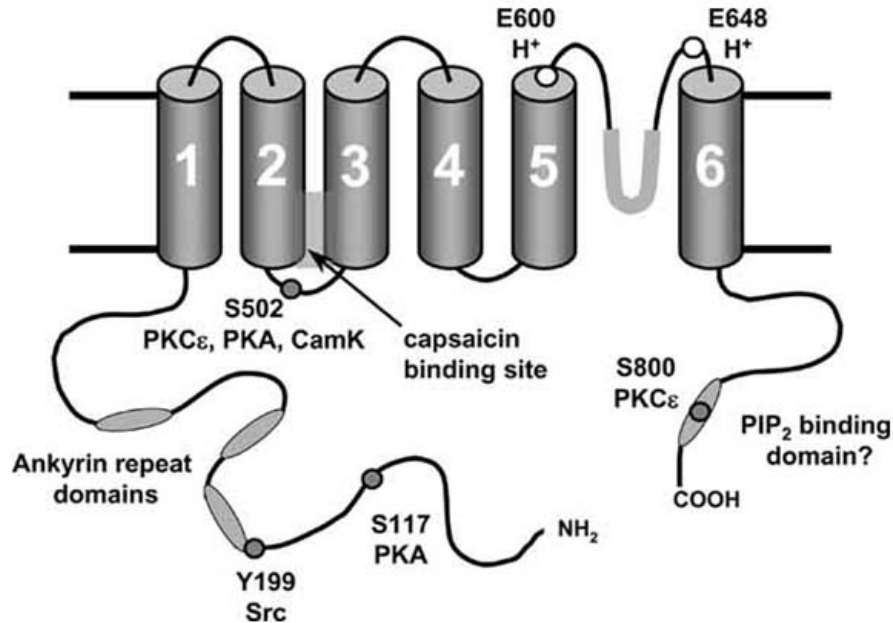


Fig. (1). Sites on TRPV1 modulating function.

The hypothesised 6-transmembrane domain structure of TRPV1 is shown, with a pore loop between TM5 and TM6. A functional homomeric channel is formed from four such subunits. Sites at which phosphorylation is known to modulate TRPV1 function are shown together with the kinases responsible. Protons can bind to two extracellular glutamates, and protonation of E600 has an apparently similar effect on TRPV1 gating to phosphorylation at S502. Ankyrin domains, the capsaicin-binding domain and the hypothesised C-terminal PIP₂ binding domain are also shown.

in vivo. Although some recent studies suggest that TRPV1 may form heteromultimers with the other TRPV family members, such as TRPV2 [60] and TRPV3 [110], the possible existence and functional roles of TRPV1 heteromers in native neurons are currently unclear.

TRPV1 functions as a polymodal sensor of physical and chemical noxious stimuli in nociceptors. Studies of the ion selectivity of TRPV1 in HEK 293 cells reveal that TRPV1 is a non-selective cation channel with a high relative permeability to calcium [19]. To date, TRPV1 is believed to be the only receptor for vanilloids such as capsaicin, based on the finding that vanilloids evoked no pain related behaviours in TRPV1 knock-out mice [18]. In addition to its response to vanilloids, TRPV1 is also directly gated by noxious heat (>42 °C) and strong acidic conditions (pH<6, ref [118]), by ethanol [121] and by a variety of endogenous lipids such as anandamide [123,138], lipoxygenase products [44] and the potent endovanilloids N-arachidonoyl-dopamine, NADA [42] and N-oleoyldopamine, OLDA [22].

