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REVIEW

Update on the Role of Spinal Cord TRPV1 Receptors in Pain Modulation

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Summary

The structure, expression and function of the transient receptor potential vanilloid 1 (TRPV1) receptor were intensively studied since the cloning in 1997 and TRPV1 receptors are now considered to act as transducers and molecular integrators of nociceptive stimuli in the periphery. In contrast, spinal TRPV1 receptors were studied less extensively and their role in pain modulation is still not fully understood. This short review is a follow up on our previous summary in this area (Spicarova and Palecek 2008). The aim was to review preferentially the most recent findings concerning the role of the spinal TRPV1 receptors, published within the last five years. The update is given on the expression and function of the spinal TRPV1 receptors, their activation by endogenous agonists, interaction between the endocannabinoid and endovanillod system and possible role of the spinal TRPV1 receptors in pathological pain states. There is now mounting evidence that TRPV1 receptors may be an important element in modulation of nociceptive information at the spinal cord level and represent an interesting target for analgesic therapy.

Key words

Hyperalgesia • Capsaicin • TRPV1 • Spinal cord

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Introduction

The expression and function of the transient receptor potential vanilloid 1 (TRPV1) receptor was intensively studied since their cloning in 1997 (Caterina *et al.* 1997) and its crucial role in nociception and peripheral pain mechanisms is unquestionable. TRPV1 receptors are expressed in both the peripheral and central branches of small and medium sized (C and Aδ) dorsal root ganglia (DRG) neurons. TRPV1 receptors on the peripheral nociceptors are considered to act as transducers and molecular integrators of peripheral nociceptive stimuli, as was demonstrated by a number of publications (for a review see e.g. Kissin and Szallasi 2011). In contrast, spinal TRPV1 receptors were not studied so extensively and their role in pain modulation is still not fully understood (Palazzo *et al.* 2010, Matta and Ahern 2011). This short review is a follow up on our previous summary in this area (Spicarova and Palecek 2008). The aim was to review preferentially the most recent findings concerning the role of the spinal TRPV1 receptors, published within the last five years.

Expression of TRPV1 receptors at the spinal cord level

The presence of TRPV1 receptors at the presynaptic endings of primary afferents in the spinal cord was clearly documented. However, there is still significant discussion concerning the presence or absence of these receptors postsynaptically in dorsal horn (DH) neurons or in glial cells (Spicarova and Palecek 2008). To

better understand the TRPV1 receptor distribution in the brain, knock-in mice were generated (Cavanaugh *et al.* 2011b). However, using this approach expression of TRPV1 receptors at the spinal cord level was documented only in the central branches of DRG neurons. This expression was more extensive during the development than in the adult mouse, especially within the nonpeptidergic population (Cavanaugh *et al.* 2011a). The selective loss of TRPV1 receptors occurred in a specific subpopulation of DRG neurons without their degeneration, suggesting developmentally regulated transient expression of the TRPV1 receptors. This study also showed that TRPV1+ and TRPV1- afferents target distinct spinal cord areas in adult mice, as a result of the TRPV1 receptor down-regulation in certain DRG neurons (Cavanaugh *et al.* 2011a).

While the genetic approach did not reveal any presence of TRPV1 receptors in the dorsal horn neurons, immunocytochemical labeling showed colocalization of TRPV1 receptor and vesicular glutamate transporter-2 (vGluT-2) in these cells (Zhou *et al.* 2009). The presence of TRPV1 protein and its mRNA in DH neurons was also confirmed by histological (immunocytochemistry, *in-situ* PCR) and biochemical (Western blott, PCR) analysis in organotypic spinal cord culture model where primary afferent fibres were degenerated (Ferrini *et al.* 2010). Kim *et al.* (2012) also brought evidence in a very detailed paper that TRPV1 receptors are expressed in a substantial subpopulation of GABAergic lamina II interneurons. Activation of these receptors by capsaicin induced long-term depression (LTD) of the evoked postsynaptic currents (EPSCs). The capsaicin induced LTD was mediated by reduced expression of AMPA receptor GluR2 subunit in the membrane, resulting in depression of inhibitory input to the projection spinothalamic neurons in the spinal cord. Intrathecal capsaicin injection did not induce mechanical hypersensitivity in TRPV1 knockout mice, but was present in animals where TRPV1+ DRG neurons were ablated by resiniferatoxin (RTX) injection, suggesting involvement of postsynaptic TRPV1 receptors expressed in the dorsal horn neurons (Kim *et al.* 2012).

