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TRPV1 antagonist, SB-366791, inhibits glutamatergic synaptic transmission in rat spinal dorsal horn following peripheral inflammation

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Abstract

The anti-hyperalgesic effects of TRPV1 receptor antagonists are well documented in animal models of pain, however, the precise site of their action is not known. Here we have examined the effects of the selective TRPV1 antagonist SB-366791 on glutamatergic synaptic transmission in substantia gelatinosa using spinal cord slices from either control rats or animals that had undergone a peripheral inflammation induced by intraplantar injection of Freund's complete adjuvant (FCA). In control animals, SB-366791 (30 μM) had no effect on spontaneous excitatory post-synaptic currents (sEPSC) or evoked EPSCs. In slices from FCA-inflamed animals, SB-366791 decreased sEPSC frequency to 66±8% of control in 5/10 neurones, and decreased miniature glutamatergic EPSCs (mEPSC) frequency to 63±4% of control, in 6/7 neurones; with no significant effect on sEPSC or mEPSC amplitude. Dorsal root evoked EPSCs at C-fibre intensity were reduced to 72±6% of control by SB-366791 (30 μM) in 3/4 neurones from FCA-treated animals. In conclusion, SB-366791 inhibited glutamatergic transmission in a subset of neurones via a pre-synaptic mechanism following peripheral inflammation. We hypothesise that during peripheral inflammation spinal TRPV1 becomes tonically active, promoting the synaptic release of glutamate. These results provide evidence for a mechanism by which TRPV1 contributes to inflammatory pain and provides a basis for the understanding of the efficacy of TRPV1 antagonists.

Keywords: TRPV1; Spinal cord; Inflammation; Glutamate; Synaptic transmission; Substantia gelatinosa

1. Introduction

Transient receptor potential vanilloid 1 (TRPV1) is a ligand-gated cation channel that is primarily found on small-diameter primary afferents, particularly unmyelinated C-fibres, and is activated by heat (>42 °C), protons and a range of both endogenous and exogenous ligands (Caterina et al., 1997; Tominaga et al., 1998; Smart et al., 2000; Van Der Stelt and Di Marzo, 2004; Calixto et al., 2005). The polymodal activation exhibited by TRPV1 makes it of particular interest in pain transduction, because it creates the potential for a single protein to integrate a range of varied noxious stimuli (Caterina et al., 1997; Hayes et al., 2000). TRPV1 null mice are devoid of the hypersensitivity to thermal stimuli which accompanies an inflammatory insult (Caterina et al., 2000; Davis et al., 2000), providing key evidence for the role of TRPV1 in inflammatory pain processing and indicating that TRPV1 function may well be upregulated by inflammatory cascades. Mediators of inflammation, such as ATP, bradykinin, acid and prostaglandins, are known to activate, modulate and sensitise ion channels and receptors causing hypersensitivity, and there is evidence that not only is the expression of TRPV1 increased by peripheral inflammation (Amaya et al., 2003; Tohda et al., 2001), but that TRPV1 function is enhanced in the presence of inflammatory mediators (Tominaga et al., 1998, 2001; Sugiura et al., 2002; Moriyama et al., 2005).

Clinically, TRPV1 agonists have been used as topical analgesics (Mason et al., 2004 for review) and have shown promise in the treatment of incontinence (Cruz, 2004 for review). The clinical efficacy of TRPV1 agonists is thought to be through desensitisation and/or downregulation of the receptor (Simone et al., 1998; Nolano et al., 1999) or a physical or functional "ablation" of nociceptive terminals. A number of TRPV1 antagonists are reported to be in clinical development for pain and related conditions. There may be potential benefits of an antagonistic approach, including a more rapid onset of action and

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a lack of the initial pain sensation that results from agonist activation of TRPV1 (Rami and Gunthorpe, 2004 for review). The analgesic potential of TRPV1 antagonists was initially studied using the tool compound capsazepine. Although efficacious, this molecule is far from an ideal TRPV1 antagonist on a number of fronts, it has well documented actions at a range of other channels (Docherty et al., 1997; Liu and Simon, 1997; Gill et al., 2004; Behrendt et al., 2004; Weil et al., 2005) and a poor pharmacokinetic profile (Lopez-Rodriguez et al., 2003). In recent years, a number of more selective TRPV1 antagonists have appeared, the first of which was Iodo-Resiniferatoxin (Wahl et al., 2001). Although Iodo-Resiniferatoxin is a potent and selective inhibitor of TRPV1 it has limited utility in vivo (Almasi et al., 2003; Rigoni et al., 2003; Seabrook et al., 2002), and has recently been shown to have partial agonist actions at TRPV1 in vitro (Shimizu et al., 2005). The effect of other antagonists of TRPV1 have been investigated using tools such as BCTC (Valenzano et al., 2003), AMG9810 (Gavva et al., 2005) and A-425619 (El Kouhen et al., 2005), as well as SB-366791 (Gunthorpe et al., 2004). All of these compounds are potent inhibitors of TRPV1 activation by heat, acid and capsaicin and a number of these have been shown to possess efficacy in in vivo animal models of pain, such as capsaicin-induced wiping, capsaicin- and FCA-induced hyperalgesia, and in neuropathic pain models including partial sciatic nerve injury and chronic constriction injury (Gavva et al., 2005; Honore et al., 2005; Kanai et al., 2005; Pomonis et al., 2003; Varga et al., 2005). Although this provides clear evidence for the utility of TRPV1 antagonists in pain, the specific site of their action is still to be determined.