SIGNALLING PATHWAYS MODULATING TRPV1

The sensitivity of TRPV1 to noxious stimuli may be influenced by a number of intracellular signalling pathways (see Fig. 1). There are a number of potential target sites on TRPV1 for phosphorylation by protein kinases. For example, several residues on rat TRPV1 (S502, T704, S744, S800 and S820) have been reported to be phosphorylated by protein kinase C (PKC), although the evidence to date suggests that only S502 and S800 play a significant functional role [8,81]. Protein kinase A (PKA) phosphorylates TRPV1 at six resi-

dues (S116, T144, T370, S502, S774 and S800), amongst which S116 appears to be the only important site for PKA modulation [10,72]. The Y199 site (Y200 in human TRPV1), which is located at the start of the first N-terminal ankyrin repeat, plays a critical role in the modulation of TRPV1 trafficking to the neuronal membrane by the tyrosine kinase Src [136]. These kinases may sensitise the channel by increasing the channel open probability, which seems to be the principal effect of phosphorylation by PKCε [126], by reversing desensitisation, in the case of PKA [10], and/or by rapid recruitment of new channels to the cell surface membrane by regulated exocytosis [74,122,136], a process which is mediated by phosphorylation by Src [136]. It has also been suggested that phosphorylation of two sites (S502 and T704) on TRPV1 by CaMKII is required for vanilloid binding to TRPV1, but has no effect on the channel response to acid [50]. Unlike PKC, PKA or Src, however, whose actions have been well studied, little is known about how phosphorylation by CaMKII may alter the function of TRPV1.

Protons are released into the external medium under conditions of anoxia or inflammation, and can interact with TRPV1 in two ways: a low external pH (<6) can directly activate TRPV1 by protonating a glutamate (E648) adjacent to the pore region, but milder acidification protonates E600, at the immediate external face of transmembrane helix 5, leading to an enhancement of the activation of TRPV1 by other stimuli without directly activating the channel [49], a mechanism which is reminiscent of the effects of phosphorylation by PKCε [20,21].

TRPV1 may also be modulated by endogenous PIP₂, which has been suggested to bind to the C-terminal of TRPV1

and to inhibit the function of the channel [23,92]. Removal of PIP₂ by application of exogenous PLC to isolated membrane patches was found to increase the current activated by capsaicin, heat and protons [23], and removal of a supposed PIP₂ binding cassette in the C-terminal domain ablated the sensitising effect of NGF, which activates PLC γ and so metabolises PIP₂ [92]. More recently, however, it has been found that the resynthesis of PIP₂ by PI4K promotes recovery of TRPV1 from desensitisation [61], an effect apparently contrary to that described above. It has also recently been reported that the removal of the supposed PIP₂ binding domain promotes phosphorylation of the Y199 site by Src [136], an action which may explain the effect of removal of this domain without the need to invoke binding of PIP₂. Thus the physiological role (if any) of PIP₂ binding to TRPV1 is currently unresolved and is a major issue to be clarified in future studies.

SENSITISATION OF TRPV1 BY INFLAMMATORY MEDIATORS

Inflammatory mediators have multiple effects on TRPV1 that lead to nociceptor sensitisation. Some pro-inflammatory mediators, such as protons and certain lipid mediators (e.g.

anandamide) are capable of directly activating TRPV1, albeit at rather high and perhaps unphysiological concentrations. Probably a more physiologically relevant mechanism is the modulation of the gating of TRPV1 by the actions of protein kinases, phosphatases, and/or lipid messengers produced by receptor-coupled intracellular signalling pathways. Many inflammatory mediators (e.g. bradykinin, prostaglandins, glutamate, chemokines, prokineticins and ATP) bind to receptors belonging to the G-protein-coupled receptor (GPCR) family, which signal to PKC and PKA to sensitise TRPV1 (see Fig. 2). Furthermore, some inflammatory mediators, such as NGF, insulin and IGF-1, may induce hyperalgesia by increasing the number of TRPV1 channels available to be activated on the nociceptor surface membrane. This process includes both transferring functional TRPV1 from intracellular vesicles to the plasma membrane [74,122,136] and up regulating the expression level of TRPV1 [46,93].

