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Differential involvement of TRPV1 receptors at the central and peripheral nerves in CFA-induced mechanical and thermal hyperalgesia

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Abstract

Transient receptor potential vanilloid 1 (TRPV1) antagonists are known to attenuate two typical symptoms of inflammatory hyperalgesia: thermal and mechanical. However, it is not clear whether the sites of participation of TRPV1 for each symptom are different. In this study, we clarified the difference between the site of TRPV1 involvement in both symptoms by analysing the anti-hyperalgesic activity of two kinds of TRPV1 antagonists given locally (i.e. intraplantarly and intrathecally) in rats with CFA (complete Freund's adjuvant)-induced inflammation. TRPV1 antagonists BCTC (*N*-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl) tetrahydropyrazine-1(2*H*)-carbox-amide, 1–300 μg) and SB-366791 (*N*-(3-methoxyphenyl)-4-chlorocinnamide, 30–300 μg) administered intraplantarly in a dose-dependent manner inhibited CFA-induced thermal hyperalgesia. In addition, CFA-induced thermal hyperalgesia was significantly reversed by intrathecal administration of 1–100 μg of BCTC and SB-366791. While intraplantar BCTC (1–300 μg) and SB-366791 (30–300 μg) did not reverse CFA-induced mechanical hyperalgesia, 1–100 μg of intrathecally administered BCTC and SB-366791 dose-dependently reduced mechanical hyperalgesia. Regression analysis showed that a correlation exists between the inhibitory effects on thermal hyperalgesia and mechanical hyperalgesia after intrathecal administration (correlation factor = 0.6521), but not after intraplantar administration (correlation factor = 0.0215). These data suggest that TRPV1 in the peripheral endings of the primary afferents plays a key role in thermal hyperalgesia, but it makes only a minor contribution in CFA-induced mechanical hyperalgesia. Furthermore, it is suggested that the spinal TRPV1 is critical in the development of both types of hyperalgesia.

Introduction

Transient receptor potential vanilloid 1 (TRPV1) is a ligand-gated non-selective cation channel that was cloned as a receptor of capsaicin, a pungent ingredient of hot chilli pepper (Caterina et al 1997). TRPV1 is primarily found on small-diameter primary afferents, particularly unmyelinated C-fibres and A-δ fires (Guo et al 1999; Valtschanoff et al 2001), and it integrates multiple pain stimuli such as noxious heat, protons and vanilloids (Caterina et al 1997; Tominaga et al 1998; Szallazi & Blumberg 1999).

A significant amount of evidence indicates that TRPV1 is important in the development of inflammatory hyperalgesia. For instance, TRPV1 knock-out mice exhibited a reduction in inflammatory thermal hyperalgesia (Caterina et al 2000; Davis et al 2000). TRPV1 protein or mRNA levels were increased in the skin, sciatic nerve and dorsal root ganglion (DRG) following complete Freund's adjuvant (CFA) (Amaya et al 2003, Carlton & Coggeshall 2001; Ji et al 2002; Luo et al 2004) or carrageenan-induced inflammation (Tohda et al 2001). Various inflammatory mediators are known to activate and sensitize TRPV1 functions (Chuang et al 2001; Tominaga et al 2001; Sugiura et al 2002). Electrophysiological studies showed that under inflammatory conditions spinal neuronal responses were attenuated by treatment with TRPV1 antagonists (Kelly & Chapman 2002; Jhaveri et al 2005). Systemically administered TRPV1 antagonists inhibit both inflammatory thermal and mechanical hyperalgesia and allodynia (Pomonis et al 2003; Walker et al 2003; Gavva et al 2005; Honore et al 2005).

Local injection studies of TRPV1 antagonists have been performed to show the peripheral site of TRPV1 involvement in inflammatory hyperalgesia (Kwak et al 1998; Menendez

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et al 2004). Recent reports demonstrated that CFA-induced thermal hyperalgesia is attenuated by both intrathecal (i.t.) and intraplantar (i.pl.) administration of TRPV1 antagonist (Honore et al 2005; Cui et al 2006). These findings indicate that TRPV1 plays a critical role at both the CNS and peripheral inflammatory site in the development of thermal hyperalgesia. On the other hand, although TRPV1 antagonists are known to attenuate mechanical hyperalgesia, another typical and therapeutically important symptom, it has not yet been demonstrated whether TRPV1 at both the CNS and peripheral site is critical for mechanical hyperalgesia.

