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The vanilloid receptor-1 is expressed in rat spinal dorsal horn astrocytes

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Abstract

The vanilloid receptor-1 (TRPV1), expressed by nociceptive fibers, is a transducer of thermal and chemical nociceptive messages. However, endogenous ligands excite TRPV1 receptors localized on central nociceptive terminals and interneurons. Using immunocytochemistry at the ultrastructural level, we show that TRPV1 is also expressed in spinal glial cells characterized as astrocyte by double labeling with glial fibrillary acid protein. Quantification of the labeling shows that the most numerous labeling is neuronal and that 7% of the total TRPV1 labeling is localized in astrocytes. The total absence of staining in TRPV1 knock out mice strongly suggests that true TRPV1 protein is present in astrocytes. The localization of TRPV1-containing astrocytes apposed to nociceptive C-terminals suggests that they may be involved in the control of pain transmission.
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The vanilloid receptor-1 (TRPV1) is a ligand-gated cation channel sensitive to capsaicin [6]. It is also activated by heat, protons [6] and inflammatory mediators [9]. TRPV1 is expressed by nociceptive primary neurons [6] where it is involved in transduction of nociceptive stimulations [6]. Several arguments led us to hypothesize that TRPV1 receptors may also be expressed by astrocytes. (1) Recent studies have shown that TRPV1 is expressed not only by primary nociceptive neurons but also by instraspinal neurons [15]. (2) TRPV1 and the cannabinoid receptor-1 (CB1), co-localized in a subpopulation of dorsal root ganglion cells [2], are linked by a common endogenous ligand anandamide [18]. And CB1 receptors have already been demonstrated in spinal astrocytes [12]. (3) Finally, TRPV1 is essential for the development of inflammatory hyperalgesia at both peripheral [5] and spinal levels [14]. And spinal astrocytes are known to be activated in experimental models that produce hyperalgesia [17]. In order to verify this hypothesis, we have investigated the presence of TRPV1 immunoreactivity in astrocytes in the dorsal horn of the rat spinal cord.

The experiments were performed on 12 adult male Wistar rats in accordance with the European Community Council Directive (86/609/EEC). Under anesthesia, rats were perfused intracardially with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4). Lumbar spinal cords were dissected out and postfixed in the same fixative. Free floating vibratome sections (50 μm thick) were preincubated in 0.02 M saline phosphate buffer (pH 7.4) containing 6% bovine serum albumin (PBS-BSA), then incubated for 15 h at 4 °C with (1/1000) goat anti-TRPV1 antibodies (Santa Cruz, USA). Sections were incubated for 1 h in 1/250 biotinylated horse anti-goat IgG (Vector, USA) and then incubated for 1 h in 1/250 avidin-biotinylated-peroxidase complex (Vector, USA). The peroxidase reaction was processed with 0.04% (w/v) 3,3′-diaminobenzidine (Sigma, France) and 0.01% (v/v) hydrogen peroxide in 0.5 M Tris–HCl (pH 7.4). The specificity of the reaction was controlled by preabsorption of the antibodies (1/1000) with 100 μg/ml immunizing peptide. The antibodies' specificity was controlled on spinal cord sections of three mice invalidated for the TRPV1 gene (produced by Caterina et al. [5] and purchased from Jackson Lab., USA). Three wild type mice were processed in parallel. For electron microscopy, immunolabeled sections were postfixed with 1% osmium tetroxide and embedded in araldite. Ultrathin sections were stained with lead citrate and examined using a Jeol EM.

The TRPV1 labeling was quantified directly under the electron microscope in the two superficial layers from 12

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ultrathin lumbar sections from six different animals. Cellular processes were classified as axon, axon terminals, perikarya, dendrites or glia according to morphological criteria previously described [11]. All elements containing a peroxidase reaction product associated with a subcellular organelle were counted, even if it was restricted to a small group of neurotubules (often seen in dendrites) or to a few synaptic vesicles. Counting was made in dorsal horn lamina I (n:3388) and lamina II (n:2395). The total surface area examined was 221 000 μm^2.