Bradykinin

Bradykinin, a nonapeptide which is generated following tissue injury and noxious stimulation, has been known for many years to be a major contributor to the inflammatory response [30]. Injection of bradykinin into human skin pro-

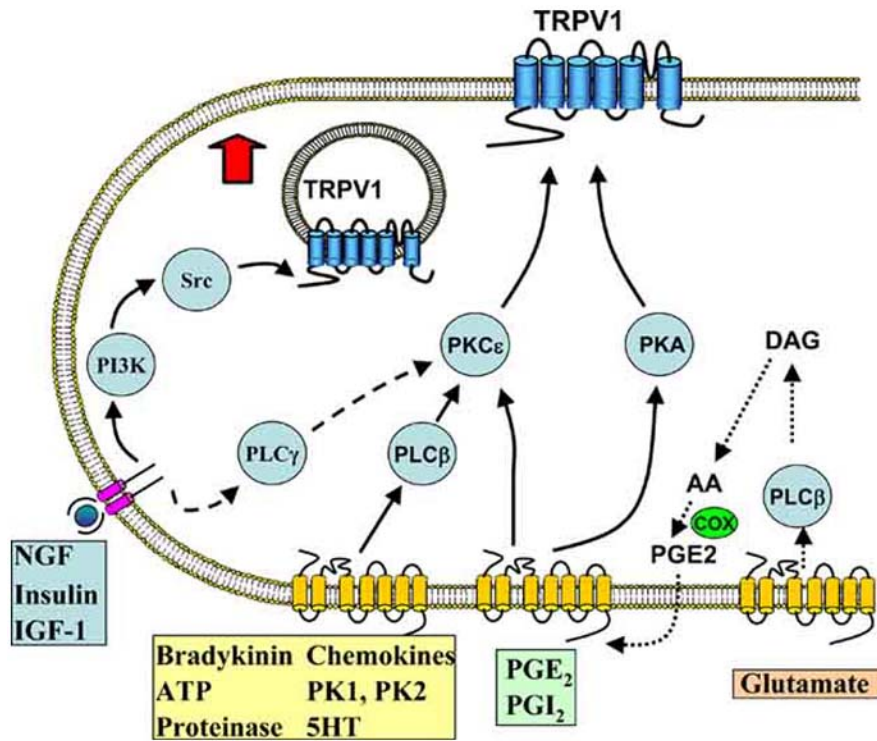


Fig. (2). Inflammatory mediators and intracellular signalling pathways modulating TRPV1.

NGF and other mediators activating tyrosine kinase-coupled receptors promote the insertion of TRPV1 into the membrane, from a pool located in subcellular vesicles, via a pathway in which PI3K plays an early role while the downstream tyrosine kinase Src activates trafficking to the membrane by phosphorylating Y199 in the N-terminal tail of TRPV1. These receptors also couple to a lesser extent to TRPV1 via PLC γ and PKC ϵ . Seven-transmembrane G-protein coupled receptors activated by a range of inflammatory mediators, including bradykinin and ATP, activate PLC β and consequently PKC ϵ , which enhances the activity of TRPV1 in the surface membrane by phosphorylating S502 and S800. Prostaglandins PGE₂ and PGI₂ activate EP and IP receptors, which couple to PKA and PKC ϵ . Glutamate (and probably other agonists such as bradykinin) appears to act at least in part via an indirect route in which the production of arachidonic acid (AA) leads to the synthesis of prostaglandins via the cyclo-oxygenase (COX) pathway and activation of prostaglandin receptors.

duces a dose-dependent pain and a heat hyperalgesia [63], suggesting that bradykinin is capable of exciting nociceptors as well as sensitising their response to heat.

The biological effects of bradykinin *in vivo* are mediated through two transmembrane G-protein-coupled receptors, the B1 and B2 receptors [65,87]. Functional B2 receptors are widely expressed in the nervous system and in other tissues, but the B1 receptor, on the other hand, is thought to be mainly absent under normal non-inflamed conditions, but to be induced and overexpressed during chronic inflammation [24,88]. Recent data, however, has demonstrated the existence of functional B1 receptors in a small proportion (~2%) of normal DRG neurons [127]. Activation of the B1 receptor sensitises the heat-evoked current in DRG neurons with a more prolonged effect than the B2 receptor, indicating that the B1 receptor may play a more important role in the maintenance of inflammatory pain [127].

B2 receptor activation initiates a number of signal transduction cascades. A major target is PLC β , which catalyses breakdown of PIP₂ into IP₃, causing a rise of calcium in the cell, and release of diacylglycerol (DAG), which in turn activates PKC. B2 receptor activation is also known to activate phospholipases A2 and D, and in addition protein tyrosine kinases, phosphatases and MAP kinases [24].