This study was aimed at clarifying the difference of the site of involvement of TRPV1 in two types of hyperalgesia, thermal and mechanical, in a CFA-induced inflammatory model. We thoroughly analysed the analgesic efficacy of local administration (i.e., intraplantar and intrathecal) of TRPV1 antagonists BCTC (*N*-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl) tetrahydropyrazine-1(2*H*)-carbox-amide) and SB-366791 (*N*-(3-methoxyphenyl)-4-chlorocinnamide) to rats with CFA-induced inflammation.

Materials and Methods

The experiments were carried out according to a protocol approved by the animal ethics committee at the Nagoya Laboratories of Pfizer Global Research and Development. Male Sprague-Dawley (SD) rats (Japan SLC, Shizuoka, Japan), 120–200 g, were used. BCTC was synthesized in Pfizer Global Research and Development's Nagoya laboratories according to the method described in patent application WO 02/08221 (Jan. 31, 2002) filed by Neurogen Corp. SB-366791 was obtained from Tocris (Bristol, UK). The in-vitro antagonistic activity of these compounds on rat TRPV1 functions have been reported previously (IC50 of BCTC = 35 nM (Valenzano et al 2003) and IC50 of SB-366791 = 7.5 nM (Gunthorpe et al 2004)).

Drugs were administered intraplantarly or intrathecally under anaesthesia with 3% isoflurane (Dainippon Seiyaku, Osaka, Japan). Immediately after drug injection, the isoflurane was stopped, and to avoid the influence of anaesthesia the analgesic tests were initiated 20 min after intrathecal or intraplantar administration. For intraplantar injection, 50 μL compound solutions were administered subcutaneously into the hind paw of rats using a Hamilton syringe with a 27-guage needle. For intrathecal injection, 10 μL compound solutions were administered directly into the spinal cord between the L4 and L5 vertebrae using a Hamilton syringe with 27-guage needle. The vehicle of BCTC and SB-366791 contained 10% Tween 80 in saline for intraplantar injection and dimethyl sulfoxide (DMSO) for intrathecal injection. The control and vehicle-treated rats received 10% Tween 80 in saline or DMSO for intraplantar or intrathecal administration, respectively.

CFA (100 μL) containing 3 mg mL^{-1} of *Mycobacterium tuberculosis* H37RA (Difco, MI) in liquid paraffin (Wako Chemical Industries, Osaka, Japan) was injected subcutaneously into the plantar skin of the left hind paw, while the control rats were injected with liquid paraffin. Two days after the CFA injection, thermal and mechanical hyperalgesia were determined. Thermal hyperalgesia was tested by the method described previously (Hargreaves et al 1988) using the plantar test apparatus (Ugo-Basil, Varese, Italy). Rats were adapted to the testing environment for 15 min before any stimulation. Radiant heat was applied to the plantar surface of the hind paw and paw withdrawal latencies (PWL) were determined (n = 6 per group). The intensity of radiant heat was adjusted to produce a stable PWL of 10–15 s.

Mechanical hyperalgesia was tested by measuring the paw withdrawal threshold (PWT) to pressure using an analgesy meter (Ugo-Basil). The rats were gently restrained, and a steadily increasing pressure was applied to the hind paw via a plastic tip (n = 6 per group).

To assess the effects of the compounds, the PWL (or PWT) was determined before and at 20, 40 and 60 min after administration, and the results were expressed as the mean PWL (or PWT) of each group. The percentage of inhibition was calculated as $[1-(B-A)/(C-A)]\times 100$, where A is the mean PWL (or PWT) in the CFA-control group, B is the drug-treated group, and C is the control (veh) group.

