Contribution of Ion Channel Trafficking to Nociceptor Sensitization

M. Camprubí-Robles[1,3], A. Ferrer-Montiel[1] and R. Planells-Cases*[,2]

[1]*Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Alicante, Spain;* [2]*Centro de Investigación Príncipe Felipe, Valencia, Spain;* [3]*Present address Division of Physiology, Department of Physiology and Medical Physics, Innsbruck Medical University, Innsbruck, Austria*

Abstract: Nociceptor sensitization is a process triggered by proinflammatory factors that result in a significant increase in neuronal excitability by affecting both the threshold and frequency of action potential firing. The increased sensory neuron activity is due to a metabolic change produced by the activation of intracellular signaling cascades that usually alter the expression and functionality of molecular sensors present in the neuronal surface, i.e. ion channels and metabotropic receptors. Cumulative evidence is showing that inflammatory sensitization of nociceptors is significantly contributed by an enhanced expression of ion channels that directly influence neuronal excitability. Furthermore, recent data indicates that mobilization and regulated exocytosis of a ready-to-go vesicular population of channels as a pivotal event underlying acute inflammatory sensitization of sensory neurons; whereas an increment in transcription and/or translation and trafficking is involved in chronic neuronal sensitization. Therefore, identification of the molecular components involved in channel trafficking and exocytosis in the inflamed terminal may provide new strategies for attenuating nociceptor sensitization and their consequences, namely hyperalgesia and allodynia.

Keywords: Hyperalgesia, nociceptors, membrane traffic, channel receptors.

INTRODUCTION

Noxious stimuli evoke numerous physiological events triggering acute pain. However, in specific pathological circumstances, an enhanced sensitivity to mild or non-noxious stimuli appears long after vanishing the original cause for pain, resulting in the development of chronic pain (for review [1]). Chronic pain can be classified as *inflammatory pain*, when tissue and/or viscera are injured or inflamed, or as *neuropathic pain*, following nervous system lesion or dysfunction. Further distinction is made depending on the stimulus intensity causing pain: the term *hyperalgesia* is applied when pain is caused by low-intensity stimuli and may be provoked by either a decrease in threshold, an increase in suprathreshold response, or both, whereas *allodynia* is defined when an innocuous stimuli causes pain (IASP). Hyperalgesia is the preferred term and can be further classified according to the nature that elicits pain as thermal or mechanical or both (polymodal).

Hyperalgesia arises either by peripheral and/or central sensitization. *Peripheral sensitization* occurs by enhanced excitability of high threshold Aδ- and C-fibres in Dorsal Root Ganglion cells (DRGs), trigeminal or nodose ganglia, is a transient phenomenon and remains constrained to the area of the injury. Noteworthy, not all Aδ- and C-fibres are nociceptive because they are responsive to low threshold stimuli. Likewise, large diameter-, Aβ fibers are also low threshold and only some of them may be nociceptive. *Central sensitization* is activity and use-dependent, and results in an increase in nociceptor synaptic efficacy and an enhanced responsiveness of dorsal horn neurons. NMDA, non-NMDA and metabotropic glutamate receptors, ligand- and voltage-gated ion channels and neuropeptides such as Substance P deeply contribute to synaptic plasticity within CNS (For review see [2, 3]). Longer lasting changes in the properties of dorsal horn neurons result from intracellular signalling cascade activation that promote either posttransductional changes (such as phosphorylation) of ion channels and neurotransmitter receptors, altering electrical properties of the neuron or/and transcriptional changes in gene expression thus producing the phenotypic switching found in chronic pain (Fig. **1**).

Various metabotropic and ionotropic receptors regulate neuronal excitability and synaptic transmission in the nociceptive sensory pathway playing key roles in both the induction and in the maintenance of nociceptor sensitization. Thus, for instance, several Transient Receptor Potential (TRP) channels, voltage-gated Ca^{2+} and Na^{+} channels, purinergic P2X receptors, Glutamate Receptors (NMDA-, Kainate- and AMPA- Receptor types) have been involved in neuronal sensitization. Whereas long term plasticity requires expression/silencing of specific genes, short term plasticity can be achieved by covalent modification of the receptor subunits (phosphorylation/dephosphorylation) thus altering the receptor biophysical properties and/or altering the number of functional receptor expressed at the cell surface. This trafficking mechanism that involves receptor expression, exocytosis, internalization and recycling underlies peripheral sensitization and is the topic reviewed in this chapter.

TRP CHANNELS

Transient Receptor Potential (TRP) channels constitute a large family of channels with a wide variety of: i) activation modes (voltage, temperature, pH, chemicals, stress, etc), ii) regulation and, iii) physiological functions. Given that six

*Address correspondence to this author at the Centro de Investigación Príncipe Felipe, Av. Autopista del Saler 16, 46012, Valencia, Spain;
Tel: +34963289680; Fax: +34963289701; E-mail: rplanells@cipf.es

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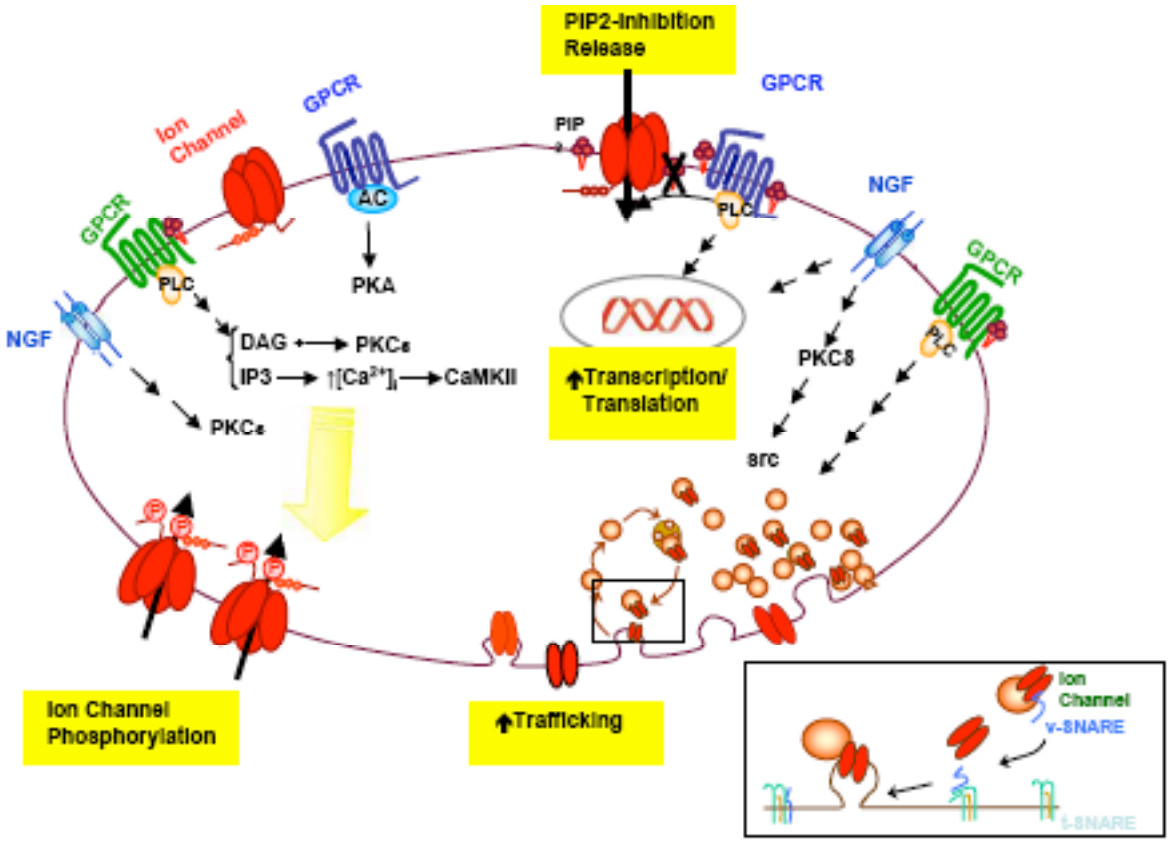