For double labeling TRPV1/glial fibrillary acidic protein (GFAP), sections were incubated in a mixture of 1/1000 goat anti-TRPV1 antibodies and 1/1000 rabbit GFAP (DAKO, Denmark) for 15 h. Sections were incubated in 1/250 biotinylated anti-goat antibodies (Vector, USA) for 1 h and then in 1/400 streptavidin-Cy3 (Sigma, France) for 1 h. Finally, sections were incubated in 1/400 donkey anti-rabbit-FITC (Santa Cruz, USA) for 1 h. The images were collected with an MRC 600 confocal microscope (Bio-Rad). For ultrastructural TRPV1/GFAP double labeling, sections were incubated in 1/1000 rabbit anti-GFAP antibodies, for 15 h and then in 0.8 nm gold-labeled anti-rabbit IgG antibodies (1/100) (Aurion, France) for 4 h in PBS-BSA. Gold was intensified by the silver intensification kit (Sigma, France). Sections were then fixed in 2.5% glutaraldehyde. TRPV1 labeling was performed as seen above using the avidin-biotin-peroxidase method. The following control experiments were performed: (1) antigen/antibody preabsorption (see above); (2) omission of the primary antisera to test the background brought by the second antibodies; (3) reciprocal labeling (gold for TRPV1, peroxidase for GFAP).

TRPV1 immunoreactivity was concentrated in the dorsal horn, particularly in lamina I. The outer part of lamina II was weakly labeled whereas the inner part was intensely labeled (Fig. 1A). Antigen/antibody preabsorbtion resulted in a total absence of staining. A total absence of staining was observed in TRPV1 knock out mice, whereas wild type mice displayed a pattern of immunoreactivity comparable to that observed in the rat (Fig. 1B,C). Double labeling TRPV1/GFAP revealed co-localization in the dorsal horn (Fig. 1D). Under the electron microscope, numerous presynaptic labeling (in axon and axon terminals) but also a consistent postsynaptic labeling (cell bodies, dendrites) was observed. TRPV1 labeling was also observed in glial cells (Fig. 2), identified by their amorphous shape, lack of vesicles and synaptic contacts, and presence of microfilaments. The glial labeling was localized in the cytoplasm; it appeared as patches of grey precipitate. Labeled astrocytes were found as foot processes on blood vessels (Fig. 2A). They might surround primary afferent terminals (Fig. 2B). Double