Statistical difference in PWL (or PWT) at each point was determined using one-way analysis of variance followed by Fisher's LSD test. $P<0.05$ was considered statistically significant.

The correlation between the inhibitory activity (%) of both antagonists in thermal and mechanical hyperalgesia was calculated by linear regression analysis using GraphPad Prism (GraphPad software Inc., CA). Analysed data were from the mean inhibition percent values of each dose of both antagonists at the time point that a peak efficacy was observed (20 min for intrathecal and 40 min for intraplantar post administration (n = 6 for intrathecal, n = 7 for intraplantar)).

Results

Two days post CFA injection, a significant decrease of PWL to thermal stimulation was observed (PWL control vs PWL inflamed: 11.6 ± 1.1 vs 4.8 ± 0.79 s, $P<0.01$, Figure 1A; 11.1 ± 1.7 vs 4.8 ± 0.8 s, $P<0.05$, Figure 1C; 13.1 ± 1.9 vs 5.8 ± 0.58 s, $P<0.01$, Figure 1B; 12.0 ± 1.5 vs 4.7 ± 0.37 s, $P<0.01$, Figure 1D), demonstrating a maintained thermal hyperalgesia.

BCTC administered intraplantarly dose-dependently reversed CFA-induced thermal hyperalgesia by 29.4 ± 8.4% and 65.0 ± 7.1% at the highest dose (300 μg) at 20 and 40 min post administration, respectively (Figure 1A). The effects were significant at 100 and 300 μg at 40 min post dosing and at 300 μg at 60 min post dosing, respectively. Intraplantar SB-366791 also dose-dependently inhibited thermal hyperalgesia by 61.3 ± 17.3% and 65.6 ± 13.5% at 300 μg at 20 and 40 min post injection, respectively (Figure 1B). The effects were significant at 300 μg at 20 and 40 min post dosing, and decreased at 60 min. The onset of action of SB-366791 was faster than that of BCTC.

To negate the possibility of systemic effects after the compounds diffused into other sites including CNS, we tested the effects of contralateral intraplantar injection of the compounds on the PWL of ipsilateral paws. The injection of both BCTC and SB-366791 (300 μg, i.pl.) to the contralateral paw