Fig. (1). Induction of nociceptor sensitization can be achieved by the functional overactivation of ion channel activity. Release of pro-inflammatory agents bind different GPCRs that activate second messenger signalling cascades, phosphorylate/dephosphorylate or alter PIP_2-induced inhibition of ion channels thus altering intrinsic channel open probability. Activation of intracellular cascades can also alter the number and sub-cellular distribution of receptor channels expressed at the cell surface, thus modulating medium and long-term sensitization. Many signalling components share common pathways for different effectors.

TRP channels are expressed in DRG neurons and given their ability to influx cations in response to diverse stimuli, they became good candidates for chemical, mechanical or temperature transducers and even to contribute to the initiation and maintenance of acute and chronic pain. These channels are TRPV1 to TRPV4, TRPM8 and TRPA1.

TRPV1 was the first TRP receptor isolated from a DRG library using an expression-functional screening with capsaicin, the pungent component in chili peppers [4], which was already known to produce a flow of cations through nociceptor membranes [5]. TRPV1 is one of the main effectors that integrate multiple noxious stimuli and its activation leads to the sensitization of peripheral sensory nerves. Physiological and behavioural studies of TRPV1 knockout animals have indicated a clear role of TRPV1 in the acute phase of thermal hyperalgesia (in the setting of inflammation) [6, 7] and also in the chronic (but not in the acute) phase of mechanical hyperalgesia [8].

TRPV1 is expressed in a wide variety of tissues although the highest levels of expression are found in small and medium diameter neurons in DRGs, trigeminal ganglia and nodose ganglia. TRPV1 is activated by different physical (T≥42ºC and voltage) and chemical stimuli, such as exogenous vanilloids like capsaicin and resiniferatoxin, by acid and basic pH, by anandamide and lipooxigenase metabolites and by proalgesic substances released during injury (for review see [9]). In normal conditions, singular mediators display low potency and efficacy on TRPV1 activation. Nonetheless, upon a lesion or under inflammatory conditions, several of these modulators are locally released and can act synergistically, leading to a reduction of the temperature threshold from 42ºC to ≈37ºC, and to nociceptor over-activation at body temperature.

TRPV1 potentiation by pro-algesic substances involves either direct phosphorylation by an interplay of kinases or phosphatases or by hydrolysis of phosphoinositides like phosphatidylinositol-4,5-bisphosphate (PIP_2) thus releasing TRPV1 from PIP_2-mediated inhibition [10, 11]. In addition, augmented nociceptor response due to enhanced cytoplasmic membrane receptor can be achieved either by mobilization of a subcellular reserve pool and/or by increasing the transcription/translation of the receptor [12, 13] . Some growth factors like Nerve Growth Factor (NGF) are upregulated in inflamed tissues inducing phenotypic changes in nociceptors and increasing peripheral heat hyperalgesia [14-16]. NGF appears to sensitize peripheral nociceptors through fast and slow mechanisms altering TRPV1 activity at different levels. The fast mechanism involves binding of NGF to its high affinity receptor *trkA* thus leading to Phospholipase C (PLC_γ) hydrolysis, membrane PIP_2 level reduction, and subsequent release of PIP_2-mediated inhibition of TRPV1 [15]. NGF also involves phospha-

tidylinositol-3-kinase (PI3K), Protein Kinase C ε (PKCε) and Ca^{2+}/calmodulin-dependent protein kinase (CAMKII) signalling cascades activation. Phosphorylation of TRPV1 in specific Serine/Threonine (Ser/Thr) residues by these kinases increases TRPV1 open probability, sensitization and thus leads to inflammatory heat hyperalgesia [17, 18]. A similar mechanism could be implicated in Bradikynin (BK)-evoked sensitization of TRPV1, since it involves PLC hydrolysis [15] and PKCε activation [19]. Interestingly, in addition to Ser/Thr phosphorylation, NGF also activates *Src* kinase through the PI3K cascade and promotes phosphorylation of Tyrosine-199 at the N-terminus domain ensuing translocation of TRPV1 to the cell surface [18]. In addition to NGF, other mediators such as insulin and insulin growth factor-I (IGF-I) promote TRPV1 potentiation in heterologous expression systems and in DRG neurons via the PI3K signalling cascade and subsequent PKC activation. Thus, the enhanced TRPV1 activity upon release of NGF, insulin or IGF-I can be accounted from a significantly increased open channel probability as well as ~2-fold receptor accumulation at the plasma membrane [20].

Regulated exocytosis seems to lie beneath TRPV1 surface receptor translocation as a regulated mechanism that contributes to nociceptor sensitization. Indeed, using the cytosolic N-terminus domain of TRPV1 as bait in a yeast-two hybrid screen, we identified two vesicular proteins involved in the Ca^{2+}-dependent neuronal exocytosis as interacting partners of TRPV1. The isolation of Snapin and Synaptotagmin IX (SytIX) suggested a vesicular reservoir of TRPV1 that may intervene in the TRPV1 trafficking and/or delivery to the plasma membrane. In heterologous expression systems, TRPV1 trafficking to the plasma membrane was promoted by PKC signalling activation and readily blocked by botulinum neurotoxins as well as overexpression of both snapin and SytIX. Thus, we concluded that PKC- sensitization of TRPV1 receptors was due at least in part by regulated exocytosis of channels located in a pool of cytosolic vesicles [21]. Evidence that botulinum neurotoxin attenuated heat hyperalgesia lent support to this hypothesis, although additional *in vivo* experiments are needed to determine the precise role of receptor exocytosis to the maintenance of neurogenic inflammation.

