

The role of spinal cord vanilloid (TRPV1) receptors in pain modulation

Diana Spicarova and Jiri Palecek

Department of Functional Morphology, Institute of Physiology,
Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Short title: THE SPINAL CORD TRPV1 RECEPTORS

Corresponding author:
Jiri Palecek M.D., Ph.D.
Department of Functional Morphology
Institute of Physiology ASCR
Videnska 1083
142 20 Praha 4
Czech Republic
e-mail: palecek@biomed.cas.cz
office: (-420) 2 4106 2664
fax: (-420) 2 4106 2488

SUMMARY

Transient receptor potential vanilloid 1 (TRPV1) receptor is a nonselective cation channel activated by capsaicin, a pungent substance from chili peppers. It is considered to act as an integrator of various physical and chemical nociceptive stimuli, as it can be gated by noxious heat ($>43^{\circ}\text{C}$), low pH (protons) and also by recently described endogenous lipids. The structure and function of TRPV1 receptors was vigorously studied, especially since its cloning in 1997. However, most of the research was pointed towards the role of TRPV1 receptors in the peripheral tissues. Mounting evidence now suggests that TRPV1 receptors on the central branches of dorsal root ganglion neurons in the spinal cord may play an important role in modulation of pain and nociceptive transmission. The aim of this short review was to summarize the knowledge about TRPV1 receptors in the spinal cord dorsal horn, preferentially from morphological and electrophysiological studies on spinal cord slices and from *in vivo* experiments.

KEYWORDS: hyperalgesia, capsaicin, TRPV1, spinal cord

Capsaicin, the pungent substance from chili peppers, was repeatedly used in pain research for its ability to induce both hyperalgesia and analgesia, depending on the concentration and route of application (Jancso *et al.* 1987, Willis 2001, Knotkova *et al.* 2008). It was in 1997 (Catarina *et al.* 1997) that the specific receptor activated by capsaicin (transient receptor potential vanilloid 1 - TRPV1) was cloned and characterized. Since then, numerous studies were published characterizing the biophysical and physiological properties, while its possible therapeutic use for pain treatment was pursued (Numazaki *et al.* 2004, Szallasi *et al.* 2007, Vennekens *et al.* 2008). The aim of this short review was to summarize the knowledge about TRPV1 receptors in the spinal cord dorsal horn, preferentially from morphological and electrophysiological studies on spinal cord slices and from *in vivo* experiments.

Vanilloid TRPV1 receptors

The TRPV1 receptors belong to a large family of TRP channels (Venkatachalam and Montell, 2007). TRPV1 receptor formerly known as the vanilloid receptor (VR1) is the founding member of the TRPV subfamily and belongs to the group of six thermosensitive channels in mammals (TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1). TRPV1 receptors are considered to be integrators of noxious chemical and physical stimuli, that can be activated by capsaicin, heat, low pH and also by recently described endogenous lipids derived from dopamine (Tominaga *et al.* 1998, Numazaki *et al.* 2004, Vennekens *et al.* 2008). There are numerous studies published that confirm the crucial role of these receptors for transduction and modulation of acute and chronic pain. Based on these studies a substantial potential for treatment of different acute and chronic pain disorders and other diseases is recently emerging (Szallasi *et al.* 2007).

TRPV1 receptors are specifically activated by capsaicin, a substance that was used frequently in the pain research to activate nociceptors in the past, when the mechanisms of its

action were not known. It was recognized that its application can have both analgesic and algesic effects based on the concentration used and the form of administration. Systemic high concentration capsaicin administration in neonatal rats is neurotoxic and leads to selective degeneration of capsaicin sensitive primary neurons (Jancso *et al.* 1977, 1987, Nagy *et al.* 1983). High concentration intradermal injection of capsaicin was also shown to induce degeneration of epidermal nerve fibers in humans (Simone *et al.* 1998) and to reduce signs of postoperative pain in rats (Pospisilova and Palecek 2006). Intradermal application of low concentration capsaicin leads to acute pain, followed by mechanical hyperalgesia in humans (Simone *et al.* 1989) and to sensitization of spinothalamic tract neurons (Simone *et al.* 1991).