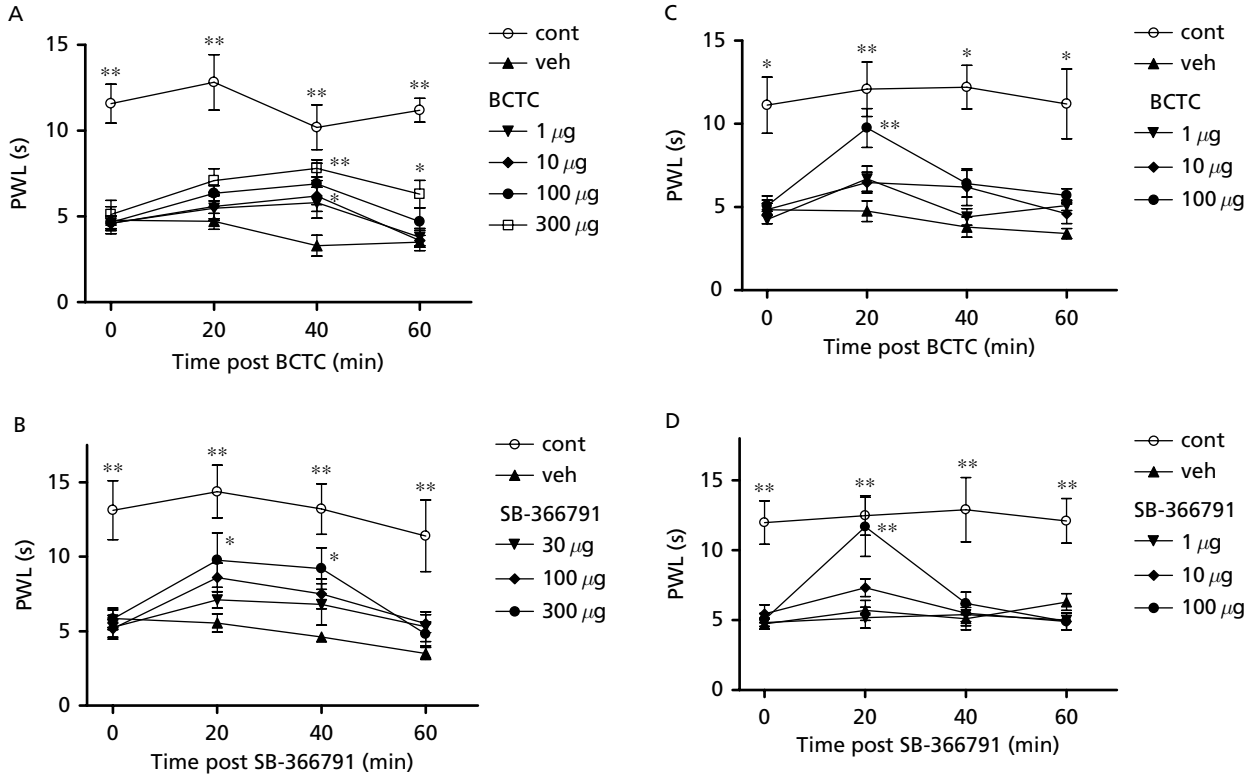

Figure 1 Effect of intraplantar and intrathecal administration of BCTC and SB-366791 on CFA-induced thermal hyperalgesia. BCTC (1–300 μg, i.pl.) (A), SB-366791 (30–300 μg, i.pl.) (B), BCTC (1–100 μg, i.t.) (C) and SB-366791 (1–100 μg, i.t.) (D) were administered to rats two days after CFA injection. The paw withdrawal latency (PWL, s) of the CFA-injected paw to heat stimulation was determined before (0) and at 20, 40 and 60 min after drug administration. Each plot represents the mean ± s.e.m. of PWL, n = 6. *$P < 0.05$, **$P < 0.01$, compared with the CFA-vehicle (veh) group (one-way analysis of variance, Fisher's LSD test).

did not attenuate thermal hyperalgesia of the inflamed paw (–3.2 ± 5.6% and 4.1 ± 7.9% inhibition at 40 min, respectively, n = 6).

Intrathecal treatment with BCTC significantly inhibited thermal hyperalgesia at 20 min post injection (68.2 ± 16.0% inhibition at 100 μg, Figure 1C). Intrathecal SB-366791 inhibited thermal hyperalgesia in a similar manner (88.2 ± 31.2% inhibition at 100 μg at 20 min post dosing, Figure 1D).

The PWTs of CFA-injected rats were significantly lower than that of control rats (PWT control vs PWT inflamed: 143.0 ± 15.0 vs 65.0 ± 4.3 g, $P < 0.01$, Figure 2A; 114.2 ± 13.0 vs 45.8 ± 2.9 g, $P < 0.01$, Figure 2B), indicating the occurrence of sustained mechanical hyperalgesia. Neither BCTC (1–300 μg) nor SB-366791 (30–300 μg) showed a significant effect on mechanical hyperalgesia until 60 min after intraplantar injection (Table 1). On the other hand, BCTC administered intrathecally significantly reversed mechanical hyperalgesia at 20 min post injection (30.5 ± 13.3% inhibition at 100 μg, Figure 2A), and the effects were significant at 10 and 100 μg. SB-366791 dose-dependently inhibited mechanical hyperalgesia by 81.9 ± 14.3% at 100 μg at 20 min post dosing. The effects were significant at 100 μg at 20 min and 60 min post administration (Figure 2B). Throughout the 60-min testing periods no abnormal behaviour, such as sedation, was observed in any rats.

The correlation between the inhibitory activity (%) of both antagonists in thermal and mechanical hyperalgesia were calculated. In the intrathecal administration study, a correlation between inhibition in thermal and mechanical hyperalgesia was observed (correlation factor = 0.6521, n = 6). On the other hand, little correlation was observed in intraplantar administration (correlation factor = 0.0215, n = 7).