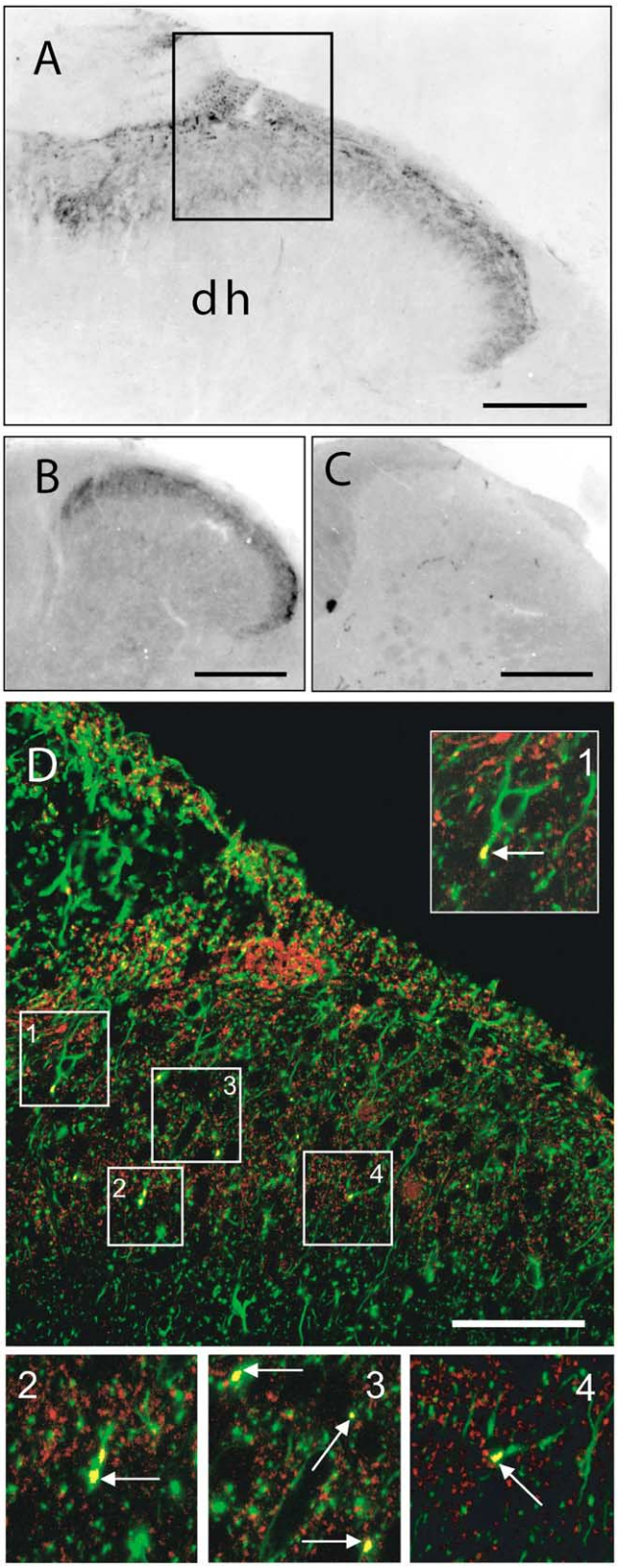


Fig. 1. (A) TRPV1 immunolabeling in the lumbar (L4) dorsal horn of the rat spinal cord. Immunoreactive fibers are dense in the two superficial layers of the dorsal horn (dh), particularly in lamina I and IIi while lamina Iio is less labeled. (B) TRPV1 immunoreactivity in the mouse lumbar spinal cord. Notice the labeling of the two superficial layers. (C) Absence of TRPV1 labeling in a mouse in which the TRPV1 gene has been invalidated. (D) Double labeling TRPV1 (red)/GFAP (green) in the region corresponding to the insert in (A). Colocalization is indicated in yellow (arrows). Four examples are shown at higher magnification. Scale bars 250 μm in (A), (B), (C) and 50 μm in (D).

labeling (silver-gold for GFAP and peroxidase for TRPV1) showed a co-localization in the same glial process (Fig. 2C). Antigen/antibodies preabsorption led to a total absence of labeling. Moreover, the reciprocal labeling (gold for TRPV1, peroxidase for GFAP) gave the same results (not shown). Finally, no labeling was present under the electron microscope in knock out mice either in neuronal or in glial cells. The quantitative analysis showed that the main labeled neuronal profiles in laminae I and II were axons and terminals.

