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# Inhibition of acute nociceptive responses in rat spinal cord by a bradykinin $B_1$ receptor antagonist

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**Abstract**

This study used behavioural and in vivo electrophysiological paradigms to examine the effects of systemic and spinal administration of a bradykinin $B_1$ receptor antagonist, compound X, on acute nociceptive responses in the rat. In behavioural experiments, compound X significantly increased the latency to withdraw the hindpaw from a radiant heat source after both intravenous and intrathecal administration, without affecting motor performance on the rotarod. In electrophysiological experiments, both intravenous and direct spinal administration of compound X attenuated the responses of single dorsal horn neurones to noxious thermal stimulation of the hindpaw. These data show that the antinociceptive effects of a bradykinin $B_1$ receptor antagonist are mediated, at least in part, at the level of the spinal cord and suggest a role for spinal bradykinin $B_1$ receptors in acute nociception.


*Keywords:* Bradykinin $B_1$ receptor; Antinociception; Spinal cord; (Rat)

## 1. Introduction

Bradykinin and related peptides are primary mediators of pain and inflammation. After tissue injury, bradykinin directly activates and sensitises nociceptors, in addition to promoting vasodilation and prostaglandin release, thereby potentiating inflammation and hyperalgesia (for reviews see Dray and Perkins, 1997; Rupniak et al., 2000). Experimentally, injection of bradykinin causes acute pain in man (Whalley et al., 1987; Jensen et al., 1991; Manning et al., 1991) and intense algesic responses in rats (Hong and Abbott, 1994) suggesting an additional role for bradykinin in acute nociception. These actions are mediated by two receptor subtypes designated $B_1$ and $B_2$; while $B_2$ receptors are widely-expressed in normal tissue and mediate acute algesic responses to bradykinin, constitutive expression of $B_1$ receptors is low and $B_1$ receptor activation does not induce pain under non-inflamed conditions (for reviews see Dray and Perkins, 1993, 1997; Hall and Morton, 1997; Marceau et al., 1998). However, bradykinin $B_1$ receptors are highly inducible by trauma and tissue injury and bradykinin $B_1$ receptor agonists can exacerbate hyperalgesia in rats (Perkins and Kelly, 1993), suggesting a more important role for bradykinin $B_1$ receptors in chronic inflammatory conditions (Dray and Perkins, 1993). Experiments with bradykinin $B_1$ receptor knockout ($BK_1R^{-/-}$) mice and the peptide bradykinin $B_1$ receptor antagonist des-Arg$^9$[Leu$^8$]bradykinin support this view: deletion of the bradykinin $B_1$ receptor reduces complete Freunds adjuvant-induced thermal hyperalgesia (Boyce et al., 2001; Ferreira et al., 2001) and des-Arg$^9$[Leu$^8$]bradykinin, which has no effect on nociceptive thresholds in naïve animals, reduces or prevents persistent mechanical or thermal hyperalgesia (e.g., Perkins et al., 1993; Davis and Perkins, 1994a, 1996; Rupniak et al., 1997; Belichard et al., 2000; see also

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Rupniak et al., 2000; Gabra and Sirois, 2003; Yamaguchi-Sase et al., 2003).

However, the conventional view that bradykinin $B_1$ receptors exclusively mediate hyperalgesic responses after induction by injury has been challenged by recent data which suggests that constitutive bradykinin $B_1$ receptors may also participate in acute nociception. This includes anatomical studies that have identified constitutively-expressed bradykinin $B_1$ receptors on primary sensory neurones and dorsal root ganglia in the rat (Ma et al., 2000; Wotherspoon and Winter, 2000; Ma, 2001) and behavioural studies that have shown $BK_1R^{-/-}$ mice are hypoalgesic to acute noxious thermal (paw flick, hot plate), mechanical (von Frey) and chemical (intraplantar formalin or capsaicin) stimuli (Pesquero et al., 2000; Boyce et al., 2001). Furthermore, some of the antinociceptive effects of des-Arg$^9$[Leu$^8$]bradykinin, which are difficult to reconcile with the de novo induction of bradykinin $B_1$ receptors (in less than 90 min), may be explained by acute receptor blockade. For example, des-Arg$^9$[Leu$^8$]bradykinin has been reported to block oedema induced by intradermal capsaicin (Mantione and Rodriguez, 1990) and to reduce responses to formalin in rats and mice assessed within 30 min of algogen injection (Shibata et al., 1989; Correa and Calixto, 1993; Sufka and Roach, 1996).