Based on the RTX radiolabelled binding, immunohistochemical and dorsal rhizotomy studies (Spicarova and Palecek 2008), it seems clear that the substantial majority of TRPV1 receptors in the spinal cord dorsal horn is present presynaptically, on the central branches of primary afferents. However, especially the functional significance of some postsynaptic TRPV1 receptors needs to be further studied and clarified.

Effect of TRPV1 receptors expressing terminals ablation in the spinal cord

Administration of high doses of potent TRPV1 receptor agonist (resiniferatoxin or capsaicin) leads to functional inactivation or ablation of neuronal processes and/or the whole cells expressing TRPV1 receptors, depending on the method and concentration of the drug administration. These approaches have beside experimental use, gained also a lot of attention for treatment of intractable pain states (Spicarova and Palecek 2008, Mitchell *et al.* 2010, Anand and Bley 2011, Iadarola and Mannes 2011).

Pharmacological ablation of spinal cord central terminals of a subpopulation of TRPV1+ peptidergic DRG neurons produced a near-complete loss of responsiveness to noxious heat (Cavanaugh *et al.* 2009). In contrast, genetic ablation of unmyelinated sensory neurons expressing the G protein coupled receptor MrgprD+ that represent approximately 90 % of cutaneous nonpeptidergic nociceptors, produced selective reduction of mechanical sensitivity (Cavanaugh *et al.* 2009). Extracellular recordings from lumbar DH neurons following elimination of the central terminals of TRPV1 expressing DRG neurons by intrathecal capsaicin injection, showed their diminished responsiveness to noxious heat with no change in their responses to noxious mechanical stimulation (Zhang *et al.* 2013). In comparison, ablation of the MrgprD+ afferents did not alter the responses to noxious heat, but reduced the responses of the superficial DH neurons to noxious mechanical stimuli. These results suggest that TRPV1+ and MrgprD+ primary afferent neurons provide modality-specific contributions to the response properties of the DH neurons (Zhang *et al.* 2013).

Intrathecal injection of resiniferatoxin (RTX) may selectively ablate central endings of TRPV1 receptors expressing nociceptors, preserving their neuronal bodies in the DRG and their peripheral terminals without effect on capsaicin-induced release of calcitonin gene-releated peptide (CGRP) in the peripheral tissues (Bishnoi *et al.* 2011b). Systemic intraperitoneal administration of RTX ablated the whole population of TRPV1-expressing DRG neurons together with significant decrease in acute thermal pain sensitivity, whereas localized intrathecal injection had only limited effect (Jeffry *et al.* 2009, Bishnoi *et al.* 2011b). RTX-induced ablation of central nociceptive terminals persisted for several months (Jeffry *et al.* 2009).

Modulation of synaptic transmission by TRPV1 receptors

Regulation of glutamate release from central terminals of nociceptive primary afferent fibers, terminating preferentially in the lamina I and II of the superficial DH, due to TRPV1 receptor activation is well established. Activation of the presynaptic TRPV1 receptors by different agonists increases the spontaneous glutamate release in a concentration dependent manner with different potency, measured as an increase of miniature or spontaneous excitatory postsynaptic current (mEPSC, sEPSC) frequency (Jeffry *et al.* 2009, Spicarova and Palecek 2009). Evoked EPSC induced by dorsal root stimulation in spinal cord slices may be abolished or depressed after capsaicin application but capsaicin application may also elicit increased firing of action potentials in the superficial DH neurons (Baccei *et al.* 2003, Jeffry *et al.* 2009). Capsaicin-induced glutamate release was shown to be reduced by activation of presynaptic somatostatin receptor 2 in lamina II of the spinal cord (Bencivinni *et al.* 2011). These results indicate that TRPV1 receptor activation dependent release of transmitter from primary nociceptive neurons may be regulated by somatostatin, suggesting its antinociceptive role.

