The VR1 Antagonist Capsazepine Reverses Mechanical Hyperalgesia in Models of Inflammatory and Neuropathic Pain

KATHARINE M. WALKER,1 LASZLO URBAN, STEPHEN J. MEDHURST,2 SADHANA PATEL, MOHANJIT PANESAR, ALYSON J. FOX, and PETER MCINTYRE

Novartis Institute for Medical Sciences, London, United Kingdom

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ABSTRACT

Vanilloid receptor type 1 (VR1) (TRPV1) is a ligand-gated ion channel expressed on sensory nerves that responds to noxious heat, protons, and chemical stimuli such as capsaicin. Herein, we have examined the activity of the VR1 antagonist capsazepine in models of inflammatory and neuropathic pain in the rat, mouse, and guinea pig. In naive animals, subcutaneous administration of capsazepine (10–100 mg/kg s.c.) did not affect withdrawal thresholds to noxious thermal or mechanical stimuli. However, pretreatment with capsazepine prevented the development of mechanical hyperalgesia induced by intraplantar injection of capsaicin, with a similar potency in all three species. Capsazepine (up to 100 mg/kg s.c.) did not affect mechanical hyperalgesia in the Freund’s complete adjuvant (FCA)-inflamed hind paw of the rat or mouse. Strikingly, capsazepine (3–30 mg/kg s.c.) produced up to 44% reversal of FCA-induced mechanical hyperalgesia in the guinea pig. Capsazepine also produced significant reversal of carageenan-induced thermal hyperalgesia in the guinea pig at 30 mg/kg s.c., but was ineffective in the rat. Similarly, in the partial sciatic nerve ligation model of neuropathic pain, capsazepine was surprisingly effective in the guinea pig, producing up to 80% reversal of mechanical hyperalgesia (1–30 mg/kg s.c.) but had no effect in the rat or mouse. These data show that VR1 antagonists have antihyperalgesic activity in animal models of chronic inflammatory and neuropathic pain, and illustrate species differences in the in vivo pharmacology of VR1 that correlate with differences in pharmacology previously seen in vitro.

The vanilloid receptor type 1 (VR1) is a pivotal molecular integrator of noxious stimuli that is expressed on somatic and autonomic primary afferent neurons. VR1 has been confirmed as a ligand-gated ion channel after its cloning from rat (Caterina et al., 1997) and human (Hayes et al., 2000; McIntyre et al., 2001). In vitro studies using recombinant VR1 have shown that, like the native vanilloid receptor, it can be activated by a variety of plant-derived compounds, including capsaicin, a pungent component from chili peppers, and resi-niferatoxin (Szallasi and Blumberg, 1999), as well as noxious heat (Caterina et al., 1997), low pH (Tominaga et al., 1998), and some lipid mediators such as anandamide (Smart et al., 2000) and the lipoygenase product 12-(S) hydroxyei-cosatetraenoic acid (Hwang et al., 2000).

Capsazepine is a VR1 antagonist that has been shown to competitively inhibit capsaicin-mediated responses in isolated dorsal root ganglion (DRG) neurons (Bevan et al., 1992a) or tissues from rat (Bevan et al., 1992b; Maggi et al., 1993; Jerman et al., 2000), mouse (Urban and Dray, 1991), and guinea pig (Lou and Lundberg, 1992; Ellis and Undem, 1994; Fox et al., 1995). In vivo, capsazepine has also been shown to inhibit the nocifensive responses to capsaicin in mice and rats (Santos and Calixto, 1997), as well as capsaicin-induced bronchoconstriction or cough in the guinea pig (Satoh et al., 1993; Laloo et al., 1995). However, early studies investigating the potential antihyperalgesic effects of capsazepine in rat models of acute and chronic pain led to the idea that capsaicin antagonists would be unlikely to be useful as analgesics (Perkins and Campbell, 1992).