Although little is known about the location of those bradykinin $B_1$ receptors that mediate acute nociception, recent work has identified the spinal cord as a likely site. Constitutively expressed bradykinin $B_1$ receptors have been localised in laminae I to V of the rat dorsal horn (Wotherspoon and Winter, 2000; Ma and Heavens, 2001) and in the substantia gelatinosa in man (Raidoo and Bhoola, 1997) and in vitro electrophysiological examination of spinal nociceptive reflexes in the $BK_1R^{-/-}$ mouse has revealed a 50% reduction in activity-dependent facilitation in the $BK_1R^{-/-}$ mouse compared with the wild type (WT; Pesquero et al., 2000). In addition, application of the bradykinin $B_1$ receptor agonist desArg$^9$bradykinin to the WT preparation facilitated nociceptive reflex activity. These observations suggest that spinal bradykinin $B_1$ receptors may contribute to acute nociceptive responses.

The main aim of the present study was to investigate further the role of spinal bradykinin $B_1$ receptors in acute nociception using a bradykinin $B_1$ receptor antagonist, known as compound X (Ferrari et al., 1997). Initially, compound X was assessed in a behavioural test of hypersensitivity to confirm a similar profile to peptide bradykinin $B_1$ receptor antagonists (see Dray and Perkins, 1993; Rupniak et al., 2000) and to identify an effective analgesic dose to test against acute nociception. Behavioural and in vivo electrophysiological paradigms were then used to examine the effects of compound X on acute nociceptive responses and to compare the effects after intravenous (i.v.) and intrathecal (i.t.) or local spinal administration. Compound X was (3R)-3-(3,4-dichlorophenyl)-*N*-{(1R)-1-[4-(4,5-dihydro-1H-imidazol-2-yl)benzyl]-2-oxo-2-pyrrolidin-1-ylethyl}-3-[(2-naphthylsulfonyl)amino]propanamide, an antagonist with high affinity at the rat bradykinin $B_1$ receptor ($K_i$=0.37 nM), selectivity for bradykinin $B_1$ over $B_2$ receptors (rat $B_2$ $K_i$=9400 nM; human $B_1$ $K_i$=0.07 nM, human $B_2$ $K_i$=6000 nM) and a half-life of 1.2 h (unpublished observations).

## 2. Methods

Experiments were performed on male Sprague Dawley rats (100–120 g in behavioural tests, 330–410 g in electrophysiological study; Bantin and Kingman or Charles River, UK) and were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. The behavioural experiments also conformed to ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983). The number of animals and intensity of noxious stimuli were the minimum necessary to demonstrate consistent effects of drug treatments. Animals received drug treatments on one occasion only and were humanely killed immediately after testing.

### 2.1. Carrageenan-induced mechanical hypersensitivity

The paw pressure threshold to a noxious mechanical stimulus was determined using a modified Ugo Basile algesiometer. The rat's hindpaw was positioned over a convex surface (radius 2.5 mm) and increasing pressure was applied to the dorsal surface until the rat vocalised. The mechanical thresholds were determined for both hindpaws to provide a baseline for subsequent comparison following injection of carrageenan into one paw. Rats then received an intraplantar injection of carrageenan (4.5 mg in 0.15 ml; Sigma) or saline (0.15 ml) into one hindpaw and mechanical thresholds of both hindpaws were re-determined 3 h later. Test compounds were administered i.t. 2.5 h after carrageenan ($n$=8–15 per group). Data are expressed as the mean difference in paw pressure thresholds to elicit vocalisation (DPPT) for each rat before and after the induction of inflammation by carrageenan (i.e., paw pressure score at 3 h—baseline score). Carrageenan hypersensitivity is defined as the difference in paw pressure thresholds for saline/vehicle- and carrageenan/vehicle-treated rats. Percentage inhibition of hyperalgesia is calculated as follows:

$$100-\frac{(\text{DPPT carrageenan/drug-treated rats}-\text{DPPT saline/vehicle-treated rats})}{(\text{DPPT carrageenan/vehicle-treated rats}-\text{DPPT saline/vehicle-treated rats})}\times 100.$$

### 2.2. Acute thermal nociception

Thermal nociception was measured using a modified Hargreaves paw flick test. Rats were habituated on a glass table (1×2 m) under perspex boxes (height 10 cm, width 20 cm, depth 12 cm) for at least 15 min to reduce exploratory activity. A mobile radiant heat source, located 2.5 cm beneath the glass surface, was pre-calibrated to give a baseline paw withdrawal latency of approximately 15 s. The latency to paw withdrawal from the heat source aimed on the plantar surface of the paw was determined for both hindpaws on three occasions (10 min apart) and the mean was recorded as the baseline response for each paw for each animal. Rats were then allocated to drug treatments ensuring that the baseline latencies were balanced across groups. Dosing was performed by a third party such that the experimenter was blinded to the treatment. Rats were anaesthetised briefly using isoflurane and test compounds were injected i.v. ($n$=4–5 per group) and the paw withdrawal

latency re-determined 30 min later. In separate experiments, rats were briefly anaesthetised with isoflurane in oxygen and test compounds were administered i.t. (10 μl; 30 min pretreatment) via freehand injection using a 10 μl Hamilton syringe at the L5 vertebra ($n=7$–9 per group). Each rat received only one i.t. injection. Data are expressed as the mean difference in paw withdrawal latency for each animal before and after the drug administration.

### 2.3. Rotarod performance

To determine the effects of the compounds on motor coordination, rats were trained to remain for 120 s on a Ugo Basile rotarod apparatus revolving at 12 r.p.m. on the morning before the test. Test compounds were administered i.v. ($n=11$–12 per group) or i.t. ($n=8$ per group) under brief isoflurane anaesthesia and 30 min later the rats were placed on an accelerating rotarod (increasing from 4 to 40 r.p.m. during a 5 min period) and the time for which the rats were able to remain on the rotarod was recorded up to a cut-off of 5 min. Latencies for drug-treated rats are expressed as a percentage of the time vehicle-treated rats were able to remain on the rotarod (typically 150 s).

### 2.4. In vivo electrophysiology

Rats were anaesthetised with isoflurane in oxygen (induction with 5% in 2 l/min, reduced as necessary) and the trachea, one femoral vein and femoral artery were cannulated to permit artificial ventilation, i.v. administration of compound X and measurement of blood pressure. Experiments were terminated if the mean blood pressure fell below 50 mmHg. The spinal cord was exposed by a laminectomy at T13-L2, the dura mater was removed and the rat was immobilised in a stereotaxic frame. Anaesthesia was maintained with 2% isoflurane in 200–300 ml oxygen delivered by a ventilation pump and adequate anaesthesia was ensured by the absence of limb withdrawal and blood pressure responses to noxious pinch of the hindpaw. Body temperature was monitored and maintained between 36 and 37.5 °C. A stabilisation period of 1 h was allowed between completion of the surgery and neuronal recording.