Majority of spinothalamic tract neurons (STT), labeled in the rat spinal cord deep DH using retrograde tracer injected into the ventral posterior lateral nucleus of the thalamus, receive direct inputs from capsaicin sensitive fibers (Kim *et al.* 2009a). It was suggested that deep DH STT neurons send dendritic projections to the superficial laminae where they receive nociceptive input from the TRPV1+ primary afferent terminals. Activation of TRPV1 receptors increased glutamate release from the presynaptic terminals and subsequently increased the neural activity of the recorded STT projection neuron (Kim *et al.* 2009a).

It was previously suggested that the presynaptic TRPV1 receptors may be tonically active only after their phosphorylation, like in a model of peripheral inflammation, but not under control conditions (Lappin *et al.* 2006, Spicarova and Palecek 2008, 2009). In a recent study by Park *et al.* (2011a), the authors demonstrated moderate tonic activity of spinal presynaptic TRPV1 receptors in control mice. Application of two TRPV1 receptor antagonists, capsazepine and AMG9810, reduced sEPSC frequency recorded in lamina II neurons. This finding was further supported by reduced sEPSC frequency in TRPV1 knock-out mice. These mice also failed to demonstrate LTP induction following tetanic stimulation in comparison to the wild type. The presence or absence of presynaptic TRPV1 receptor tonic activity in different studies is most likely due to dissimilar experimental conditions used, like bath temperature and experimental species.

Regulation of inhibitory synaptic transmission by capsaicin-induced activation of TRPV1 receptors was demonstrated in organotypic spinal cord cultures (Ferrini *et al.* 2010). In cultures where the primary afferent fibres were degenerated, capsaicin did not affect the excitatory synaptic transmission. Capsaicin-induced increase of spontaneous inhibitory postsynaptic current (sIPSC) frequency was dramatically reduced by tetrodotoxin (TTX) application (Ferrini *et al.* 2010). This observation suggests that activation of local spinal cord circuitry is needed to evoke the capsaicin-induced increase of the sIPSC frequency.

Neuroinflammatory changes, activation of glial cells and release of different proinflammatory cytokines and chemokines at the spinal cord level were shown to play an important role in different pain states, especially of neuropathic origin (Marchand *et al.* 2005, Old and Malcangio 2012, Schomberg and Olson 2012). One of the cytokines, tumor necrosis factor (TNFα), was shown to potentiate TRPV1 mediated responses of DRG neurons to capsaicin application in cultured DRG neurons *in vitro* (Constantin *et al.* 2008). In our experiments, we have used incubation of spinal cord slices with TNFα to modulate activity of the presynaptic TRPV1 receptors. TNFα application induced increased frequency of spontaneous and miniature EPSC in the superficial DH neurons and increased sensitivity to endogenous TRPV1 agonist in the control animals (Spicarova and Palecek 2010) and in a model of peripheral neuropathy (Spicarova *et al.* 2011). Similar effect of the TNFα application was seen in the superficial DH neurons identified as vGluT-2 excitatory neurons (Park *et al.* 2011a). The TNFα-elicited increase of sEPSC frequency was present only in the wild-type mice but not in the TRPV1 knock-out animals, suggesting that the presynaptic effect of cytokine TNFα was mediated by the TRPV1 receptors (Park *et al.* 2011a).

Endogenous activators of spinal TRPV1 receptors

Activation and/or sensitization of peripheral TRPV1 receptors by temperature increase, low pH or by

different molecules released due to tissue injury is well documented. At the spinal cord level, activation of these receptors seems to be more complex, but a number of different molecules of endogenous origin that could activate TRPV1 receptor was already identified (see the reviews Spicarova and Palecek 2008, De Petrocellis and Di Marzo 2009, Di Marzo and De Petrocellis 2010, 2012, Starowicz and Przewlocka 2012).