Breakthrough in the TRPV1 receptor research came with the cloning of cDNA encoding the receptor by Caterina *et al.* in 1997 and construction of TRPV1 $-/-$ knockout mice three years later. Mice lacking TRPV1 receptors had normal responses to noxious mechanical stimuli, but detection of noxious heat was impaired, inflammatory thermal hypersensitivity was attenuated and pain behavior induced by capsaicin application was abolished (Caterina *et al.* 2000) and the development of carrageenan-mediated thermal hyperalgesia was completely absent (Davis *et al.* 2000).

The majority of the research was oriented to study the function of TRPV1 receptors in the peripheral nervous system and to define the biophysical properties in different expression systems using cell cultures (Cortright and Szallasi 2004, Szallasi *et al.* 2007, Vennekens *et al.* 2008). It was well established that peripheral TRPV1 receptors, acting as integrators of nociceptive stimuli, can be sensitized or activated by inflammatory mediators such as bradykinin, prostaglandins or protons (Cortright & Szallasi, 2004). The number of peripheral receptors is also increased by transport of TRPV1 to the peripheral terminals of small diameter sensory neurons by NGF action under inflammatory conditions (Ji *et al.* 2002).

In contrast, the role of central TRPV1 receptors in the spinal cord dorsal horn was not studied extensively and the function of these receptors in pain transmission and modulation is not fully understood. The activation of spinal TRPV1 receptors is not as straightforward as in the periphery, where temperature increase, low pH or release of different molecules due to tissue damage can gate or sensitize the receptor. A great effort was thus employed to detect possible endogenous agonists of central TRPV1 receptors (Starowicz *et al.* 2007). Anandamide (N-arachidonylethanolamine), an endogenous ligand of cannabinoid receptors was one of the first substances suggested to be an endogenous ligand of TRPV1 receptors (Zygmunt *et al.* 1999). Also several products of lipoxygenases (12- and 15-HPETE, 5- and 15-HETE) were found to directly activate TRPV1 receptors in isolated membrane patches of sensory neurons (Hwang *et al.* 2000). Endogenous agonists of TRPV1 were found between unsaturated *N*-acyldopamines isolated from bovine striatal extract. *N*-arachidonoyldopamine (NADA) potently activated native TRPV1 in neurons from rat DRG thereby inducing release of substance P and calcitonin gene-related peptide (CGRP) from spinal cord slices (Huang *et al.* 2002). *N*-oleoyldopamine (OLDA) induced calcium influx in TRPV1-transfected HEK cells and dose-dependent thermal hyperalgesia when injected subcutaneously in plantar surface of the paw (Chu *et al.*, 2003). Direct interaction of omega-3 polyunsaturated fatty acids with TRPV1 was demonstrated by Matta *et al.* (2007). For example docosahexaenoic acid can act as an agonist, whereas eicosapentaenoic and linolenic acid exhibit inhibitory effect on the TRPV1 receptors.

Spinal cord TRPV1 receptors distribution

High density of TRPV1 receptors was detected and visualized in the superficial dorsal horn of the spinal cord using radiolabelled resiniferatoxin ($[^3\text{H}]\text{RTX}$), an ultra potent analog of capsaicin, in the rat, pig and man (Szallasi *et al.* 1994). Staining for TRPV1 receptors was

absent or highly reduced following administration of high, neurotoxic doses of capsaicin and ipsilaterally after unilateral dorsal rhizotomy, suggesting that the majority of the TRPV1 receptors in the spinal cord are present on the central branches of dorsal root ganglion (DRG) sensory neurons (Szallasi *et al.* 1994, Guo *et al.* 1999, Valtschanoff *et al.* 2001). DRG neurons express TRPV1 not only on both central and peripheral terminals, but also on the cell bodies (Acs *et al.* 1994). The expression of TRPV1 receptors in DRG neurons is restricted to small and medium sized cells with C and A- δ axons, corresponding to nociceptors. It was demonstrated by *in situ* hybridization for TRPV1 mRNA (Caterina *et al.* 1997) and by TRPV1-immunoreactivity (Guo *et al.* 1999).