Nevertheless, other studies using capsazepine indicated possible species differences in the pharmacology of the vanilloid receptor. For example, although low pH solutions were shown to mimic the effect of capsaicin in several species, capsazepine inhibited low pH-evoked depolarization of sensory nerves in guinea pig airways (Lou and Lundberg, 1992; Satoh et al., 1993; Fox et al., 1995), but not in rat DRG neurons (Bevan et al., 1992b; Habelt et al., 2000). More recently, studies in our group
using recombinant VR1 have also demonstrated clear species selectivity in the activity of capsazepine. Thus, we have found that capsazepine inhibited responses to noxious heat and noxious heat and noxious cold stimuli in a dose-dependent manner, as well as capsain, at the cloned human or the cloned guinea pig VR1 expressed in Chinese hamster ovary cells, whereas at the rat VR1, it inhibited responses to capsazepine but not to low pH (McIntyre et al., 2001; Savidge et al., 2002).

The accumulating evidence for the activation of VR1 by several stimuli, together with the demonstration of species differences in the pharmacology has prompted us to reevaluate the activity of capsazepine in animal models of pain. Herein, we examine the effects of capsazepine in guinea pig, rat, and mouse models of persistent inflammatory and chronic neuropathic pain.

Materials and Methods

Compounds and Administration Procedures. Capsazepine (Tocris Cookson, Bristol, UK), Diclofenac and carbamazepine (Sigma Chemical, Poole, Dorset, UK) were administered subcutaneously in a volume of 10 μl; Sigma Chemical) into one hind paw. Capsaicin (Tocris Cookson) was dissolved in dimethyl sulfoxide to a stock solution of 100 mM with dilutions in saline and administered into the plantar region of the hind paw in a volume of 10 μl. Compounds were stored under blind experimental conditions. Carageenan and Freund's complete adjuvant (FCA) were obtained from Sigma Chemical.

Animals. Male Wistar rats (180–220 g), C57BL mice (25–30 g), and Dunkin-Hartley guinea pigs (250–300 g) were housed in groups of six and had free access to food and water at all times. All experiments were carried out according to the Declaration of Helsinki and the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, with approval from the Novartis Animal Welfare and Ethics Committee. For comparison with compound-treated groups, animals treated with the appropriate drug vehicle were included in each experiment. The volume of administration was identical for vehicle- and compound-treated rats. Vehicle-treated rats were identical to compound-treated rats with respect to all other experimental procedures.

Capsaicin-Induced Hyperalgesia. Many studies have demonstrated a dose-dependent sensitization to mechanical stimulation and the development of mechanical hyperalgesia after intradermal injections of capsain in human (Simone et al., 1989; LaMotte et al., 1992) and rodent pain assays (Ghilchrist et al., 1996). Herein, capsacin-induced mechanical hyperalgesia was assessed in rats, mice, and guinea pigs by measuring hind paw withdrawal thresholds to an increasing pressure stimulus using an analgesymeter (7200; Ugo Basile, Comerio, Italy) with a wedge-shaped probe (area 1.75 mm²). The cut-off was set to 250 g and the endpoint was taken as paw withdrawal or vocalization. In preliminary experiments, paw withdrawal thresholds were measured before and up to 30 min after an intraplantar injection of 1, 10, or 100 nmol of capsain into one hind paw. These experiments showed that 10 nmol of capsain produced a submaximal, reproducible mechanical hyperalgesia in all three species, and this dose was used for further studies. To examine the inhibition of capsacin-induced hyperalgesia, capsazepine (10–100 mg/kg) or vehicle was administered s.c. 30 min before capsain injection, and withdrawal thresholds were then measured 30 min after capsain injection. Percentage of inhibition of development of capsain-induced hyperalgesia was calculated according to the following formula:

% inhibition = \( \frac{(\text{naive + vehicle} - \text{postCAPS + vehicle}) - (\text{naive + CPZ - postCAPS + CPZ})}{(\text{naive + vehicle - postCAPS + vehicle})} \times 100\% \)

where CAPS refers to capsaicin administration and CPZ refers to capsazepine administration.