Discussion

In this study, we attempted to clarify the site of participation of TRPV1 in mechanical and thermal hyperalgesia in CFA-evoked inflammation by testing the effects of TRPV1 antagonists BCTC and SB-366791. We demonstrated that BCTC and SB-366791 administered intraplantarly inhibited CFA-induced thermal hyperalgesia but not mechanical hyperalgesia. On the other hand, BCTC and SB-366791 administered intrathecally inhibited both CFA-induced thermal and mechanical hyperalgesia. These findings suggest that peripheral TRPV1 receptors play a key role in the expression of thermal hyperalgesia, but not in mechanical hyperalgesia. In contrast, central TRPV1 are involved in both thermal and mechanical hyperalgesia.

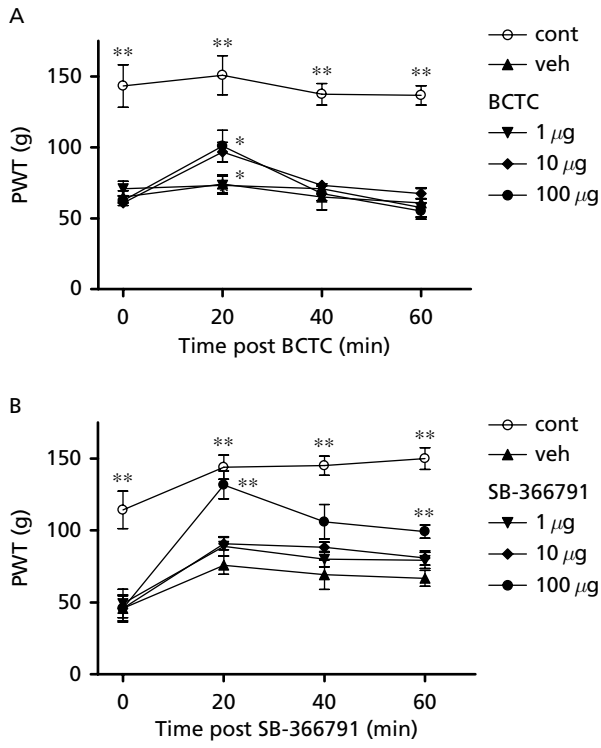

Figure 2 Effect of intrathecal administration of BCTC and SB-366791 on the CFA-induced mechanical hyperalgesia. BCTC (1–100 μg, i.t.) (A) and SB-366791 (1–100 μg, i.t.) (B) were administered to rats two days after CFA injection. Paw withdrawal threshold (PWT, g) of the CFA-injected paw to the mechanical stimulation was determined before (0), 20, 40 and 60 min after drug administration. Each plot represents the mean ± s.e.m. of PWT, n = 6. *$P < 0.05$, **$P < 0.01$ compared with CFA-vehicle (veh) group (one-way analysis of variance, Fisher's LSD test).

Recent reports have discussed the involvement of TRPV1 in thermal hyperalgesia using other compounds (Honore et al 2005; Cui et al 2006). In this study, we additionally found that BCTC and SB-366791 dosed at the contralateral paw did not reverse thermal hyperalgesia of the ipsilateral (CFA-injected) paw. This result indicates that the analgesic effect produced by ipsilaterally administered BCTC and SB-366791 is due to the direct inhibition of TRPV1 at the inflammatory site, not by their systemic effect after diffusing into other sites including the CNS. Thus, these data support the concept that TRPV1 at the inflammatory site participates in thermal hyperalgesia.

The main findings of this report are that TRPV1 antagonists administered intraplantarly failed to attenuate mechanical hyperalgesia. This result suggests that TRPV1 in the peripheral endings of the primary afferents make little contribution to CFA-evoked mechanical hyperalgesia. Regression analysis demonstrates that there is quite a difference in the contribution of peripheral TRPV1 in thermal and mechanical hyperalgesia. Thus, the contrasting effects of intraplantarly administered TRPV1 antagonists on thermal and mechanical hyperalgesia is interesting.