Capsaicin sensitive primary afferent fibers terminate predominantly in lamina I and II of the superficial dorsal horn, the inner part of lamina II (II_i) receiving substantial input compared to outer part (II_o) (Guo *et al.* 1999, Valtschanoff *et al.* 2001). Notable TRPV1 expression was also found along the lateral collateral path, where the majority of visceral afferents terminate and sporadic TRPV1-immunostaining was detected in laminae III-V (Hwang and Valtschanoff, 2003). Study by Valtschanoff *et al.* (2001) demonstrated also occurrence of postsynaptic TRPV1 in the superficial dorsal horn in rat. They observed patchy TRPV1 immunostaining in cell bodies in lamina II, which was dependent on presynaptic input, as it was lost after dorsal rhizotomy. Spinal neurons expressing TRPV1 receptors were confirmed in another study, where it was shown that some of them were also immunopositive for NK1 receptor (Doly *et al.* 2004).

The density of TRPV1 receptors changes longitudinally in the spinal cord as was shown by [³H]RTX autoradiography. The receptor labeling was comparable in the cervical and thoracic segments (approximately 70 fmol/mg protein) but it was twice as high in the lumbar segment, while membranes obtained from all spinal cord segments showed similar affinities for RTX (Szallasi *et al.* 1995). Immunofluorescent and confocal microscopy showed

that the density of TRPV1-positive fibers and buttons in the dorsal horn increases progressively from spinal segments L4 to L6 (Hwang and Valtschanoff, 2003). This study also suggested that TRPV1 receptors are preferentially expressed by visceral afferents.

The TRPV1 receptors density and their localization in the spinal cord are changing during development. Neonatal rats at postnatal day 2 (P2) have three-fold higher expression of TRPV1 than have adult rats (Acs *et al.* 1994). The shift of appearance of TRPV1 protein in the terminals of sensory neurons over postnatal period was demonstrated by Guo *et al.* (2001). As early as embryonic day (E) 16 and P2, TRPV1 immunoreactivity was only observed in lamina I and hardly visible in lamina II. However, at P10 the expression zone became wider and appeared to diffuse into lamina II. This pattern was enhanced at P20, when TRPV1 immunoreactivity was further developed and more prominent in lamina II. By P30 TRPV1 immunoreactivity appeared as two distinctive zones notably in lamina I and II_i corresponding to the pattern present in adult animals.

Recently, TRPV1 receptors were observed not only in neurons but also in glial cells in the rat dorsal horn of the spinal cord. Using immunocytochemistry at the ultrastructural level, TRPV1 receptors were detected in astrocytes by double labeling with glial fibrillary acid protein in both laminae I and II, where TRPV1 positive astrocytes corresponded to 7% of the total TRPV1 labeling in this area (Doly *et al.* 2004).

Colocalization of TRPV1 positive fibers with peptides in the spinal cord

Capsaicin administration on the spinal cord slice elicits simultaneous release of neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) in the dorsal horn (Saria *et al.* 1986). As well, intrathecal capsaicin application results in depletion of SP from primary sensory neurons and increased thresholds for thermal and chemical stimuli (Yaksh *et al.* 1979). It was shown that about 30% of VR1 positive DRG neurons co-express also SP,

while about 75% of SP positive neurons are also VR1 positive and this remains quite constant during development (Guo *et al.* 2001). In contrast, the extent of colocalization of VR1 and SP immunoreactivity in the superficial dorsal horn was much less than anticipated, given their coexistence in DRG cell bodies (Guo *et al.* 1999, Valtshanoff *et al.* 2001). While the colocalization of TRPV1 with SP in lamina I and along the lateral collateral path was unfrequented at L4, it was much more robust at L6 level, which is consistent with a higher degree of colocalization of TRV1 and SP in visceral than in cutaneous afferents (Hwang and Valtschanoff, 2003). There is significant coexpression of TRPV1 with pain related P_2X_3 purinoceptor and with glycoprotein that binds the lectin IB4 in lamina II_i (Guo *et al.* 1999).