Inflammatory Hyperalgesia. Mechanical hyperalgesia was examined in a model of inflammatory pain in the rat, mouse, and guinea pig using the paw pressure technique as described above. Paw withdrawal thresholds were measured in naïve animals before an intraplantar injection of FCA (25 μl; Sigma Chemical) into one hind paw. Withdrawal thresholds were then measured 24 h later, before (predose) and up to 6 h after drug or vehicle administration (postdose). The nonsteroidal, anti-inflammatory drug diclofenac was used as a positive control in all experiments. Reversal of mechanical hyperalgesia was calculated according to the following formula:

% reversal = \( \frac{\text{postdose threshold} - \text{predose threshold}}{\text{naive threshold} - \text{predose threshold}} \times 100\% \)

Carageenan (Sigma Chemical)-induced thermal hyperalgesia was assessed in all three species using a Plantar Test Apparatus (Ugo Basile). During tests, rats, mice or guinea pigs were placed individually and unrestrained in 15 × 10.7 × 13.8 cm (length × width × height) Perspex boxes situated on a glass platform. Withdrawal latencies to an infrared source positioned underneath the mid-plantar hind paw were measured using a digital timer that stopped automatically upon paw withdrawal with an accuracy of 0.1 s. Withdrawal latencies were measured with a beam intensity of 22.9 for rats and mice and 50 for guinea pigs, with a cut-off of 32 s. Measurements were made twice on each paw, and the mean of the readings was used for data analysis. Latencies were determined in naïve animals and then 24 h after carageenan injection before (predose) and at 1 and 3 h after capsazepine or vehicle administration. Morphine was used as a positive control in all experiments.

Neuropathic Hyperalgesia. Mechanical hyperalgesia was examined in a model of neuropathic pain induced by partial ligation of the sciatic nerve, in the rat, mouse, and guinea pig. Briefly, animals were anesthetized, the left sciatic nerve exposed at mid-thigh level through a small incision, and one-third to one-half of the nerve thickness was tightly ligated with a 7.0 silk ligature. The wound was closed with a single muscle suture and skin clips and the animals allowed to recover for 11 to 15 days postligation. Withdrawal thresholds were measured on both the ipsilateral and the contralateral hind paws before (predose) and up to 6 h after drug or vehicle administration. The antiepileptic drug carbamazepine (30 mg/kg p.o.) was used as a positive control. We have previously determined that partial nerve ligation does not affect contralateral withdrawal thresholds and that sham surgery does not affect ipsilateral thresholds. Reversal of hyperalgesia was therefore calculated according to the following formula that uses the contralateral paw as a reference rather than using additional groups of naïve or sham animals:

% reversal = \( \frac{\text{ipsilateral threshold postdose} - \text{ipsilateral threshold predose} - \text{contralateral threshold predose} - \text{ipsilateral threshold predose} \times 100\% \)

Statistical Analyses. For all experiments, data analyses were performed on the untransformed paw withdrawal threshold or latency data by ANOVA with repeated measures, followed by Tukey's HSD post hoc analysis.

Results

Capsaicin-Induced Mechanical Hyperalgesia. Intraplantar injection of capsaicin (1–100 nmol) produced a rapid, dose-dependent mechanical hyperalgesia indicated by a decrease in paw withdrawal thresholds in mice, rats, and guinea pigs, which was maximal 30 min after injection (Fig. 1A). From this experiment, a dose of 10 nmol of capsaicin was used as a positive control in all experiments. Reversal of mechanical hyperalgesia was calculated according to the following formula: % reversal = \( \frac{\text{ipsilateral threshold postdose} - \text{ipsilateral threshold predose} - \text{contralateral threshold predose} - \text{ipsilateral threshold predose} \times 100\% \)
selected to investigate the effects of capsazepine. Administration of capsazepine (10–100 mg/kg s.c.) 30 min before capsaicin injection prevented development of capsaicin-induced mechanical hyperalgesia in a dose-dependent manner, with similar potency in rats, mice, and guinea pigs (Fig. 1B).