In our experiments we used patch-clamp recordings from superficial DH neurons in acute spinal cord slices to test the effect of the endogenous TRPV1 receptor agonist N-oleoyldopamine (OLDA) application on the mEPSCs frequency (Spicarova and Palecek 2009). In the control experiments, high concentration of OLDA (10 μM) solution was needed to increase the glutamate release from primary afferent nociceptors *via* TRPV1 receptor activation and we did not see any effect on the mEPSC frequency after low concentration OLDA (0.2 μM) treatment. However, application of the same low concentration of OLDA solution increased the mEPSC frequency following protein kinase C (PKC) activation by phorbol ester (phorbol 12-myristate 13-acetate) and after bradykinin application (Spicarova and Palecek 2009), after TNFα incubation (Spicarova and Palecek 2010), in a model of peripheral inflammation induced by carrageenan (Spicarova and Palecek 2009) and in a model of peripheral neuropathy (Spicarova and Palecek 2010). These experiments suggest that endogenous agonists of TRPV1 receptors like OLDA could have a considerable impact on the synaptic transmission in the spinal cord DH, especially under pathological conditions when TRPV1 receptors are phosphorylated and sensitized.

The list of the endogenous substances capable of TRPV1 receptor activation is growing larger with more attention and experiments designed to study this issue. Depolarization of the spinal cord triggered release of oxidized linoleic acid metabolites such as 9-HODE (9-hydroxyoctadecadienoic acid), which activated spinal TRPV1 receptors, leading to development of mechanical allodynia (Patwardhan *et al.* 2009). Intrathecal injection of 9-HODE (5 μg) induced a profound mechanical allodynia with a time course that appeared to extend beyond the one induced by i. t. capsaicin (5 μg). Oxidized linoleic acid metabolites represent a family of endogenous TRPV1 receptor agonists including 9-HODE, 13-HODE, 9oxo-ODE and 13-oxoODE (Patwardhan *et al.* 2009).

It was recently demonstrated that intrathecal delivery of hepoxilin A_3 (HXA_3), a metabolite of the 12-lipoxygenases (12-LOX), induced mechanical allodynia mediated by spinal TRPV1 receptor activation while the thermal hyperalgesia induced was only modest at doses exceeding those required to elicit mechanical allodynia (Gregus *et al.* 2012).

Whole-cell recordings in lamina II neurons in spinal cord slices showed that reactive oxygen species (ROS) enhanced spontaneous release of glutamate from presynaptic terminals due to TRPV1 receptor activation (Nishio *et al.* 2013). The effects of ROS and HXA_3 were mediated also by the ankyrin type (TRPA1) of the TRP receptor family (Gregus *et al.* 2012, Nishio *et al.* 2013).

Functional membrane delimited coupling of metabotropic glutamate receptor 5 (mGluR5) and the TRPV1 receptors was suggested as another mechanism of possible TRPV1 receptor activation at the presynaptic endings of primary afferent fibers in the spinal cord (Kim *et al.* 2009b). Activation of mGluR5 receptors in acute rat spinal cord slice (P8-P12) increased the mEPSC frequency due to TRPV1 receptors activation (Kim *et al.* 2009b). It was suggested that this effect could be mediated by mGluR5 activation of phospholipase C (PLC) and hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2) to inositol (1,4,5) trisphosphate (IP3) and diacylglycerol. TRPV1 receptors are tonicaly blocked by PIP2 and following its hydrolysis they could become activated (Chuang *et al.* 2001). During activation of primary afferent terminals, glutamate is released that in turn could activate presynaptic TRPV1 receptors *via* mGluR5 and further enhance glutamate release as a positive feedback. Intrathecal injection of DHPG, a selective group I mGluR (GluR1/5) agonist, induced spontaneous pain behaviors and mechanical allodynia that was partially mediated by TRPV1 receptors, as was shown using TRPV1 knock-out mice (Kim *et al.* 2009b).