Bradykinin was found to enhance the heat-activated current in isolated DRG neurons [21]. The bradykinin-induced sensitisation of the neuronal response to heat could be mimicked by directly activating PKC, and PKC ϵ was observed to be translocated to the membrane after application of bradykinin [20]. These findings indicate that PKC ϵ plays a vital role in bradykinin-evoked thermal hyperalgesia. Subsequent experiments have confirmed the idea that enhancement of TRPV1 activity through the PLC β /PKC ϵ pathway is a major molecular mechanism for bradykinin-induced sensitisation of nociceptors [116]. Activation of PKC ϵ by bradykinin *via* the B2 receptor leads to phosphorylation of TRPV1 at two serine residues, S502 and S800, which potentiates the gating of TRPV1 by noxious stimuli [57,81,91,126]. Another mechanism which may be involved in bradykinin-induced acute sensitisation of TRPV1 is hydrolysis of PIP₂ by PLC, which has been proposed to sensitise the channel by relieving TRPV1 from PIP₂ inhibition [23], though as mentioned above, the current status of this proposal is uncertain.

In addition to sensitising the nociceptor response to heat, bradykinin is able to activate these neurons and so produce pain. TRPV1 may contribute to the excitation of neurons by bradykinin, based on the observation that bradykinin-evoked action potentials were reduced by the TRPV1 antagonist capsazepine [17,56], and that the response of C-fibres to bradykinin was significantly less in TRPV1 knockout mice than that in wild type mice [53]. However, the mechanism by which bradykinin activates TRPV1 seems to be different from that by which it sensitises the channel. The number of bradykinin-induced action potentials in C-fibres was reduced by lipoxigenase inhibitors, suggesting that lipoxigenase products may be involved in this process [17]. One likely hypothesis is that activation of PLA₂ induces mobilisation of arachidonic acid (AA) and generation *via* the lipoxigenase pathway of 12-HPETE, which has been shown to activate TRPV1 directly [105].

ATP

Another important inflammatory mediator released from damaged tissues is ATP, which both produces pain by directly exciting nociceptors and induces hyperalgesia by sensitisation of nociceptors to other noxious stimuli [16,31,102]. The ATP receptors expressed in neurons are divided into two groups: ionotropic P2X receptors and metabotropic P2Y receptors [94]. The current gated by extracellular ATP in sensory neurons is believed to be mediated by P2X receptors, while P2Y receptors play an important role in the ATP-induced sensitisation of nociceptors to noxious stimuli. In TRPV1 knock-out mice, extracellular ATP fails to induce thermal hyperalgesia, suggesting a critical role of TRPV1 in mediating the sensitising effect of extracellular ATP [76, 119]. Patch clamp experiments confirmed that extracellular ATP enhanced the TRPV1-dependent capsaicin and proton-evoked response and reduced the temperature threshold for TRPV1 activation [120]. The potentiating effects of extracellular ATP on TRPV1 activity were almost completely abolished by PKC inhibitors, indicating the activation of PKC *via* a PLC β -coupled signalling cascade as a possible mechanism [119,120]. Initial experiments suggested that the P2Y1 receptor for ATP was important [120], but more recent data show that it is P2Y2 which initiates the downstream pathways leading to TRPV1 sensitisation [54,76].

NGF

Nerve growth factor (NGF), a member of the neurotrophin family, is essential for the development and maintenance of the central and peripheral nervous systems [33,58]. The interest in NGF as a mediator of inflammatory pain was originally stimulated by the finding that it was upregulated after inflammation or injury [131]. Injection of NGF into adult rat paw produced a rapid and prolonged hypersensitivity to noxious thermal stimulation [59], confirming an important role for NGF in inflammatory pain. This thermal hyperalgesia became noticeable within a few minutes and was maintained for several days [100].