Whether TRPV1 translocation by SNARE-dependent exocytosis is a general mechanism promoted by some or all the different inflammatory ingredients released in the "Inflammatory Soup" was analyzed in cultured DRG nociceptors using a specific blocker of SNARE-dependent, Ca^{2+}-activated neuronal exocytosis, namely a 6-mer peptide patterned after the N-terminus of the SNAP25 protein [22]. These experiments substantiated that neuronal exocytosis of TRPV1 channels play a key role in nociceptor sensitization and, additionally show that regulated secretion was not a general mechanism used by all pro-inflammatory mediators. Hence, NGF, IGF-I and ATP promoted both nociceptor sensitization and TRPV1 surface translocation by a SNARE-dependent exocytosis, translocation that was readily prevented by inhibiting regulated exocytosis with the 6-mer peptide patterned after the SNAP25 protein. In contrast, IL-1β, BK and Artemin (a neurotrophin from the Glial Cell Derived Neurotrophic Factor (GDNF) family) promoted sensitization by a mechanism that was independent of regulated exocytosis since their neuronal potentiation was fully insensitive to the inhibitory action of the 6-mer peptide. Therefore, this study indicates that TRPV1 translocation to the neuronal surface under inflammatory conditions is an important but not general mechanism of nociceptor sensitization [23].

The slower and sustained inflammatory response evoked by NGF injection is mediated by changes in sensory neurons that involve retrograde transport of the neurotrophin to the cell soma where it activates the p38 mitogen-activated protein kinase (MAPK) and increase the translation and transport of TRPV1 to the peripheral nociceptor terminal, where it contributes to enhance heat hypersensitivity [13]. It is interesting to note that the p38-mediated signalling pathway has been also involved in the inflammatory hyperalgesia evoked through activation of BK B_1 receptors. Thus, an increment in the expression of TRPV1 channels in the peripheral nociceptor terminals seems fundamental not only for the induction but also for the maintenance of hyperalgesic conditions.

Furthermore, altered distribution of TRPV1 has been reported in different animal models of chronic pain. Thus, for instance, a shift of TRPV1 from small unmyelinated C fibers to myelinated Aβ-fibers has been reported in the nodose ganglia of a chronic airway inflammation rat model [24] and in DRGs of a diabetes-induced animal model [25]. In addition, an increase in TRPV1 immunoreactivity has been reported for human conditions such as vulvodynia [26]. Therefore, an increase of channel activity along with a change in nociceptor neurons that express the receptor gives rise to a sensitization and phenotypic change that results in the development of chronic pain associated to disease.

TRPV2, TRPV3, TRPV4, TRPA1 and TRPM8 are also widely expressed in non neuronal and neuronal cells and most remarkably in nociceptors. Besides TRPV1, other TRP channels such as TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1 are classified as thermoreceptors. Additionally, TRPV4 and TRPA1 are also considered mechanoreceptors. Nonetheless they are polymodal receptors since they specifically respond to different irritant and aromatic compounds, to endocannabinoids, and to a wide temperature range (from noxious cold to noxious heat). Studies in mice lacking any of these TRP channels suggest that they might play important roles in either thermal or/and mechanical hyperalgesia and even to neuropathic pain. Although much lesser information is available regarding trafficking of these receptors during nociceptor sensitization, some similarities are shared with those reported for TRPV1. For instance, TRPV2, which shares ~50% homology with TRPV1, activates with a threshold T≥ 52ºC, by cell swelling and by 2- aminoethoxydiphenylborate (2-APB). In contrast to TRPV1, TRPV2 is highly expressed in Aδ fibers and in to a lesser extent in C-fibers. Little is known about TRPV2 regulation but IGF-I and PI3K signalling seem to translocate TRPV2 to the plasma membrane [27, 28]. Its role in thermal nociception is not clear but TRPV2 upregulation reported in medium size diameter neurons in the nociception-induced test model of intraplantar Complete *Freund's* Adjuvant (CFA) injection suggested a role in thermal hyperalgesia during inflammation [29]. Nonetheless, because TRPV2 appears to associate with TRPV1 subunits [30], the increased

TRPV2 expression observed during sensitization may be the consequence of TRPV1-TRPV2 heterooligomerization.

TRPA1 receptor is also a candidate sensor regulated in inflammatory conditions. TRPA1 is expressed in free endings from small diameter peptidergic C-fibers and highly coexpressed with TRPV1. Because TRPA1 is also expressed in inner hair cells and its fly homologue is a mechanoreceptor, TRPA1 was initially considered to be involved in mechanical nociception. Whereas the role of TRPA1 in mechanosensitivity and in detection of noxious cold-stimuli in TRPA1 knockout mice has resulted in controversial results, a clear role of TRPA1 has been demonstrated in sensing irritating substances such as mustard oil, acrolein and garlic [31, 32]. Regarding TRPA1 regulation, BK promotes TRPA1 potentiation through activation of PLC. A transgenic mouse model overexpressing the growth factor Artemin, generated hypertrophied DRGs with high expression of both TRPV1 and TRPA1 receptors. The heat threshold in C-fibres from these mice was reduced whereas the mechanical threshold was unaltered [33]. Interestingly, a close functional interaction/cooperativity between both TRPV1 and TRPA1 receptors has been reported for the activation and internalization of TRPA1 [34]. Recently, the biophysical properties of native TRPA1 in DRGs were shown to better compare with heterologously TRPA1/TRPV1 expressing cells than those expressing TRPA1 alone suggesting a functional cooperation between both TRP receptors [35]. Indeed, it may be possible that both channels co-localize in the same vesicular population and that inflammatory mediators promote the surface expression of the receptors, thus linking thermal and mechanical hypersensitivity. Additional experimental evidence is required to prove this exciting hypothesis.

ASIC CHANNELS

Acid-sensing ion channels (ASICs), which belong to the degenerin/epithelial Na^+ channel protein superfamily, activate upon an extracellular pH reduction by triggering Na^+ influx and membrane depolarization. ASICs are expressed in peripheral sensory neurons where they primarily function in nociception [36-40], mechanosensation [41-43] and taste transduction [44, 45]. Four of the seven cloned subunits have been reported in nociceptors: ASIC1a, ASIC1b, ASIC2b and ASIC3 [40, 46, 47]. In DRG neurons, ASIC1a is often associated with the ASIC2 subunit thus forming heteromers, although ASIC1a is also found as part of homomers. Interestingly, ASIC channels have been reported both in the soma and in the peripheral endings of DRG neurons but not in the central projection [41, 46]. Furthermore, in inflammatory conditions, the transcript levels of different ASIC isoforms are highly increased and Aβ neurons acquire a pain fibre resembling phenotype by increasing number of acid responding neurons [48]. In contrast, ASIC1a transcripts decrease in spinal nerve ligation models [49].