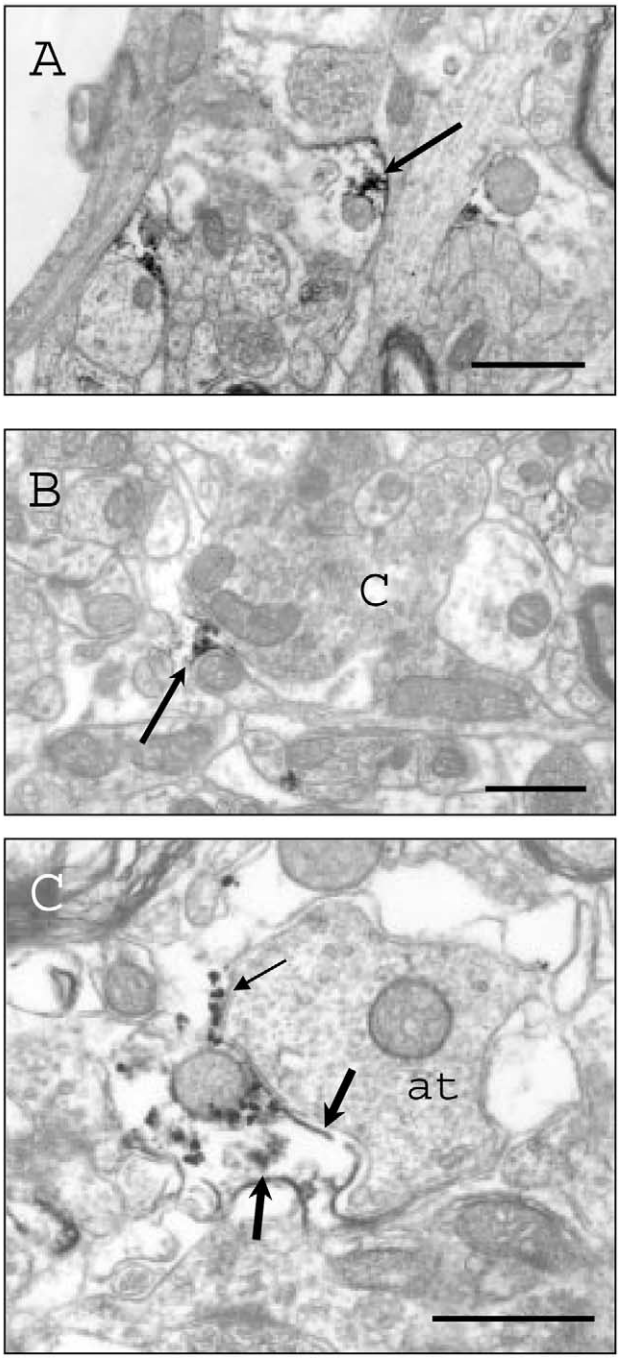


Fig. 2. TRPV1 labeling in glial cells at the electron microscope level. In (A), a labeled astrocytic foot is apposed to a capillary. In (B), a labeled glial process surrounds a primary afferent axon terminal (C), presumably the center of a C1 glomerulus. In (C) a double labeling TRPV1/GFAP is shown. GFAP labeling (gold, pointed by thin arrows) appears as dense black particles, peroxidase-TRPV1 labeling (large arrows) appears as grey precipitate. Scales bars: 250 nm.

In laminae I and II axons represented 42.1% and 39.4% of the total immunoreactive elements, respectively; axon terminals represented 35.1% and 22.5%. The postsynaptic labeling, in dendrites and cell bodies, was less frequent in lamina I than in lamina II. In lamina I, 16.3% of the total labeling was localized in dendrites, whereas in lamina II the labeled dendrites represented up to 30% of the total labeling. In contrast, the percentage of glial labeling was nearly constant over the two laminae (7.4% in lamina I and 7% in lamina II).

Our results show that TRPV1 immunoreactivity in the rat dorsal horn is observed not only in neuronal but also in glial cells. The absence of immunoreactivity in the dorsal horn of TRPV1 knock out mice (at both light and electron microscope levels) suggests that true TRPV1 is expressed in glial cells. Double labeling with GFAP shows that TRPV1 glial cells are astrocytes. The quantification of TRPV1 labeling at the ultrastructural level shows, in agreement with previous data, that the most numerous immunoreactive processes were neuronal (in axons and axon terminals). It also shows that the post-synaptic labeling, in dendrites and cell bodies, is substantial, particularly in lamina II. Finally it demonstrates that the glial labeling corresponds to a relatively high percentage (about 7%) of the total labeling in both laminae. Such a non-neuronal localization of TRPV1 has been previously described in peripheral tissues for example in cardiomyocytes [8] or epithelial cells [4]; we demonstrate here that a non-neuronal localization of TRPV1 also occurs in the central nervous system. The presence of immunoreactive astrocytic feet apposed to blood vessels suggests that endogenous vanilloids may play a role in the control of blood pressure at the spinal level. Several data already shows that anandamide is involved in the control of blood pressure through TRPV1 and/or CB1 receptors [10].

Some TRPV1-labeled astrocytes are apposed to C fiber terminals suggesting that they could be involved in pain modulation. Anandamide, a potential TRPV1 endogenous ligand [13], has been shown to inhibit astrocytic gap junctions in the striatum, and hence modulates glutamate synapses, through a non-CB1 mechanism [16]. It remains to determine whether such a mechanism also occurs in the spinal cord and whether TRPV1 is involved. Astrocytic TRPV1 receptors in the spinal cord may be also involved in inflammation and central sensitization, and actually, it has been shown that capsaicin stimulates NO release from isolated dorsal root ganglion neurons [3]. Moreover, in several models of pathological pain a ‘glial reaction’ consisting of a drastic swelling of astrocytes leading to the release of proinflammatory substances is observed [17].

CB1 and TRPV1 receptors are co-expressed in numerous C-fibers [2] and a physiological balance between the two

receptors has been suggested. Anandamide may regulate CGRP release from capsaicin sensitive fibers in vivo through TRPV1 or CB1 receptors, depending on anandamide concentration but also on the state of the receptors [1]. At the spinal level, anandamide has been shown to modulate capsaicin-sensitive fibers in a concentration-dependent manner, inhibitory through CB1 and excitatory through TRPV1[13]. In addition, several allosteric interactions by pro-inflammatory substances may enhance the effect of anandamide on TRPV1 (see Ref. [7]). In order to bring further insights into the role of CB1/ TRPV1 receptors in pain modulation we are currently investigating their expression after acute and chronic inflammation. Are TRPV1 mRNAs up-regulated only in the central end of primary fibres [14] or also in astrocytes and/or spinal neurons? Are CB1 receptors modified in the same conditions?

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