NGF interacts with two types of cell surface receptors, the tyrosine kinase receptor A (TrkA) and the pan-neurotrophin p75^{NTR} receptor, each of which activates different sets of signalling pathways [26,78,98]. The effect of NGF in promoting inflammatory hyperalgesia is thought to be predominantly mediated *via* TrkA receptors [66].

NGF-mediated thermal hyperalgesia results principally from sensitisation of TRPV1, because in TRPV1 null mice, the thermal hyperalgesia induced by injection of NGF is largely absent [23]. Short term (~10 minutes) administration of NGF acutely enhances the response of DRG neurons to heat and capsaicin [13,34,107], while long term (4~6 days) application of NGF increases the number of capsaicin-sensitive DRG neurons in culture [132,133], indicating that NGF enhances both the activity of existing TRPV1 and the expression level of TRPV1 in DRG neurons.

The short-term sensitisation of TRPV1 by NGF occurs so rapidly that it must be mediated by post-translational processing. Several groups have investigated the mechanisms underlying the acute effect of NGF on TRPV1, but the intracellular signalling involved in this sensitisation process is

still controversial. Phosphorylation of TRPV1 *via* PKA was at first suggested to be involved in the sensitisation of DRG neurons by NGF [107]. Later, experiments using calcium imaging rather than patch clamp, in order to measure sensitisation in a larger number of neurons and so improve the statistics, discounted PKA as a participant but found that partial or total inhibition of the action of NGF was produced by inhibitors of PI3 kinase (PI3K), PKC and CaMKII but not of the Ras-MEK-Erk cascade, at least on a short-term time scale [13]. Later studies, however, have suggested an involvement of the Ras-MEK-Erk cascade [3,137]. A different mechanism was proposed by Chuang and colleagues [23], who suggested that NGF sensitises TRPV1 by activating PLC γ , which hydrolyses PIP₂, thereby releasing TRPV1 from PIP₂-mediated inhibition. A region was identified in the C-terminal domain of TRPV1 (777-820), which was proposed to be essential for PIP₂-TRPV1 interaction [92]. However, this mechanism was questioned by calcium imaging experiments using isolated DRG neurones [13] and patch clamp and behavioural experiments in adult rats [137] which demonstrated the participation of PI3K and not PLC γ in both the enhancement of TRPV1 function and the NGF-induced hyperalgesia *in vivo*.

More recent data have shown that sensitisation of TRPV1 by NGF is mediated by two pathways [136]. The major pathway is activated by the Y760 site on TrkA, which stimulates PI3 kinase, with Src kinase being activated downstream of PI3K. Src kinase phosphorylates TRPV1 at a single tyrosine residue, Y199 (Y200 in human TRPV1), leading to trafficking and insertion of the channel into the surface membrane and thus enhancing membrane ionic currents carried by TRPV1 (see Fig. 2). A second and more minor pathway is the PLC γ / PKC ϵ signalling pathway, which causes phosphorylation of TRPV1 at the S502 and S801 sites. Furthermore, deletion of the proposed PIP₂ binding cassette [92] increased hTRPV1 tyrosine phosphorylation at the Y200 site and sensitised the basal activity of the channel, suggesting that the change in behaviour of TRPV1 following the 777-820 deletion is a consequence of sensitisation of the channel by Src, and not of removal of a PIP₂ binding site *per se* [136].

In addition to its short-term action, NGF is responsible, at least in part, for the upregulation of TRPV1 expression following inflammation [6,46]. However, in DRG neurons, neither an increase in TRPV1 mRNA levels was detected after the injection of NGF [46], nor was there any change in the TRPV1 mRNA level found in PC12 cells treated with NGF [93]. In contrast, increased axonal transport of TRPV1 mRNA was observed after carrageenan treatment [117], and expression of TRPV1 protein was promoted by NGF in both DRG neurons and PC12 cells [46,93]. Therefore, TRPV1 does not show parallel NGF-induced changes in mRNA and protein levels, indicating that NGF regulates TRPV1 expression by increasing translation and transport of the channel without changing the degree of transcription. The increase in TRPV1 expression induced by NGF was found to be mediated by the TrkA-dependent MAPK pathways [15]. Small GTP binding regulatory proteins, Ras and Rac, are believed to play an important role in initiating the signalling pathway [15]. It has recently been suggested that Rac activates NADPH oxidase, which subsequently generates reactive

oxygen species (ROS) leading to stimulation of p38 MAPK activity [93]. Activation of p38 MAPK results in upregulation of TRPV1 expression and increases pain perception [46]. Other studies show that Erk, another member of the MAPK family, and PI3K may also be involved in this process [15].