**Inflammatory Hyperalgesia.** FCA injection produced a pronounced mechanical hyperalgesia in all three species, with ipsilateral paw withdrawal thresholds in naive animals of approximately 100 g reduced to 55 to 65 g 24 h after treatment (data not shown).

Capsazepine (3–30 mg/kg s.c.) produced a marked reversal of inflammatory mechanical hyperalgesia in the guinea pig, with a maximal 44% reversal observed 1 h after administration (Fig. 2A). The efficacy of capsazepine was comparable with that of the nonsteroidal anti-inflammatory drug diclofenac, which produced up to 48% reversal of hyperalgesia, although the effect of capsazepine was more short-lived, with a loss of antihyperalgesic activity by 3 h postadministration. In contrast, capsazepine administered at doses up to 100 mg/kg did not affect mechanical hyperalgesia in rats or mice (Fig. 2B), although diclofenac (30 mg/kg s.c.) administered as a positive control in the same experiments produced 51 and
40% reversal in rats and mice, respectively (data not shown). There was no effect of capsazepine on contralateral paw withdrawal thresholds to mechanical stimulation in any of the species, and no other overt behavioral effects, such as motor defects or sedation, were observed.

Carrageenan injection into the hind paw produced thermal hyperalgesia in rats and guinea pigs (Urban et al., 2000) that was maximal 24 h after treatment. Capsazepine did not affect established inflammatory thermal hyperalgesia in the rat or mouse. Data are presented as percentage of reversal of hyperalgesia measured 1 h after capsazepine administration. In A and B, each point represents mean ± S.E.M. from six animals per group. *, P < 0.05 compared with vehicle by ANOVA followed by Tukey’s HSD test.

Neuropathic Hyperalgesia. After partial sciatic nerve ligation in rats, guinea pigs, and mice, ipsilateral paws exhibited marked mechanical hyperalgesia, with paw withdrawal thresholds averaging approximately 60 g compared with contralateral thresholds of approximately 100 g. Capsazepine (3–30 mg/kg s.c.) produced a dose-related reversal of mechanical hyperalgesia in the guinea pig. This effect was rapid in onset, with a maximal 80% reversal seen 30 min after administration, and was absent by 3 h postdose (Fig. 4A). There were no significant changes in contralateral paw withdrawal thresholds up to 3 h after capsazepine administration. The anticonvulsant carbamazepine was less efficacious than capsazepine in this experiment, producing approximately 40% reversal of hyperalgesia 3 h after administration. As observed in the inflammatory model, capsazepine did not affect ipsilateral (Fig. 4B) or contralateral (data not shown) paw withdrawal thresholds in neuropathic rats or mice at doses up to 30 mg/kg.
Discussion

We have studied the potential role of VR1 in chronic pain conditions by examining the antihyperalgesic activity of the VR1 antagonist capsazepine in models of persistent neuropathic and inflammatory pain. Experiments were carried out in rats, mice, and guinea pigs test the species selectivity of capsazepine indicated by in vitro studies (Savidge et al., 2002). As expected, capsazepine acts as an antagonist of capsaicin-induced mechanical hyperalgesia over the same dose range in the three species tested (Fig. 1). However, the most striking finding of this study is that capsazepine produces effective reversal of mechanical hyperalgesia associated with partial sciatic nerve ligation (Fig. 4) and both mechanical and thermal hyperalgesia associated with hind paw inflammation in the guinea pig (Figs. 2 and 3). The antihyperalgesic effects of capsazepine in the guinea pig models of persistent inflammatory and neuropathic pain were produced over the same dose range that inhibited the development of capsaicin-induced hyperalgesia and is, therefore, consistent with its VR1 receptor antagonist activity. Paw withdrawal thresholds from the noninjured contralateral paw were not altered after capsazepine administration, indicating that the antihyperalgesic effects of capsazepine in the guinea pig were restricted to hyperalgesia associated with inflammation or nerve injury, rather than reflecting an acute analgesic activity.