Modulation of inhibitory synaptic transmission by N-arachidonoyl-dopamine, an endovanilloid/endocannabinoid agonist of TRPV1 receptor and N-palmitoyl- with N-stearoyl-dopamine, two naturally occurring N-acyldopamines was shown in organotypic spinal cord cultures. These endogenous substances increased the recorded sIPSC frequency (Ferrini *et al.* 2010).

Interaction between the endovanilloids and endocannabinoids in the spinal cord

Endovanilloids and endocannabinoids are mostly represented by numerous groups of lipid molecules

derived from arachidonic acid. This group includes extensively studied substances such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) and other molecules (oleoylethanolamide, N-oleoyldopamine, O-arachidonoylethanolamine, noladin ether, N-arachidonoyl dopamine) where the role in signaling remains less certain. Some of these endogenous substances activate just cannabinoid receptors (CB1 and CB2), like 2-AG, and noladin ether (Di Marzo 2008) and some show significantly higher affinity to the vanilloid receptor (TRPV1) than to CB receptors, like oleoylethanolamide and N-oleoyldopamine (Jara-Oseguera *et al.* 2008). There are few endovanilloids/ endocannabinoids such as AEA and N-arachidonoyl dopamine that activate CB receptors or TRPV1 receptors in a concentration dependent manner (Pertwee 1999, Zygmunt *et al.* 1999, Ross 2003). Some studies also described TRPV1 as an ionotropic cannabinoid receptor (Akopian *et al.* 2009, Borroto-Escuela *et al.* 2013). The preferential activation of the CB and TRPV1 receptors is mediated also by localization of these receptors and compartmentalization of their activation sites (Millns *et al.* 2006). The agonist binding site of the CB1 receptor is extracellular and on the TRPV1 receptor it is located intracellularly (Shire *et al.* 1996, Jung *et al.* 1999).

Endovanilloids/endocannabinoids play a very important role in nociception, either in periphery or in the CNS. Here we will focus mainly on their function at the spinal cord level. The endovanniloids/endocannabinoids are synthesised on demand by several metabolic pathways (Ahluwalia *et al.* 2003, van der Stelt *et al.* 2005, Vellani *et al.* 2008). There is also evidence of interaction among different signaling pathways of the main endovanniloids/endocannabinoids system. In the mouse striatum, the elevated level of AEA mediated by TRPV1 receptor activation reduced concentration, metabolism and physiological effects of the 2-AG (Maccarrone *et al.* 2008). This effect may be specific for just some brain regions, although it displayed some of possible cross-talk mechanism between the CB1 and TRPV1 receptors (Di Marzo and Cristino 2008). Anandamide, as the major endogenous endovanniloid/ endocannabinoid, is mainly synthesised *via* calcium dependent N-acylphosphatidyl-ethanolamine phospholipase D, NAPE-PLD (Okamoto *et al.* 2004). NAPE-PLD is expressed in nociceptive DRG neurons, where it is also co-expressed with TRPV1 receptors and also in spinal cord neurons (Nagy *et al.* 2009, Moreno-Martet *et al.* 2012). Recently described calcium insensitive pathway of AEA synthesis in DRG cultured cells was suggested to play a role in TRPV1-mediated excitation of primary sensory neurons (Varga *et al.* 2013).

Endovanilloids act at short distance (autocrinne or paracrinne, respectively) due to their on-demand synthesis and fast subsequent degradation by fatty amino acid hydrolase (FAAH). Tonic activity of CB1 receptors at the spinal cord level was suggested (Richardson *et al.* 1998), which pointed to possible continuous synthesis of endovanniloids/endocannabinoids such as AEA. As an indirect support of this hypothesis, permanent activity of the degradation enzyme FAAH and its up-regulation in neuropathies with lowered level of AEA was reported (Alkaitis *et al.* 2010, Okine *et al.* 2012, Guindon *et al.* 2013).