Formation and trafficking of functional receptors to and from the plasma membrane and the receptor/channel activity often relay in regulatory proteins such as chaperones, adaptor and/or cytoskeleton proteins. Using a yeast two-hybrid screen, annexin II light chain p11 was found to bind to ASIC1a N-terminal domain and modulate its trafficking to the plasma membrane [50]. Other proteins have been identified as ASIC-interacting proteins. For instance, PICK-1, a PSD-95 Drosophila discs-large protein Zonula occludens protein 1 (PDZ) domain-containing protein, was found to interact with ASIC1 subunit, depending on the ASIC phosphorylation state, thus determining ASIC1 subcellular distribution [51]. On the other hand, ASIC3 subunit has been illustrated to interact with CIPP, a multivalent PDZ domain-containing protein, significantly increasing ASIC3 current density [52]. Heterologous expression of ASIC3 with PDZ domain-containing proteins PSD-95 and Lin-7b has shown interaction between these proteins and ASIC3. However, while PSD-95 decreases ASIC3 cell-surface expression, Lin-7b accumulates ASIC3 at the cell membrane [53].

VOLTAGE-GATED SODIUM CHANNEL (NAV)

Four voltage-gated Na^+ (Na_v) channels have been reported as being specifically expressed in DRGs: $Na_v1.1$ and $Na_v1.3$ which constitute the tetrodotoxin (TTX)-sensitive Na^+ current, and $Na_v1.8$ and $Na_v1.9$, that originate the TTX-resistant currents. Human mutation of Na_v in different pain disorders as well as specific gene knockout in mice have provided great insight of nociceptor Na_v channels in chronic pain conditions (for review see [54]). For instance, $Na_v1.3$ is highly expressed during development although becomes over-expressed in adults upon inflammation or axotomy (a model of neuropathic pain) and reverses with GDNF treatment [55]. Different studies exploiting the knockdown or knockout of this gene have suggested a role in neuropathic pain although the exact contribution remains controversial. Among TTX-resistant channels, $Na_v1.8$ has been shown to support action potential electrogenesis in small and medium diameter DRG neurons [56]. Furthermore, several human and rodent neuropathies seem accompanied by an increased $Na_V1.8$ immunoreactivity in peripheral axons near the injury site [57] suggesting a functional peripheral upregulation of $Na_V1.8$ [58]. However, discrepancies between these findings and the observed reduction of $Na_V1.8$ protein and mRNA expression in the cell bodies of injured sensory neurons as well as the normal development of neuropathic pain behaviour in $Na_V1.8$–null mice [59] raises controversy about its role in the development of neuropathic pain. In contrast, $Na_v1.9$ null mice displayed an almost absent response to proalgesic substances such as formalin, CFA or to inflammatory mediators such as ATP or bradikynin [60, 61].

Inflammatory mediators such as PGE_2 and serotonin modulate TTX- resistant currents via PKA and PKC signalling cascades. Interestingly, several groups found an upregulation of TTX-resistant currents in rat models of acute inflammation [62-64]. In chronic inflammatory pain $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ have been shown to be up-regulated shortly and rapidly upon a single injection of CFA [65, 66] as well as after longer periods (up to 28 days) [67] suggesting that increased Na_v channels expression contributes to the initiation and maintenance of nociceptor hyperexcitability.

PURINERGIC P2X RECEPTORS

ATP can be released from injured tissue and locally depolarize DRGs and dorsal horn neurons from spinal cord through P2X receptors. P2X receptors are expressed in DRGs both at peripheral terminals, where they may initiate

nerve impulse, and in central terminals modulating sensory transmission to spinal cord. Seven P2X subunit have been identified that can form at least eleven functional forms [68]. $P2X_4$ and $P2X_7$ receptors have been proposed as being involved in chronic pain but the underlying mechanism are yet unclear. For instance, $P2X_5$ subunit expression is overexpressed in DRGs following spinal nerve ligation (a model for neuropathic pain) [69]. Furthermore, $P2X_7$ deletion in mice reduced both neuropathic and chronic inflammatory pain [70]. However, $P2X_3$ are the most extensively studied and can form both $P2X_3$ homomers or $P2X_{3/2}$ heteromers, with different kinetics upon infusion with α,β-methylene ATP. $P2X_3$ is expressed on a subset of primary afferent neurons and, whereas homomers are highly expressed in TRPV1-containing DRG neurons and seem to mediate thermal pain, $P2X_{3/2}$ heteromers are expressed in non-TRPV1 expressing DRGs and intervene in mechanical pain sensation [57, 58].

Several inflammatory mediators modulate P2X receptors through subunit phosphorylation. One of the mechanisms proposed to link P2X receptors and nociceptor sensitization is up-regulation of $P2X_3$ and $P2X_4$ on DRG neurons upon inflammation. Indeed, CFA treatment induced a two- to three-fold increase in ATP current density and changed the voltage-dependence and enhanced ~2-fold total receptor protein expression [71]. The neuropeptide CGRP, a vasodilator agent with pro-inflammatory ability, produced an increased peak current after 1h treatment as well as faster recovery from desensitization. Interestingly, biotinylation of surface proteins demonstrated a $P2X_3$ receptor increase 2-5h after CGRP infusion due to an enhanced transcription and translation after the neuropeptide treatment [72]. The mechanism of CGRP on the overexpression of ATP receptors on trigeminal neurons could underlie the mechanism of increased plasma CGRP release and migraine. Alternatively, P2X receptor sensitization was obtained after infusion with growth factors such as NGF and Glia-cell line-derived Factor (GDNF). While GDNF up-regulates $P2X_3$ in regions where it is normally localized, NGF induced an abnormal *de novo* immunoreactivity of axons projecting to innapropiate lamina in spinal cord, so that peripheral ATP would now be able to activate a distinct population of nociceptive dorsal horn neurons from that normally activated [73].

METABOTROPIC RECEPTORS

Although these receptors have no intrinsic ion channel activity, they usually modulate the functionality of ion channels present in sensory neurons. Thus, we will briefly expose the current knowledge on the alteration of their levels and functionality upon inflammatory sensitization of nociceptors.