The antihyperalgesic activity of capsazepine observed in guinea pigs is in marked contrast to its lack of effect in mice and rats, where no antihyperalgesic activity was observed in either the neuropathic (Fig. 4B) or inflammatory models (Fig. 2B). This is in agreement with the findings of Perkins and Campbell (1992) who found that capsazepine administered alone did not have antinociceptive activity in several models of acute and chronic pain in rats (tail-flick, hot-plate, and carrageenan-induced inflammatory mechanical hyperalgesia). In contrast to these findings, other groups have reported that capsazepine prevents the early phase of formalin-induced mechanical hyperalgesia when administered intradermally in rats (Kwak et al., 1998) or mice (Santos and Calixto, 1997) and inhibits carrageenan-induced inflammatory hyperalgesia in the rat (Kwak et al., 1998). However, in both these studies, extremely high concentrations of capsazepine (>10 μM) were injected directly into the paw, which may have resulted in nonspecific actions of capsazepine on other ion channels (Docherty et al., 1997; Liu and Simon, 1997).

Interestingly, normal mice and mice lacking VR1 show comparable behavioral responses to formalin injection (Caterina et al., 2000), showing that VR1 activation is not essential for the formalin response.

The observation of species differences in the in vivo antihyperalgesic activity of capsazepine between guinea pig and rodents is consistent with previous in vitro and in vivo findings. As discussed in the Introduction, capsazepine is a potent antagonist of capsaicin effects in a variety of in vitro and in vivo assays in rats, mice, and guinea pigs. However, there are clear differences in the activity of capsazepine against other VR1 stimulants. Thus, it blocks responses of native VR1 to
low pH in the guinea pig airways (Satoh et al., 1993; Fox et al., 1995; Laloo et al., 1995), as well responses of both guinea pig and human VR1 to low pH and noxious heat (McIntyre et al., 2001; Savidge et al., 2002). In contrast, capsazepine does not affect responses of rat DRG neurons to low pH (Bevan et al., 1999b) or of the recombinant rat VR1 to low pH or heat (McIntyre et al., 2001; Savidge et al., 2002). The present findings therefore support the apparent species differences in the responses of VR1 uncovered by capsazepine, and further suggest that compounds that can block capsaicin, low pH and heat stimulation of human VR1, will be antihyperalgesic in humans.

Capsazepine was found to be weakly active against thermal hyperalgesia in guinea pigs (Fig. 3). This activity is in agreement with the role of VR1 in thermal hyperalgesia observed in studies of VR1 knockout mice. Thus, VR1 null mice show marked deficits to noxious thermal stimuli (Caterina et al., 2000) and a complete absence of carrageenan-induced thermal hyperalgesia (Davis et al., 2000). However, the hyperalgesic effects of heat cannot be explained by the action of VR1 alone, because mice lacking VR1 retain normal sensation of noxious heat (Caterina et al., 2000; Davis et al., 2000), suggesting the involvement of additional thermal receptors. In keeping with this hypothesis two VR1 homologues, VRL-1 (TRPV2) and TRPV3, have been reported to be insensitive to capsaicin or protons but respond to either higher or low-threshold heat stimulation and neither is blocked by capsazepine (Caterina et al., 1999; Peier et al., 2002).