The small-diameter primary afferent fibres, on which TRPV1 is located, carry nociceptive information from the periphery to the superficial dorsal horn of the spinal cord. It is here, in particular within substantia gelatinosa (lamina II of dorsal horn), where the initial modulation and integration of nociceptive information occurs (Sugiura et al., 1986; Willis and Coggeshall, 1991). To date, the post-inflammatory role of TRPV1 has yet to be extensively investigated at this first level of integration of peripheral transmission. Here we report that following peripheral inflammation, a tonic excitatory presynaptic activity appears in the spinal cord that is sensitive to the TRPV1 antagonist SB-366791. This provides further validation for the role of TRPV1 in inflammatory pain, and an insight into the potential site of action that may underlie the therapeutic potential of TRPV1 antagonists.

2. Materials and methods

2.1. Freund's complete adjuvant (FCA) model of inflammatory pain

All animal procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. Lister-hooded rats (17–29 days old) were injected intraplantar with a 25 μl Freund's complete adjuvant (FCA, 1 mg/ml solution of Mycobacterium tuberculosis; Sigma, St. Louis, MO), in their left hind paw. This procedure produced a peripheral inflammation and pain hypersensitivity that was characterised by a reduction in paw withdrawal thresholds, peaking 24 h post-injection (Walker et al., 2001). 24 h after receiving the FCA treatment animals underwent euthanasia and removal of the spinal cords, as described below.

2.2. Slice preparation

These methods have been previously described in detail (Morisset and Nagy, 1989). Briefly, Lister-hooded rats, aged 17 to 29 days old, were anaesthetised with halothane and subsequently decapitated in accordance with UK Home Office guidelines. A dorsal laminectomy was performed to remove the spinal cord, and 400 μm transverse slices were obtained from the lumbar enlargement (L4–L6), using a Leica VT1000S automatic slicer. In animals that had undergone an FCA injection in the left hind paw, the spinal cord was orientated such that a small incision could be made through the tissue on the right ventral side of the cord, therefore allowing identification of the left dorsal horn and substantia gelatinosa to enable recording ipsilateral to the injected side. Spinal cord slices were transferred to a submerged recording chamber where they were superfused at 2–3 ml/min with an artificial cerebrospinal fluid (aCSF) equilibrated with 95% O_2–5% CO_2, at a temperature of 30 °C. The aCSF contained (in mM) 124 NaCl, 2.4 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 KH_2PO_4, 26 $NaHCO_3$, and 10 glucose. Small deviations in the pH of the solution were adjusted back to pH 7.4. Slices were allowed to recover for at least 1 h before recordings were initiated.

2.3. Electrophysiological recordings

Lamina II neurones were visually identified using infrared differential interference contrast microscopy (BX50WI, Olympus) and patch-clamp recordings were made in the whole cell configuration with pipettes (6–10 MΩ) containing (in mM) 120 K-Gluconate, 20 KCl, 0.1 $CaCl_2$, 1.3 $MgCl_2$, 1 EGTA, 10 HEPES, 0.1 GTP, 0.2 cAMP, 0.1 leupeptin, and 3 Na_2-ATP, pH 7.3 (KOH). Membrane currents were recorded using an Axopatch 200B amplifier (Axon Instruments) and filtered at 2 kHz. Signals were digitized at 20 kHz using a Digidata 1200 interface (Axon Instruments) and the pClamp 9 software package. Excitatory postsynaptic currents (EPSCs) were recorded in all neurons at a holding potential of −60 to −70 mV. The addition of 30 μM 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) abolished all synaptic events, indicating the involvement of non-NMDA glutamate receptors (data not shown). Synaptic responses were evoked using a concentric bipolar electrode, inner diameter 12.5 μm, outer diameter 125 μm, (FHC, Bowdoinham, ME) placed on the dorsal root. The stimulus intensity used to activate Aδ- and C-fibres was >500 μA and 500 μs (Morisset and Nagy, 1989). The stimulus was applied every 20–30 s, to avoid any activity-dependent modification of synaptic responses.

2.4. Analysis

All data were captured using pClamp 9 software (Axon Instruments Ltd) and analysed off-line using the programmes