Metabotropic Receptors constitute a large and functionally diverse receptor family with a common architecture of seven trans-membrane (7-TM) domains that signal through activation of intracellular heterotrimeric G proteins, and are referred to as G-protein coupled receptors (GPCRs). Agonist binding classically occurs in the receptor extracellular protein domain, inducing a protein conformational change that translates into Receptor-G protein coupling and activation. GPCRs modulate ion channel activity either by second messenger cascade activation, kinase activation and channel phosphorylation or by direct $G\beta\gamma$ subunits binding to the ion channel. Several GPCRs underlie pain modulation (for review [74]), here we will focus on those whose trafficking is regulated upon injury or in inflammatory conditions.

OPIOID RECEPTORS

Opiates are among the most effective drugs currently available for treating from acute to chronic pain. Small-medium- and large-diameter DRG neurons express opioid receptors and opioid peptides and the excitability of these neurons can be modulated by both endogenous opioids as well as by opiate drugs. These receptors directly or indirectly inhibit voltage-gated Ca^{2+}-channels, TTX-resistant Na^{+}-channels or open K^{+}-channels, or other ion channels such as P2X and TRPV1 receptors.

The three classical opioid receptor subfamilies described: mu-, delta- and kappa-opioid receptors (MOR, DOR and KOR, respectively) seem to be involved upon tissue lesion or inflammation, since the systemic or local application of specific agonists for each of the receptor subfamilies elicits pronounced pain attenuation [75, 76]. Interestingly, inflammation induced in a CFA animal model increased MOR and DOR expression [77, 78] while KOR remained unaltered. MOR upregulation is caused both by an increase of the number of MOR-expressing neurons, by augmenting MOR density per neuron [79] and by enhancing G-protein coupling efficacy while the affinity for its specific agonist DAMGO remains unaltered [79]. This increase in MOR positive cells and receptor density may be related to mediators of inflammation [80]. Bradykinin and capsaicin activation of DRGs also promotes membrane recruitment of opioid receptors to the plasma membrane [81-83].

Nowadays, most of the clinically used opioids are specific for MORs. Sistemic administration of opioids display unwanted central side effects (like tolerance and dependence) while a large proportion of the analgesic effects seem to be mediated by peripheral opioid receptors. An important line of pharmacological developments includes opioids that do not cross the blood-brain barrier. Alternatively, specific DOR agonists are currently been explored because their specific analgesic efficacy and much lower incidence of dependence. Nevertheless, DOR selective agonists have been shown effective in preclinical studies in various pain models such as cancer, neuropathic and inflammatory pain. Several groups have shown that DOR analgesic activity arise by a translocation of DOR to the plasma membrane which, in DRG neurons is mediated by a regulated secretory mechanism that depends on neuronal activity or noxious stimulation [82, 84-86].

Patients with either long term- or high dose- treatment with opioid agonists described tolerance and sometimes hyperalgesia. High dose of agonist leads to receptor desensitization which is associated to receptor phosphorylation, to agonist-mediated receptor internalization and to downregulation. Although receptor internalization was initially correlated to opioid tolerance, nowadays is a subject of controversy. Indeed, recent studies have shown an inverse correlation between the potency of drugs to induce internalization/recycling and tolerance generation This internaliza-

tion mechanism, therefore, has been now proposed to prevent that inactivated receptors become internalized and further degraded but rather recycled to the cell surface in a reactivated state, thus preventing long term desensitization (for Review see [87]). On the other hand, opioid-induced hyperalgesia has been shown to elicit thermal and mechanical hyperalgesia by an enhanced TRPV1 receptor activity [88, 89] that, at least in part, is consistent with a moderate increase in TRPV1 positive cells within the spinal cord [89].

CANNABINOID RECEPTORS

In addition to the use of opioids, cannabinoids are considered nowadays either as alternative or supplementary pharmacological therapeutic candidates for the treatment of some chronic pain conditions. Unfortunately, cannabinoid use in humans is accompanied by central side effects such as sedation and clumsiness as well as transient memory loss and dependence. The Cannabinoid (CB) receptor family is formed by two members: CB1 and CB2, and possibly by the orphan receptor GPR55, all belonging to the 7-TM GPCR type of receptors signalling through the Gi/o proteins. CB1 and CB2 receptors are widely expressed in non-neuronal cells as well as in peripheral and central neurons. Parallel to peripherically injected opioids, peripheral administration of specific CB1 or CB2 agonists induce analgesia in several pain models. As previously mentioned, some endogenous cannabinoids operate as partial agonists of different receptors involved in nociception (such as TRPV1 and TRPA1). Nevertheless, CB receptor activity may indirectly activate the above TRP receptors plus TRPV2 and P2X receptors. In addition, CBs have been reported to inhibit different voltage gated channels (K_v, Na_v and Ca_v) as well as voltage insensitive channels TASK K^+ channels (For review see [90]). CB1 receptors are expressed in nociceptive DRG and trigeminal ganglia, specially medium- and large-diameter neurons [91-94]. Interestingly, upon inflammation, CB1 receptor expression became upregulated in C-fiber nociceptors, increasing labelling at nociceptors soma and free endings [95]. Although surface CB1 receptors have been located in both the axonal compartment and in the presynaptic terminal, only in the synaptic compartment undergoes constitutive receptor endocytosis and recycling in the chronic presence of agonist, suggesting that agonist-induced desensitization is correlated to the mobilization of a specific receptor from the synaptic terminal [96]. CB2 upregulation has also been observed in nociception states. Specifically, an increased reactivity was detected in afferent fibres at the spinal cord level in a fibre ligation model of neuropathic pain [97].

GLUTAMATE RECEPTORS

Peripheral and central sensitization involves activation of both ionotropic glutamate (NMDA, AMPA and/or kainate subtypes) receptors as well as metabotropic glutamate receptors (mGluRs). Released inflammatory mediators activate protein kinases PKC, PKA and CaMKII and Src that in turn phosphorylate these receptor subunits altering the channel open probability as well as trafficking to the synaptic membrane. Thus, for instance, intraplantar noxious stimulation with capsaicin or by heat leads to phosphorylation of NR1 PKA- and PKC-dependent phosphorylation [98-100]. NR1 phosphorylation is important for the suppression of an ER retention signal [100]. Furthermore, a rapid and sustained recruitment of GluR1 AMPA receptor subunit to plasma membrane was detected in spinal cord upon intracolonic capsaicin infusion, by a mechanism dependent, at least in part, of CaMKII activation [101]. Eight mGluR are classified in three groups depending on the activated cellular cascade and their pharmacology. Different mGluRs are expressed in DRGs and spinal cord neurons where they primarily function as autoreceptors to provide feedback regulation of glutamate release. Furthermore, mGluRs can also be found in GABAergic interneurons. Therefore, metabotropic receptors seem to regulate firing activity of spinal dorsal horn neurons in acute and chronic pain models both by controlling and by integrating excitatory and inhibitory inputs. Upregulation of specific mGluRs has been reported in some animal models of inflammatory an neuropathic pain [102, 103].