The finding that capsazepine inhibits both thermal and mechanical hyperalgesia in guinea pig models of inflammatory and chronic pain was unexpected. Although no direct link between the mechanical stimulation and VR1 activation has previously been demonstrated, stimuli that activate VR1 have been shown to induce mechanical hypersensitivity. It is known that activation of the VR1 by capsaicin causes both thermal and mechanical hyperalgesia in rats, primates, and human subjects (Simone et al., 1988; LaMatte et al., 1991; Torebjork et al., 1992). In fact, the mechanical hyperalgesia observed after capsaicin injection is more robust than the thermal hyperalgesia both in terms of duration of action and the size of the area of secondary hyperalgesia (Gilchrist et al., 1996). It has been shown that an intradermal capsaicin injection facilitates the responses of dorsal horn neurons due to input of low-threshold mechanoreceptors and nociceptors (LaMatte et al., 1991; Simone et al., 1991). Thus, in inflamed tissue, local pH decreases may ultimately activate VR1, sensitizing nociceptors and low-threshold mechanoreceptors, decreasing the threshold at which mechanical stimuli result in the detection of noxious stimuli.

A role for VR1 in neuropathic and inflammatory pain is supported by several recent studies indicating that VR1 is up-regulated in both inflammatory and neuropathic conditions. Peripheral inflammation has been shown to increase the sensitivity of isolated DRG to capsaicin (Nicholas et al., 1999). Moreover, VR1 can be sensitized or activated by molecules such as prostaglandin E2 and bradykinin (Vyklicky et al., 1998), which are present in inflamed tissues and activation of PKC can either directly activate (Premkumar and Ahern, 2000) or sensitize (Vellani et al., 2001) VR1. After peripheral nerve injury VR1 protein is up-regulated in undamaged neurons and down-regulated in damaged neurons (Hudson et al., 2001). Taken together, these studies show that the expression and function of VR1 are regulated under conditions of altered noxious sensory input, which may explain why VR1 blockers can affect the sensation of noxious stimuli under pathophysiological, but not under normal conditions.

It is not clear why capsazepine was more effective in blocking the mechanical hyperalgesia associated with nerve injury (Fig. 4) than inflammation (Fig. 2) in the guinea pig. Perhaps it has to do with different levels of VR1 up-regulation or sensitization in these two conditions. The ability of capsazepine to reduce mechanical hyperalgesia after partial ligation of the guinea pig sciatic nerve may be attributed in part to altered expression of VR1. Partial ligation of rat sciatic nerve results in decreased VR1 immunoreactivity in damaged (ligated) neurons, but increased VR1 immunoreactivity in undamaged neurons (Hudson et al., 2001). It is interesting that the increased VR1 immunoreactivity was present only in C fibers in the L5 DRG but also in myelinated A fibers (Hudson et al., 2001), which provides evidence for the involvement of VR1 in mechanical sensitivity after nerve injury and supplies a rationale for why a VR1 antagonist is effective in reversing the pain behavior associated with mechanical hyperalgesia. The increased levels of VR1 may then also prime the sensory neurons to respond to other physiological consequences of nerve damage that occur postinjury, such as the release of inflammatory mediators from macrophages during Wallerian degeneration (Syritowitz et al., 1999).

The discovery of a role for VR1 in models of persistent and chronic pain may have been delayed by the fact that previous studies investigating capsazepine’s effects were only carried out in rat or mouse models of chronic pain. In this study, we provide evidence that capsazepine is an effective antihyperalgesic in guinea pig models of chronic pain and that this correlates with the effectiveness of this compound in blocking VR1 activation by low pH and capsaicin (Savidge et al., 2002). In contrast, capsazepine does not reverse chronic pain in rats and mice, where it blocks only capsaicin-induced VR1 activation. These data therefore suggest that the antinociceptive effects of VR1 antagonists are predicted by their ability to block noxious heat- and proton- as well as capsaicin-induced activation of VR1 and provide evidence for a potential therapeutic benefit of VR1 antagonists in the treatment of chronic neuropathic and inflammatory pain conditions.

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References


Address correspondence to: Dr. Peter McIntyre, Molecular Biology Laboratory, Novartis Institute for Medical Sciences, Gower Place, London WC1E 6BN, UK. E-mail: peter.mcintyre@pharma.novartis.com