The main role of the cannabinoid system in spinal nociceptive transmission seems to lie in attenuation of the nociceptive signaling and its changes under pathological pain states are considered an important mechanism in spinal cord synaptic modulation of nociceptive signaling (Starowicz *et al.* 2012). The function of the spinal TRPV1 receptors in nociceptive transmission seems to be different and the interaction between the endovanilloid and endocannabinoid system may play an important role in the pain mechanisms. The necessary concentration of the endogenous endovanilloids/endocannabinoids to activate the TRPV1 receptors under control conditions is relatively high (Zygmunt *et al.* 1999, Spicarova and Palecek 2009). Several studies suggested increased sensitivity of spinal TRPV1 receptor to its agonists under pathological conditions, in models of neuropathic and inflammatory pain (Singh Tahim *et al.* 2005, Spicarova and Palecek 2009, Spicarova *et al.* 2011, Starowicz *et al.* 2012). Recently, a new role for the TRPV1 receptors in the endovannilloid metabolism was suggested in a neuropathic pain model (Guindon *et al.* 2013). Upregulation of FAAH in this model induced behavioral hypersensitivity, allodynia and hyperalgesia, due to lowered AEA level. Antagonists of FAAH displayed significant anti-allodynic effect, which could be abolished by antagonists of TRPV1 and CB1 receptors (Guindon *et al.* 2013). These results suggest that TRPV1 receptors might have antinociceptive role due to endocannabinoid activation in some pathological pain states although the underlying mechanisms remain unclear. The elevated level of andandamide may have several physiological effects beside activation of the cannabinoid system, e.g.

desensitisation of TRPV1 receptors and allosteric influences on other non-cannabinoid receptors (5-HT_2 and 5-HT_3 serotonin receptors, nicotin acetycholine receptor) and others (Starowicz and Przewlocka 2012).

The possible interactions between the endovanilloid and endocannabinoid system, due to sharing of the common endogenous agonists, represent a complex system that may play an important role in the modulation of nociception and pain and may represent a promising target for possible analgesic treatment not only at the spinal cord level.

The role of the spinal TRPV1 receptors in pathological pain states

Spinal cord TRPV1 receptors may play a principal role in the pain mechanisms of different acute and chronic pain states. Studying their function may help us to reveal possible approaches how to exploit them for pain therapy. In theory, several approaches are possible. Application of potent TRPV1 receptor agonists, such as capsaicin or RTX intrathecally, may lead to functional inactivation of the whole central nociceptive ending and/or the DRG neuron. Recent evidence suggests that positive allosteric modulation of TRPV1 receptors that would further increase their sensitivity to capsaicin, could be used to produce a selective analgesia through calcium overload of neurites (Kaszas *et al.* 2012, Lebovitz *et al.* 2012). Antagonists of the TRPV1 receptors prevent their activation and if given intrathecally, it should not affect the normal thermal sensitivity profoundly, while their systemic administration proved to be difficult (Xia *et al.* 2011). Pharmacological interaction with the spinal TRPV1 receptors preventing their incorporation into the presynaptic membrane and/or their phosphorylation may provide pain relief without significant side effects, similar to the experiments done in the periphery (Fischer *et al.* 2013). Also other methods leading to reduction of TRPV1 receptor expression (siRNA, viral vectors) should provide analgesic effect. The role of the spinal TRPV1 receptors in pain mechanisms gained more attention recently, since the original review (Spicarova and Palecek 2008). Some of the published findings are presented here.

Intrathecal administration of RTX or other TRPV1 receptor agonists is a promising method to treat pain by ablation and/or inactivation of nociceptor central terminals. This method could be especially suitable for patients treated with large doses of potent opiate analgesics, which may have severe side effects (Jeffry *et al.* 2009). Intrathecal administration of RTX resulted in only modest thermal analgesia (Mishra and Hoon 2010) or did not alter the acute thermal sensitivity (Jeffry *et al.* 2009) but profoundly reduced carrageenan induced thermal hypersensitivity, while mechanical sensitivity was unaffected (Jeffry *et al.* 2009, Mishra and Hoon 2010).