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REFERENCES

[1] Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science 2000; 288: 1765-69.

[2] Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol Dis 2001; 8: 1-10.

[3] Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 2003; 26: 696-705.

[4] Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997; 389: 816-24.

[5] Wood JN, Winter J, James IF, Rang HP, Yeats J, Bevan S. Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. J Neurosci 1988; 8: 3208-20.

[6] Caterina MJ, Leffler A, Malmberg AB, *et al.* Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000; 288: 306-13.

[7] Davis JB, Gray J, Gunthorpe MJ, *et al.* Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 2000; 405: 183-7.

[8] Szabo A, Helyes Z, Sandor K, *et al.* Role of transient receptor potential vanilloid 1 receptors in adjuvant-induced chronic arthritis: *in vivo* study using gene-deficient mice. J Pharmacol Exp Ther 2005; 314: 111-9.

[9] Planells-Cases R, Garcia-Sanz N, Morenilla-Palao C, Ferrer-Montiel A. Functional aspects and mechanisms of TRPV1 involvement in neurogenic inflammation that leads to thermal hyperalgesia. Pflugers Arch 2005; 451: 151-9.

[10] Prescott ED, Julius D. A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. Science 2003; 300: 1284-8.

[11] Klein RM, Ufret-Vincenty CA, Hua L, Gordon SE. Determinants of molecular specificity in phosphoinositide regulation. Phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) is the endogenous lipid regulating TRPV1. J Biol Chem 2008; 283: 26208-16.

[12] Obata K, Yamanaka H, Kobayashi K, *et al.* Role of mitogen-activated protein kinase activation in injured and intact primary afferent neurons for mechanical and heat hypersensitivity after spinal nerve ligation. J Neurosci 2004; 24: 10211-22.

[13] Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 2002; 36: 57-68.

[14] Amaya F, Shimosato G, Nagano M, *et al.* NGF and GDNF differentially regulate TRPV1 expression that contributes to development of inflammatory thermal hyperalgesia. Eur J Neurosci 2004; 20: 2303-10.

[15] Chuang HH, Prescott ED, Kong H, *et al.* Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 2001; 411: 957-62.

[16] Woolf CJ, Safieh-Garabedian B, Ma QP, Crilly P, Winter J. Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. Neuroscience 1994; 62: 327-31.

[17] Bonnington JK, McNaughton PA. Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. J Physiol 2003; 551:433-46.

[18] Zhang X, Huang J, McNaughton PA. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 2005; 24: 4211-23.

[19] Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA. Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. Neuron 1999; 23: 617-24.

[20] Van Buren JJ, Bhat S, Rotello R, Pauza ME, Premkumar LS. Sensitization and translocation of TRPV1 by insulin and IGF-I. Mol Pain 2005; 1: 17.

[21] Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A. Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 2004; 279: 25665-72.

[22] Blanes-Mira C, Merino JM, Valera E, *et al.* Small peptides patterned after the N-terminus domain of SNAP25 inhibit SNARE complex assembly and regulated exocytosis. J Neurochem 2004; 88: 124-35.

[23] Camprubi-Robles M, Planells-Cases R, Ferrer-Montiel A. Differential contribution of SNARE-dependent exocytosis to inflammatory potentiation of TRPV1 in nociceptors. FASEB J 2009; 23(11): 3722-33.

[24] Zhang G, Lin RL, Wiggers M, Snow DM, Lee LY. Altered expression of TRPV1 and sensitivity to capsaicin in pulmonary myelinated afferents following chronic airway inflammation in the rat. J Physiol 2008; 586: 5771-86.

[25] Hong S, Wiley JW. Early painful diabetic neuropathy is associated with differential changes in the expression and function of vanilloid receptor 1. J Biol Chem 2005; 280: 618-27.

[26] Tympanidis P, Casula MA, Yiangou Y, Terenghi G, Dowd P, Anand P. Increased vanilloid receptor VR1 innervation in vulvodynia. Eur J Pain 2004; 8: 129-33.

[27] Penna A, Juvin V, Chemin J, Compan V, Monet M, Rassendren FA. PI3-kinase promotes TRPV2 activity independently of channel translocation to the plasma membrane. Cell Calcium 2006; 39: 495-507.

[28] Kanzaki M, Zhang YQ, Mashima H, Li L, Shibata H, Kojima I. Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. Nat Cell Biol 1999; 1: 165-70.

[29] Shimosato G, Amaya F, Ueda M, Tanaka Y, Decosterd I, Tanaka M. Peripheral inflammation induces up-regulation of TRPV2 expression in rat DRG. Pain 2005; 119: 225-32.

[30] Liapi A, Wood JN. Extensive co-localization and heteromultimer formation of the vanilloid receptor-like protein TRPV2 and the capsaicin receptor TRPV1 in the adult rat cerebral cortex. Eur J Neurosci 2005; 22: 825-34.

[31] Bautista DM, Jordt SE, Nikai T, *et al.* TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 2006; 124: 1269-82.

[32] Kwan KY, Allchorne AJ, Vollrath MA, *et al.* TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 2006; 50: 277-89.

[33] Elitt CM, McIlwrath SL, Lawson JJ, *et al.* Artemin overexpression in skin enhances expression of TRPV1 and TRPA1 in cutaneous sensory neurons and leads to behavioral sensitivity to heat and cold. J Neurosci 2006; 26: 8578-87.

[34] Akopian AN, Ruparel NB, Jeske NA, Hargreaves KM. Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization. J Physiol 2007; 583: 175-93.

[35] Salas MM, Hargreaves KM, Akopian AN. TRPA1-mediated responses in trigeminal sensory neurons: interaction between TRPA1 and TRPV1. Eur J Neurosci 2009; 29: 1568-78.

[36] Mamet J, Baron A, Lazdunski M, Voilley N. Proinflammatory mediators, stimulators of sensory neuron excitability via the expression of acid-sensing ion channels. J Neurosci 2002; 22: 10662-70.

[37] Mamet J, Lazdunski M, Voilley N. How nerve growth factor drives physiological and inflammatory expressions of acid-sensing ion channel 3 in sensory neurons. J Biol Chem 2003; 278: 48907-13.

[38] Sluka KA, Price MP, Breese NM, Stucky CL, Wemmie JA, Welsh MJ. Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC1. Pain 2003; 106: 229-39.

[39] Ugawa S, Ueda T, Ishida Y, Nishigaki M, Shibata Y, Shimada S. Amiloride-blockable acid-sensing ion channels are leading acid sensors expressed in human nociceptors. J Clin Invest 2002; 110: 1185-90.