Lipid Mediators

Prostaglandins are generated in response to noxious stimuli and inflammatory insults [12]. Several prostanoid receptor subtypes are reported to be expressed in DRG neurons, including the PGE₂ receptors EP(1-4) [84,114] and the PGI₂ receptor IP [64]. Critical actions of EP and IP receptors in inflammation and pain were later demonstrated by behavioural studies of mice lacking IP, EP1 or EP3 receptors, where in all three cases, mice lacking one of these receptors showed a significant reduction in pain perception and thermal hyperalgesia [71,75,77,113]. At the cellular level, when TRPV1 was knocked out, the PGI₂-induced thermal hyperalgesia was found to almost completely disappear, indicating that TRPV1 is an essential contributor to prostaglandin-mediated thermal hyperalgesia [75]. Two second-messenger pathways are implicated in potentiation of TRPV1 by PGE₂ and PGI₂ (see Fig. 2): the PKC-dependent pathway [75] and the cAMP / PKA signalling cascade, both of which sensitise TRPV1 by phosphorylating specific sites on the channel [35,62,111].

Prostaglandins work not only as mediators that directly activate downstream cascades leading to sensitisation of TRPV1, but also function as second messengers in the potentiation of TRPV1 by other inflammatory mediators. One such mediator is glutamate, which contributes to the development of inflammatory hyperalgesia through the group I metabotropic glutamate receptors, of which mGluR1 and mGluR5 are expressed in sensory neurons [9,128]. Application of an mGluR1/5 agonist DHPG enhanced the capsaicin response in DRG neurons, indicating that TRPV1 is involved in the hyperalgesia evoked by mGluR1/5 activation [41]. mGluR1/5 receptors primarily couple to the PLC pathway, which activates PKC downstream [14]. However, the DHPG-induced enhancement of the capsaicin response does not seem to depend on the activation of PKC but instead on the activation of cyclooxygenases (COXs) and PKA, suggesting that mGluR1/5 activation sensitises TRPV1 by a novel mechanism: PLC activation results in generation of lipid messengers (e.g. arachidonic acid), which are metabolised by COX into prostaglandins, ultimately activating the PKA pathway through prostaglandin receptors [41]. Thus, prostaglandins may play a crucial role in glutamate-induced TRPV1 sensitisation (Fig. 2).

Anandamide (*N*-arachidonoyl-ethanolamide) is another potential lipid mediator in inflammatory hyperalgesia [86, 138]. The role of anandamide in nociception is potentially complex, because two receptors are activated by anandamide in DRG neurones: TRPV1 itself, and the cannabinoid 1 (CB1) receptor, both of which show a high degree of colocalisation [1,2]. The significant structural similarity between anandamide and some of the synthetic vanilloids, such as olvanil, suggested that anandamide may be an endogenous agonist of TRPV1 [28]. However, the potency of anandamide for

TRPV1 is 5 ~ 10 fold lower than capsaicin in various native tissues [99]. For, example, in DRG neurons, the EC₅₀ of anandamide is 6 ~ 10 μ M [44,85,109]. Thus micromolar concentrations of anandamide are required to activate TRPV1 and it is unclear whether endogenous concentrations of anandamide will be sufficient to activate the channel. Some evidence suggests that during inflammation, other inflammatory mediators, such as bradykinin and PGE₂, may activate pathways that convert anandamide to a potent TRPV1 activator [108]. However, at much lower concentrations anandamide activates the CB1 receptor, which has a dual effect on the sensitivity of TRPV1 [40]. Firstly, activation of the CB1 receptor was observed to enhance the TRPV1 response to capsaicin, an effect suggested to be mediated by PI3K and PLC pathways [40]. On the other hand, CB1 receptor activation has been found to attenuate rather than enhance TRPV1 activation when PKA is stimulated by forskolin [40]. Such an effect might explain the findings that cannabinoids can reduce hyperalgesia and inflammation, at least in part by inhibition of TRPV1-mediated release of transmitters from terminals of sensory neurones [96]. Thus, anandamide may play a dual role on TRPV1 activity, directly and *via* the CB1 receptor. However, it has been suggested that during inflammation or other pathological conditions, because of the increase in TRPV1 expression in neurons, an increased anandamide concentration in terminals may lead to an enhancement rather than a decrease in inflammatory heat hyperalgesia [108]. This hypothesis was supported by the earlier findings that anandamide increases release of CGRP in diabetic animals but inhibits it in normal animals [32].