For the use of TRPV1 agonists and antagonists in pain therapy are also important recent findings of Mogg *et al.* (2013) suggesting that effect of both TRPV1 receptor agonist and antagonist may be differentially altered by PKC modulation of TRPV1 receptors. Using a spinal cord CGRP release assay they pharmacologically characterized TRPV1 receptors under the basal and phosphorylated conditions. Interestingly, N-hydroxy-4-(3-phenylpropanamido)benzamide was identified as antagonist of the capsaicin-evoked CGRP release, but it potentiated the phorbol 12,13-dibutyrate evoked CGRP release (Mogg *et al.* 2013).

Peripheral inflammation was shown to increase the level of 12-lipoxygenases (12-LOX) metabolites, particularly hepoxilins (HXA_3 and HXB_3) in the spinal cord (Buczynski *et al.* 2010). Intrathecal delivery of the LOX inhibitor prevented the carrageenan-evoked increase in spinal HXB_3 and attenuated the associated hypersensitivity. Evidence is provided that HXA_3 produced mechanical allodynia by activating spinal TRPV1 and TRPA1 receptors (Gregus *et al.* 2012).

Complete Freund's adjuvant-induced inflammatory thermal hyperalgesia is significantly diminished in the TRPV1 knock-out mice (Caterina *et al.* 2000). Recently evidence was presented that intrathecal post-treatment with neuroprotectin-D1 (NPD1) in this model rapidly reduced the developed hyperalgesia (Park *et al.* 2011a). NPD1 is an anti-inflammatory lipid mediator biosynthesized from ω-3 polyunsaturated fatty acid DHA (docosahexaenoic acid), which potently inhibits capsaicin-induced currents in dissociated dorsal root ganglion neurons at ~500 times lower concentration than the commonly used TRPV1 receptor antagonist AMG9810 (Park *et al.* 2011a). NPD1 acts on G protein-coupled receptors (GPCR) and might block the TRPV1 signaling *via* inhibiting the adenylylcyclase (AC), protein kinase A (PKA) and the extracellular signal-regulated kinase (ERK) signaling pathways. The intrathecal injection of very low doses of the NPD1 blocked spinal LTP and reduced the TRPV1 receptor dependent inflammatory pain, without affecting the baseline pain thresholds (Park *et al.* 2011a).

Resolvins are bioactive products of ω-3 polyunsaturated fatty acids derived from DHA and eicosapentaenoic acid (EPA) produced after aspirin treatment that counteracts the proinflammatory signaling (Serhan *et al.* 2002). Specific resolvins may differentially regulate distinct TRP channels (TRPA1, TRPV1, TRPV4) to control selective pain modalities and may offer novel analgesic approaches (Ji *et al.* 2011). Intrathecal administration of resolvin E1 (RvE1) reduced thermal hyperalgesia induced by i. t. application of TNFα that was missing in TRPV1 knock-out mice. RvE1 also blocked TNFα and capsaicin-induced increase of sEPSC frequency in lamina II neurons (Xu *et al.* 2010). Resolvin D2 (RvD2) blocked capsaicin induced increase of sEPSC frequency in lamina II neurons (Park *et al.* 2011b).

TRPV1 receptors were suggested to play an important role in the diabetic peripheral neuropathy. In a streptozotocin (STZ) model of diabetes thermal hyperalgesia was present together with increased expression of TRPV1 receptors in the nociceptive DRG neurons (Pabbidi *et al.* 2008). In the spinal cord DH, microglial cells were activated and the level of proinflammatory mediators IL-1β, IL-6 and TNFα was increased (Bishnoi *et al.* 2011a). Intrathecal administration of RTX significantly attenuated STZ-induced thermal hyperalgesia but not the mechanical allodynia, suggesting an important role of spinal TRPV1 receptors in the diabetes associated thermal hyperalgesia (Bishnoi *et al.* 2011a).

Recently the role of spinal TRPV1 receptors was recognized in central neuropathic pain that could develop after spinal cord injury (SCI). Contusive SCI in the thoracic region increased expression of TRPV1 receptor protein in the lumbar DRG 1 month after the injury and enhanced the capsaicin evoked currents in dissociated, small diameter DRG neurons from the experimental animal. TRPV1 antagonist AMG9810 application and intrathecal application of oligonucleotide antisense to TRPV1 receptor reversed the increased sensitivity to heat and mechanical stimuli without affecting the detection of noxious heat (Wu *et al.* 2013).