[40] Voilley N, de Weille J, Mamet J, Lazdunski M. Nonsteroid anti-inflammatory drugs inhibit both the activity and the inflammation-induced expression of acid-sensing ion channels in nociceptors. J Neurosci 2001; 21: 8026-33.

[41] Price MP, McIlwrath SL, Xie J, *et al.* The DRASIC cation channel contributes to the detection of cutaneous touch and acid stimuli in mice. Neuron 2001; 32: 1071-83.

[42] Price MP, Lewin GR, McIlwrath SL, *et al.* The mammalian sodium channel BNC1 is required for normal touch sensation. Nature 2000; 407: 1007-11.

[43] Page AJ, Brierley SM, Martin CM, *et al.* Different contributions of ASIC channels 1a, 2, and 3 in gastrointestinal mechanosensory function. Gut 2005; 54: 1408-15.

[44] Lin W, Ogura T, Kinnamon SC. Acid-activated cation currents in rat vallate taste receptor cells. J Neurophysiol 2002; 88: 133-41.

[45] Ugawa S. Identification of sour-taste receptor genes. Anat Sci Int 2003; 78: 205-10.

[46] Garcia-Anoveros J, Samad TA, Zuvela-Jelaska L, Woolf CJ, Corey DP. Transport and localization of the DEG/ENaC ion channel BNaC1alpha to peripheral mechanosensory terminals of dorsal root ganglia neurons. J Neurosci 2001; 21: 2678-86.

[47] Chen CC, England S, Akopian AN, Wood JN. A sensory neuron-specific, proton-gated ion channel. Proc Natl Acad Sci USA 1998; 95: 10240-5.

[48] Neumann S, Doubell TP, Leslie T, Woolf CJ. Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. Nature 1996; 384: 360-4.

[49] Hokfelt T, Zhang X, Wiesenfeld-Hallin Z. Messenger plasticity in primary sensory neurons following axotomy and its functional implications. Trends Neurosci 1994; 17: 22-30.

[50] Donier E, Rugiero F, Okuse K, Wood JN. Annexin II light chain p11 promotes functional expression of acid-sensing ion channel ASIC1a. J Biol Chem 2005; 280: 38666-72.

[51] Leonard AS, Yermolaieva O, Hruska-Hageman A, *et al.* cAMP-dependent protein kinase phosphorylation of the acid-sensing ion channel-1 regulates its binding to the protein interacting with C-kinase-1. Proc Natl Acad Sci USA 2003; 100: 2029-34.

[52] Anzai N, Deval E, Schaefer L, Friend V, Lazdunski M, Lingueglia E. The multivalent PDZ domain-containing protein CIPP is a partner of acid-sensing ion channel 3 in sensory neurons. J Biol Chem 2002; 277: 16655-61.

[53] Hruska-Hageman AM, Benson CJ, Leonard AS, Price MP, Welsh MJ. PSD-95 and Lin-7b interact with acid-sensing ion channel-3 and have opposite effects on H+- gated current. J Biol Chem 2004; 279: 46962-8.

[54] Momin A, Wood JN. Sensory neuron voltage-gated sodium channels as analgesic drug targets. Curr Opin Neurobiol 2008; 18: 383-8.

[55] Boucher TJ, Okuse K, Bennett DL, Munson JB, Wood JN, McMahon SB. Potent analgesic effects of GDNF in neuropathic pain states. Science 2000; 290: 124-7.

[56] Renganathan M, Cummins TR, Waxman SG. Contribution of Na(v)1.8 sodium channels to action potential electrogenesis in DRG neurons. J Neurophysiol 2001; 86: 629-40.

[57] Thakor DK, Lin A, Matsuka Y, *et al.* Increased peripheral nerve excitability and local NaV1.8 mRNA up-regulation in painful neuropathy. Mol Pain 2009; 5: 14.

[58] Gold MS, Weinreich D, Kim CS, *et al.* Redistribution of Na(V)1.8 in uninjured axons enables neuropathic pain. J Neurosci 2003; 23: 158-66.

[59] Zimmermann K, Leffler A, Babes A, *et al.* Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. Nature 2007; 447: 855-8.

[60] Amaya F, Wang H, Costigan M, *et al.* The voltage-gated sodium channel Na(v)1.9 is an effector of peripheral inflammatory pain hypersensitivity. J Neurosci 2006; 26: 12852-60.

[61] Priest BT, Murphy BA, Lindia JA, *et al.* Contribution of the tetrodotoxin-resistant voltage-gated sodium channel NaV1.9 to sensory transmission and nociceptive behavior. Proc Natl Acad Sci USA 2005; 102: 9382-7.

[62] Okuse K, Chaplan SR, McMahon SB, *et al.* Regulation of expression of the sensory neuron-specific sodium channel SNS in inflammatory and neuropathic pain. Mol Cell Neurosci 1997; 10: 196-207.

[63] Villarreal CF, Sachs D, Cunha FQ, Parada CA, Ferreira SH. The role of Na(V)1.8 sodium channel in the maintenance of chronic inflammatory hypernociception. Neurosci Lett 2005; 386: 72-7.

[64] Coggeshall RE, Tate S, Carlton SM. Differential expression of tetrodotoxin-resistant sodium channels Nav1.8 and Nav1.9 in normal and inflamed rats. Neurosci Lett 2004; 355: 45-8.

[65] Amir R, Argoff CE, Bennett GJ, *et al.* The role of sodium channels in chronic inflammatory and neuropathic pain. J Pain 2006; 7: S1-29.

[66] Ekberg J, Adams DJ. Neuronal voltage-gated sodium channel subtypes: key roles in inflammatory and neuropathic pain. Int J Biochem Cell Biol 2006; 38: 2005-10.

[67] Strickland IT, Martindale JC, Woodhams PL, Reeve AJ, Chessell IP, McQueen DS. Changes in the expression of NaV1.7, NaV1.8 and NaV1.9 in a distinct population of dorsal root ganglia innervating the rat knee joint in a model of chronic inflammatory joint pain. Eur J Pain 2008; 12: 564-72.

[68] North RA, Surprenant A. Pharmacology of cloned P2X receptors. Annu Rev Pharmacol Toxicol 2000; 40: 563-80.

[69] Kim C, Chung JM, Chung K. Changes in the gene expression of six subtypes of P2X receptors in rat dorsal root ganglion after spinal nerve ligation. Neurosci Lett 2003; 337: 81-4.

[70] Chessell IP, Hatcher JP, Bountra C, *et al.* Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 2005; 114: 386-96.

[71] Xu GY, Huang LY. Peripheral inflammation sensitizes P2X receptor-mediated responses in rat dorsal root ganglion neurons. J Neurosci 2002; 22: 93-102.