We demonstrated that intrathecally administered TRPV1 antagonists reversed mechanical hyperalgesia. This result is in line with a recent report that an intrathecally administered TRPV1 antagonist inhibited CFA-induced mechanical allodynia (Cui et al 2006); regarding mechanical hypersensitivity, mechanical allodynia was assessed with low-threshold stimuli (using von Frey hairs). In this study we used a paw pressure test to investigate high-threshold mechanical hyperalgesia. Our findings demonstrate that central TRPV1 participates in inflammatory mechanical hyperalgesia as well as in allodynia.

The mechanisms underlying the role of CNS TRPV1 in inflammatory hyperalgesia are still unclear. However, a recent interesting report of an electrophysiological study demonstrated that SB-366791 inhibited glutamatergic transmission in a subset of neurons via a pre-synaptic mechanism following peripheral inflammation (Lappin et al 2006). Enhancement of glutamate release could be a mechanism underlying the involvement of spinal TRPV1 in the transduction of inflammatory hyperalgesia.

Intraplantar SB-366791 showed a faster onset of action than BCTC in a thermal hyperalgesia study. In addition, intrathecal SB-366791 reversed thermal and mechanical

Table 1 Effect of intraplantarly administered TRPV1 antagonists on CFA-induced mechanical hyperalgesia in rats

Antagonist	Dose (μg)	PWT (g)			
		Time after administration			
		Before	20 min	40 min	60 min
BCTC	Control	126.7 ± 14.3**	154.2 ± 12.9**	154.2 ± 5.6**	128.3 ± 7.3**
	Vehicle	61.7 ± 7.8	61.7 ± 10.5	56.7 ± 11.9	50.0 ± 5.8
	1	57.2 ± 7.2	70.8 ± 8.2	64.2 ± 10.4	60.0 ± 7.5
	10	54.2 ± 7.2	67.5 ± 11.7	54.2 ± 8.4	48.3 ± 8.7
	100	61.7 ± 8.0	62.5 ± 8.7	58.3 ± 13.1	56.7 ± 9.2
	300	50.0 ± 6.6	60.8 ± 8.9	60.8 ± 9.3	46.7 ± 5.7
SB-366791	Control	125.0 ± 3.3**	138.3 ± 8.0**	130.0 ± 7.9**	127.0 ± 12.8**
	Vehicle	58.3 ± 5.4	72.5 ± 6.5	77.5 ± 10.5	70.8 ± 6.9
	30	59.2 ± 9.2	73.3 ± 7.9	69.2 ± 6.4	59.2 ± 7.9
	100	61.7 ± 6.6	75.0 ± 7.7	80.8 ± 4.2	71.7 ± 7.3
	300	62.5 ± 7.6	79.2 ± 12.9	73.3 ± 7.9	75.8 ± 6.3

Paw withdrawal threshold (PWT, g) of the CFA-injected paw to the mechanical stimulation was determined before and at 20, 40 and 60 min after administration. Data are expressed as mean ± s.e.m., n = 6. **$P < 0.01$ vs vehicle (one-way analysis of variance, Fisher's LSD test)

hyperalgesia more effectively than BCTC at the maximal dose. According to the reports, the in-vitro functional activity of SB-366791 against rat TRPV1 is more potent than that of BCTC (Valenzano et al 2003; Gunthorpe et al 2004). Our results could reflect their intrinsic in-vitro activity.

In this study, we used Tween 80 and DMSO as solvents for the compounds. Although these chemicals are known to produce non-specific pharmacological action, no major difference was observed between PWL or PWT before and after vehicle injection.

Conclusions

This study was performed to clarify the site of involvement of TRPV1 receptors in inflammatory hyperalgesia by testing the effects of locally administered TRPV1 antagonists. Our data demonstrated that TRPV1 receptors at the central and peripheral nerves are involved in CFA-induced inflammation. The peripheral TRPV1 plays a key role in the expression of thermal, but not mechanical, hyperalgesia. In contrast, the spinal TRPV1 are involved in both thermal and mechanical hyperalgesia.

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