It was shown before that about 80% of lamina I projection neurons express receptor for SP (neurokinin receptor NK1) (Todd 2002) and that these neurons play a major role in thermal hyperalgesia (Mantyh *et al.* 1997). Immunohistochemical study showed that there are direct contacts of TRPV1 containing afferents with NK1-positive dorsal horn neurons in lamina I. By retrograde tracing, it was also suggested that most of these synapses represented a monosynaptic pathway from periphery to the lateral parabrachial nucleus (Hwang *et al.* 2003). It was confirmed that the TRPV1 positive primary afferents are glutamatergic and contact spinal neurons that express main classes of ionotropic glutamate receptors (Hwang *et al.* 2004). The multiple immunofluorescent staining for TRPV1, AMPA and NMDA receptors also showed that a larger fraction of NK1/NMDA receptors positive sites were contacted by TRPV1 positive buttons, than those expressing TRPV1/AMPA receptors (Hwang *et al.* 2004).

TRPV1 impact on synaptic transmission in the spinal cord dorsal horn

Evidence of capsaicin evoked glutamate release from primary afferent fibers in rat was given by Ueda *et al.* (1994). The release of glutamate was measured using a fluorometric on-line monitoring system connected to an in vitro superfusion system. Administration of

capsaicin produced an increased outflow of glutamate and depletion of SP from dorsal horn slices. The capsaicin induced glutamate and SP release was concentration dependent in the range of 0.1-3 μ M and 1-10 μ M, respectively. Dorsal rhizotomy counteracted capsaicin induced increase of glutamate release from the DH of the spinal cord. Furthermore, it was shown that SP and CGRP, which are depleted by capsaicin, enhanced release of glutamate and aspartate from the rat spinal dorsal horn slice (Kangrga and Randic, 1990). Immunohistochemistry revealed depletion of not only SP but also cholecystokinin (CCK) following intrathecal capsaicin administration in concentration eliciting analgesia in rat (Micevych *et al.* 1983). In contrast, depletion of CCK was not confirmed by radioimmunoassay and in vivo superfusion of the rat spinal cord, where capsaicin elicited release of SP but not CCK (Jhamandas *et al.* 1984).

The effect of increased glutamate release on excitatory synaptic transmission in superficial dorsal horn following capsaicin application was shown also by electrophysiological methods. Capsaicin (2 μ M) administration evoked dramatic increase of spontaneous and miniature excitatory postsynaptic current (mEPSC) frequency in slices from adult rats. This activity was prevented by pretreatment with capsazepine, a competitive TRPV1 antagonist, and blocked by CNQX, a non-NMDA glutamate receptor antagonist (Yang *et al.* 1998). Capsaicin elicited increase in mEPSC frequency was demonstrated already from P0 with dramatic enhancement of the effect between P6 and P10. However, capsazepine failed to block the increase of mEPSC frequency elicited by the same concentration of capsaicin (2 μ M) in rat pups (P8-P11), while it was completely abolished by pretreatment with ruthenium red, a non-competitive TRPV1 antagonist (Baccei *et al.* 2003). Differential role of TRPV1 receptors on central branches of C and A- δ primary afferent fibers was suggested by experiments, in which C-fibers evoked EPSCs were inhibited by capsaicin administration in approximately 80% of substantia gelatinosa (SG) neurons, while A- δ fibers

elicited excitatory transmission was unaffected in adult rats (Yang *et al.* 1999). Capsaicin abolished dorsal horn neurons EPSCs evoked by electrical stimulation of attached dorsal root in pups (P7-P10), but facilitation of spontaneous glutamate release by capsaicin was sufficient to produce bursts of action potentials in superficial DH neurons in early postnatal age (P1-P5) (Baccei *et al.* 2003).