Other Inflammatory Mediators

As the role of different inflammatory mediators in modulating the activity of TRPV1 is more extensively studied, more candidates have been added to the list of mediators that may regulate the TRPV1 response during inflammation (see Fig. 2). For example, following a similar pattern to NGF (discussed above), IGF-1 and insulin have been found to enhance TRPV1-mediated membrane currents both by enhancing the channel gating properties and by translocation of TRPV1 to the plasma membrane [122]. As with NGF, both PI3K and PKC-mediated TRPV1 phosphorylation were found to be essential for the enhancement of the TRPV1 response by IGF-1 / insulin [122].

Phosphorylation of TRPV1 by PKC is involved in the sensitisation of TRPV1 by several other inflammatory mediators. Chemokines, in particular CCL3 (formerly known as macrophage inflammatory protein 1 α), play an important role in mediating inflammation [83,101]. In a similar manner to bradykinin or ATP, CCL3 sensitises TRPV1 by activation of its major receptor CCR1, which leads to activation of the PLC / PKC pathway [135]. The protease-activated receptor-2 (PAR-2), whose activation is associated with inflammation in the lung [103], was found to promote TRPV1 function through both the PKC-dependent and the PKA-dependent pathways [4,5]. Serotonin (5HT) also appears to sensitise TRPV1 *via* both PKC and PKA pathways [115]. Recent data indicate that PKC ϵ is also responsible for the sensitisation of TRPV1 by the prokineticin/prokineticin receptor system

[125]. Prokineticins (or their anuran homolog, Bv8) induce hyperalgesia in rats through binding to the prokineticin receptors 1 and 2 (PKR1 and PKR2, refs [73,79,80]; see article by L. Negri in this issue). Activation of PKRs by Bv8 induces translocation of PKC ϵ to the membrane in DRG neurons, indicating that Bv8 potentiates TRPV1 *via* phosphorylation of the channel by PKC ϵ [125]. Finally, sensitisation of TRPV1 by PKC appears to underlie the IL-1 β - induced potentiation of heat-evoked currents in rat DRG neurons, and tyrosine phosphorylation of TRPV1 may also be involved [47,82].

CONCLUSION

We have reviewed here the role of inflammatory mediators in modulating activities of TRPV1 following tissue injury and inflammation. The available evidence suggests that inflammatory mediators alter the sensitivity of TRPV1 by various signalling pathways (including PLC/PKC, PKA, ERK/MAPK, and PI3K/Src), suggesting a complex network *in vivo* for modulation of channel function under both physiological and pathological conditions. Sensitisation of TRPV1 *via* phosphorylation by PKC and/or PKA is by now a well-established mechanism of sensitisation of the gating of TRPV1, which is employed by a number of inflammatory mediators. Insertion of new TRPV1 channels into the nociceptor membrane is a recently-discovered and novel mechanism for the development and maintenance of inflammatory hyperalgesia. Recent work has shown that the trafficking of TRPV1 to the neuronal membrane is upregulated by phosphorylation of TRPV1 by Src. While this pathway was first elucidated for NGF-induced hyperalgesia, it seems likely that other inflammatory mediators activating tyrosine kinase receptors will employ the same route to induce hyperalgesia. Modulation of TRPV1 may be further complicated by cross-talk between the various signalling pathways.

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