Spinal TRPV1 receptors were recognized to be involved in the maintenance of mechanical allodynia that developed after peripheral nerve injury. Mechanical hypersensitivity induced by chronic constriction injury (Kim *et al.* 2012) or spinal nerve ligation (Watabiki *et al.* 2011) was reversed by intrathecal administration of TRPV1 receptor antagonist (BCTC or AS1928370). This method of drug administration avoided the hyperthermic side effect normally seen after systemic treatment with some TRPV1 antagonists (Kim *et al.* 2012).

Activation of spinal glial cells has been implicated in the development and maintenance of several pathological pain states. Lately the role of TRPV1 receptors in the activation of spinal microglia and astrocytes in models of adjuvant-induced inflammatory pain and in neuropathic pain developed following partial sciatic nerve ligation was recognized (Chen *et al.* 2009). It was suggested that the TRPV1 receptor dependent activation of microglia was partially mediated by bradykinin (B1) receptors. Activation of spinal TRPV1 receptors by capsaicin leads to upregulation of B1 receptor mRNA and its protein level in rat spinal cord, mostly in microglia cells. Induction mechanism involved oxidative stress, proinflammatory cytokines and the NF-κB pathway. The newly synthesized B1 receptors were functional, as their activation with an agonist caused thermal hyperalgesia (Talbot *et al.* 2012). Activated microglia cells generate ROS and this process is dependent on TRPV1 receptors (Schilling and Eder 2009). It is not clear if activation of TRPV1 receptors on the glia cells and/or neurons was crucial for the subsequent microglia activation and ROS generation.

Morphine is a highly potent opiate analgesic drug, but a long-term treatment with morphine leads to tolerance and associated hypersensitivity. After chronic morphine treatment an increase in TRPV1 receptor immunoreactivity and mRNA level in the spinal cord was documented. Intrathecal pretreatment with selective TRPV1 receptor antagonist SB366791 attenuated both the morphine tolerance and the associated thermal hyperalgesia. Chronic morphine application activated TRPV1 receptors *via* MAPK signaling pathways including p38 MAPK, ERK and c-Jun N-terminal kinase (Chen *et al.* 2009). Chronic use of opioids can repeatedly stimulate TRPV1-expressing primary afferents and thus augment the nociceptive input, resulting in sensitization of spinal dorsal horn neurons and hyperalgesia (Zhou *et al.* 2010). This sensitization can gradually overcome and mask the analgesic effects of the opioids. Brief application of the μ-opioid receptor agonist (DAMGO) caused an initial decrease followed by a large and long-lasting increase in the amplitude of EPSCs evoked by dorsal root stimulation in approximately 50 % of the recorded lamina I and II neurons. DAMGO-induced LTP was associated with an increase in the paired-pulse depression ratio. Furthermore, DAMGO application and washout induced an initial decrease followed by a

persistent increase in the frequency of the mEPSCs. Ablation of TRPV1 receptor expressing primary afferents not only eliminated DAMGO-induced LTP, but also prolonged DAMGO-induced inhibition of the mEPSC and eEPSC. The critical role of TRPV1-expressing primary afferents in opioid-induced LTP has not been recognized previously and this subpopulation of sensory neurons could be targeted to prevent or minimize opioid-induced hyperalgesia and tolerance (Zhou *et al.* 2010).

Conclusion

There is now clear evidence that TRPV1 receptors play an important role in modulation of nociceptive information synaptic transmission at the spinal cord level. Presynaptic TRPV1 receptors at the central branches of primary afferents may act as integrators of different influences on the synaptic modulation. The exact process of their activation and the consequences under different pathological conditions needs to be further studied and clarified to enable TRPV1 receptors based analgesic therapy in the future.

Conflict of Interest

There is no conflict of interest.

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