[72] Fabbretti E, D'Arco M, Fabbro A, Simonetti M, Nistri A, Giniatullin R. Delayed upregulation of ATP P2X3 receptors of trigeminal sensory neurons by calcitonin gene-related peptide. J Neurosci 2006; 26: 6163-71.

[73] Bradbury EJ, Burnstock G, McMahon SB. The expression of P2X3 purinoreceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. Mol Cell Neurosci 1998; 12: 256-68.

[74] Pan HL, Wu ZZ, Zhou HY, Chen SR, Zhang HM, Li DP. Modulation of pain transmission by G-protein-coupled receptors. Pharmacol Ther 2008; 117: 141-61.

[75] Stein C. The control of pain in peripheral tissue by opioids. N Engl J Med 1995; 332: 1685-90.

[76] Stein C, Schafer M, Machelska H. Attacking pain at its source: new perspectives on opioids. Nat Med 2003; 9: 1003-8.

[77] Puehler W, Rittner HL, Mousa SA, *et al.* Interleukin-1 beta contributes to the upregulation of kappa opioid receptor mrna in dorsal root ganglia in response to peripheral inflammation. Neuroscience 2006; 141: 989-98.

[78] Puehler W, Zollner C, Brack A, *et al.* Rapid upregulation of mu opioid receptor mRNA in dorsal root ganglia in response to peripheral inflammation depends on neuronal conduction. Neuroscience 2004; 129: 473-9.

[79] Zollner C, Shaqura MA, Bopaiah CP, Mousa S, Stein C, Schafer M. Painful inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. Mol Pharmacol 2003; 64: 202-10.

[80] Kraus J, Borner C, Giannini E, *et al.* Regulation of mu-opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. J Biol Chem 2001; 276: 43901-8.

[81] Patwardhan AM, Berg KA, Akopain AN, *et al.* nin-induced functional competence and trafficking of the delta-opioid receptor in trigeminal nociceptors. J Neurosci 2005; 25: 8825-32.

[82] Gendron L, Lucido AL, Mennicken F, *et al.* Morphine and pain-related stimuli enhance cell surface availability of somatic delta-opioid receptors in rat dorsal root ganglia. J Neurosci 2006; 26: 953-62.

[83] Zhang X, Bao L, Guan JS. Role of delivery and trafficking of delta-opioid peptide receptors in opioid analgesia and tolerance. Trends Pharmacol Sci 2006; 27: 324-9.

[84] Cahill CM, Morinville A, Lee MC, Vincent JP, Collier B, Beaudet A. Prolonged morphine treatment targets delta opioid receptors to neuronal plasma membranes and enhances delta-mediated antinociception. J Neurosci 2001; 21: 7598-607.

[85] Morinville A, Cahill CM, Aibak H, *et al.* Morphine-induced changes in delta opioid receptor trafficking are linked to somatosensory processing in the rat spinal cord. J Neurosci 2004; 24: 5549-59.

[86] Lucido AL, Morinville A, Gendron L, Stroh T, Beaudet A. Prolonged morphine treatment selectively increases membrane recruitment of delta-opioid receptors in mouse basal ganglia. J Mol Neurosci 2005; 25: 207-14.

[87] Koch T, Hollt V. Role of receptor internalization in opioid tolerance and dependence. Pharmacol Ther 2008; 117: 199-206.

[88] Suzuki R, Porreca F, Dickenson AH. Evidence for spinal dorsal horn hyperexcitability in rats following sustained morphine exposure. Neurosci Lett 2006; 407: 156-61.

[89] Vardanyan A, Wang R, Vanderah TW, *et al.* TRPV1 receptor in expression of opioid-induced hyperalgesia. J Pain 2009; 10: 243-52.

[90] Kress M, Kuner R. Mode of action of cannabinoids on nociceptive nerve endings. Exp Brain Res 2009; 196: 79-88.

[91] Hohmann AG, Herkenham M. Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. Neuroscience 1999; 90: 923-31.

[92] Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. Neuroscience 2000; 100: 685-8.

[93] Salio C, Fischer J, Franzoni MF, Conrath M. Pre- and postsynaptic localizations of the CB1 cannabinoid receptor in the dorsal horn of the rat spinal cord. Neuroscience 2002; 110: 755-64.

[94] Price TJ, Helesic G, Parghi D, Hargreaves KM, Flores CM. The neuronal distribution of cannabinoid receptor type 1 in the trigeminal ganglion of the rat. Neuroscience 2003; 120: 155-62.

[95] Amaya F, Shimosato G, Kawasaki Y, *et al.* Induction of CB1 cannabinoid receptor by inflammation in primary afferent neurons facilitates antihyperalgesic effect of peripheral CB1 agonist. Pain 2006; 124: 175-83.

[96] Leterrier C, Laine J, Darmon M, Boudin H, Rossier J, Lenkei Z. Constitutive activation drives compartment-selective endocytosis and axonal targeting of type 1 cannabinoid receptors. J Neurosci 2006; 26: 3141-53.

[97] Wotherspoon G, Fox A, McIntyre P, Colley S, Bevan S, Winter J. Peripheral nerve injury induces cannabinoid receptor 2 protein expression in rat sensory neurons. Neuroscience 2005; 135: 235-45.

[98] Zou X, Lin Q, Willis WD. Role of protein kinase A in phosphorylation of NMDA receptor 1 subunits in dorsal horn and spinothalamic tract neurons after intradermal injection of capsaicin in rats. Neuroscience 2002; 115: 775-86.

[99] Zou X, Lin Q, Willis WD. Enhanced phosphorylation of NMDA receptor 1 subunits in spinal cord dorsal horn and spinothalamic tract neurons after intradermal injection of capsaicin in rats. J Neurosci 2000; 20: 6989-97.

[100] Brenner GJ, Ji RR, Shaffer S, Woolf CJ. Peripheral noxious stimulation induces phosphorylation of the NMDA receptor NR1 subunit at the PKC-dependent site, serine-896, in spinal cord dorsal horn neurons. Eur J Neurosci 2004; 20: 375-84.

[101] Galan A, Laird JM, Cervero F. *In vivo* recruitment by painful stimuli of AMPA receptor subunits to the plasma membrane of spinal cord neurons. Pain 2004; 112: 315-23.

[102] Dolan S, Kelly JG, Monteiro AM, Nolan AM. Up-regulation of metabotropic glutamate receptor subtypes 3 and 5 in spinal cord in a clinical model of persistent inflammation and hyperalgesia. Pain 2003; 106: 501-12.

[103] Hudson LJ, Bevan S, McNair K, *et al.* Metabotropic glutamate receptor 5 upregulation in A-fibers after spinal nerve injury: 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reverses the induced thermal hyperalgesia. J Neurosci 2002; 22: 2660-8.

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