Originally it was assumed that inhibitory synaptic transmission in superficial DH of the spinal cord is not affected by TRPV1 receptors. GABAergic and glycinergic spontaneous IPSCs, and also A- δ fibers evoked IPSCs were unaffected by capsaicin administration in adult rats (Yang *et al.* 1998, Yang *et al.* 1999). However, based on immunohistochemistry it was hypothesized that SP released from primary nociceptors could activate GABAergic interneurons in the DH of cat spinal cord (Wei and Zhao, 1996). The increase of Fos expression in GABAergic neurons following intradermal capsaicin injection was observed in rat (Zou *et al.* 2001). Recently, Ferrini *et al.* (2007) detected increase of spontaneous inhibitory postsynaptic currents (sIPSCs) frequency induced by capsaicin administration (2 μ M) in lamina II neurons in spinal cord slice from mice pups (P8-P12). About 75% of sIPSCs were GABAergic and 25% were both GABA- and glycinergic. Capsaicin induced increase of sIPSCs frequency was attenuated by pretreatment with SB366791, a competitive high selective antagonist of TRPV1. Effect of capsaicin on sIPSCs in lamina II neurons was substantially dependent on activation of NK1 receptors and largely blocked by tetrodotoxin application, suggesting an action potential mediated mechanism. The authors propose TRPV1 dependent release of SP which excites inhibitory neurons in laminae I, III and IV resulting in increased release of GABA and glycine in the area of lamina II. Increase of glycinergic sIPSCs in superficial dorsal horn induced by capsaicin administration in rat spinal cord slice was reported also by Zhou *et al.* (2007) in about 70% of lamina II neurons.

Direct functional connection between capsaicin sensitive fibers and NK1-expressing spinal cord neurons was indicated by Labrakakis and Mac Dermott (2003). Using fluorescence conjugated SP they labeled NK1 receptor-expressing neurons which are prominent in lamina I and sporadic in lamina III and IV. Labeled neurons were patch clamped and the increase of mEPSCs frequency was observed in about 73% of lamina I and 43% of lamina III/IV neurons expressing NK1 receptor following capsaicin administration. Labeling of spinal cord slices from rats (P12-P25) by voltage sensitive dye revealed that capsaicin administration and increase in bath temperature inhibited the excitation evoked by C-fibers intensity stimulation, this effect was abolished by capsazepine and suramin, a purinoreceptor P₂X and P₂Y antagonist, but was facilitated by P₂Y agonist (Kusudo *et al.* 2006).

The role of spinal TRPV1 in pathological pain states

Intrathecal injection of capsaicin leads to transient hyperalgesia that can switch to long-term analgesia. Thermal antinociception following intrathecal injection of capsaicin which correlated with SP depletion in the rat and mice DH was demonstrated using hot-plate and tail-flick tests (Yaksh *et al.* 1979, Gamse 1982). Capsaicin injected intrathecally produced acute nocifensive behavior in mice, quantified as number of biting and scratching behavior. This response to capsaicin application was greatly reduced by intrathecal capsaicin or SP NH₂-terminal fragment (SP1-7) administration 24h before (Mousseau *et al.* 1994). Acute nocifensive behavior following intrathecal capsaicin application was present in rat as early as postnatal day 10 (P10) but was of longer duration in adult animals, while the intensity of the pain related behavior was the same in both age groups (Guo *et al.* 2001). Intrathecal application of TRPV1 agonist resiniferatoxin (RTX) led to ablation of TRPV1 expressing DRG neurons and to significant relief of hyperalgesia induced by inflammation in rats and arthritic pain in dogs (Karai *et al.* 2004).

Direct intrathecal application of TRPV1 antagonists displays antinociceptive effect in chronic pain states. Kanai *et al.* (2005) showed that intrathecal N-(4-tertiarybutylphenyl)-4-(3-cholorphyrudin-2-yl)tetrahydropyrazine-1(2H)-carbox-amide (BCTC), a selective antagonist of TRPV1 receptors, reduced mechanical allodynia present in model of chronic constriction injury. BCTC blocked also capsaicin stimulated release of neuropeptides in acute spinal cord slices. The analgesic effect of TRPV1 antagonist in the model of neuropathic pain could be partially mediated by up-regulation of TRPV1 receptors in the spinal cord as was documented by western blot analysis. Other recently developed TRPV1 receptors antagonists, A-784168 and A-795614, reduced inflammatory thermal hyperalgesia, mechanical allodynia and osteoarthritic pain following intrathecal administration (Cui *et al.* 2006).

Wilson-Gerwing *et al.* (2005) demonstrated that intrathecal infusion of neurotrophin-3 (NT-3), a negative modulator of nerve growth factor (NGF) expression, prevented or abolished the development of thermal hyperalgesia, but not mechanical hypersensitivity in the model of chronic constriction injury in rat. Thermal hyperalgesia in this experiment correlated with increased expression of TRPV1 mRNA and protein in DRG neurons. Thermal hypersensitivity and up-regulation of TRPV1 expression was reversed by infusion of NT-3. Increased TRPV1 expression was shown in the spinal cord dorsal horn of rats that had thermal hyperalgesia after spinal cord injury when compared to those without hyperalgesia (DomBourian *et al.* 2006). Carrageenan induced peripheral inflammation increased transport of TRPV1 mRNA from the cell body of DRG neurons to their dorsal horn terminals as was demonstrated by Tohda *et al.* (2001). The change in TRPV1 mRNA distribution was blocked by inhibitor of axonal transport colchicine. Capsaicin stimulated increased glutamate release in the dorsal horn during peripheral inflammation was abolished by cyclohexamide, an inhibitor of RNA translation and also by intrathecal injection of antisense oligonucleotides

against TRPV1. The later method was also effective in reducing mechanical hypersensitivity in rats with spinal nerve ligation (Christoph *et al.* 2007).

Anandamide, an endogenous cannabinoid (CB) receptor and TRPV1 agonist, at high concentration can excite TRPV1-positive afferents in the dorsal horn leading to depletion of neuropeptides, while low concentration of anandamide gate the CB receptors linked to dorsal horn inhibition (Tognetto *et al.* 2001). Carrageenan induced thermal hyperalgesia (with acute transient pain behavior) was shown to be reduced by intrathecal administration of anandamide, while this effect was modulated by capsazepine (Horvath *et al.* 2008). However, the highest dose of anandamide (100 µg) induced nocifensive behavior.

Lappin *et al.* (2006) demonstrated decrease of sEPSCs or mEPSCs frequency and reduction of amplitude of EPSCs evoked by C-fibers electric stimulation by bath administration of SB-366791 in dorsal horn neurons recorded in spinal cord slices from rats (P17-P29) after peripheral inflammation, while there was no effect of the antagonist in neurons recorded in slices from control animals. Based on these results authors suggest, that TRPV1 receptors in spinal cord DH become tonically active in animals with peripheral inflammation and enhance glutamate release. This is in good correspondence with findings, that capsaicin can induce higher concentration glutamate release from laminae I, II and X spinal cord located ipsilaterally to peripheral inflammation (Sasaki *et al.* 1998).

Conclusion

There is now mounting evidence suggesting that TRPV1 receptors present in the spinal cord dorsal horn play an important modulatory role in nociceptive and pain transmission. It needs to be further determined to what extent this role is important under control conditions and in different chronic pain states. Spinal cord TRPV1 receptors may represent an attractive target for pain therapy in the future.

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