Extracellular single unit recordings were made from neurones in the spinal dorsal horn (L5-S2) using glass microelectrodes filled with 4% pontamine sky blue in 0.5 M sodium acetate. Action potentials were amplified, filtered and counted using a level discriminator. Neurones were located using mechanical stimuli (prod, tap, pinch) applied to the hindpaw and, once identified, neurones were tested for responses to a 20 s thermal stimulus of 48 °C applied to the receptive field with a Peltier-driven thermode. After delineation of the receptor field, the thermode was fixed in place for the duration of the experiment and neurones were stimulated every 4 min. The effects of compound X on cell firing were examined after a minimum of 3 control responses with less than 10% variability. In 5 rats 1–10 mg/kg compound X was given i.v. in cumulative doses and in 6 rats 30–300 fmol compound X was applied directly onto the spinal cord (cumulative doses were administered every 12 min). A 10 μl Hamilton syringe was used for topical application with the syringe needle positioned at the spinal surface immediately above the recording site in a well surrounded by a pool of mineral oil. Dosing was performed in an open label manner. The 3 responses after each dose were counted, averaged and expressed as a percentage of the pre-vehicle control response. At the end of the experiment, the recording site was marked with pontamine sky blue, the spinal cord was removed and fixed in 10% formalin and then the tissue was frozen and sectioned to recover the dye spot.

### 2.5. Preparation of test compounds

Compound X (synthesised in-house) was dissolved in 50% polyethylene glycol 300 (in saline) for i.v. and i.t. dosing and in saline for local spinal administration in the electrophysiological study. Morphine HCl (Sigma) and indomethacin HCl (Merck Frosst, Montreal) were also dissolved in saline. Compounds were administered in a dose volume of 1 or 2 ml/kg i.v. or 10 μl spinally and all doses refer to free base.

### 2.6. Statistical analysis

All data were expressed as means ± S.E.M. Values were compared using a one-way ANOVA and Dunnett's post-test ($P<0.05$ indicated statistical significance), where appropriate.

## 3. Results

### 3.1. Carrageenan-induced mechanical hypersensitivity

Intraplantar injection of carrageenan caused paw swelling and hypersensitivity to mechanical compression of the hindpaw. Initial studies demonstrated that 3 mg/kg i.v. compound X completely reversed the hypersensitivity (data not shown). After i.t. administration, compound X also attenuated the hypersensitivity by about 40% at 3 nmol (2.4 μg, Fig. 1) but was less effective than morphine which reversed the hypersensitivity at 10 nmol (2.9 μg). No further inhibition of hypersensitivity was observed after higher doses of compound X.

### 3.2. Acute thermal nociception

A single dose of 3 mg/kg compound X i.v. significantly increased the latency to withdraw the paw from a noxious heat stimulus from about 4 s after vehicle treatment to about 9 s. In the same test, 1 mg/kg morphine increased the latency to about 14 s, while 3 mg/kg indomethacin was ineffective (see Fig. 2A). After i.t. administration, 0.3 (0.24 μg) and 3 nmol (2.4 μg) compound X increased the latency to paw flick to about 6 s compared with 2 s after vehicle. This effect was maximal and significant after 0.3 nmol (0.24 μg, see Fig. 2B). Intrathecal administration of morphine at 3 nmol (2.4 μg) increased the latency to about 12 s.

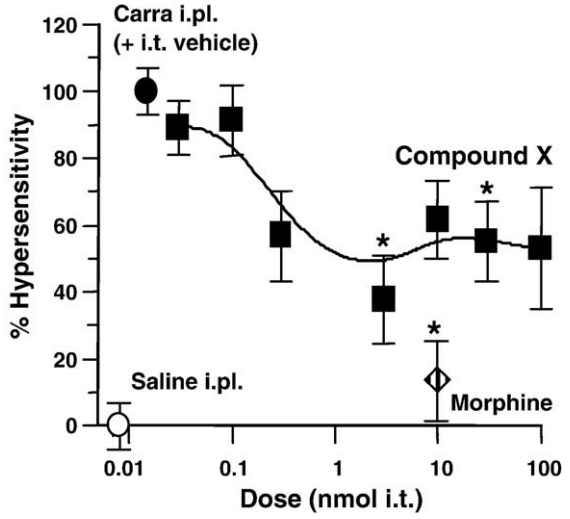


Fig. 1. Intrathecal administration of compound X attenuates carrageenan-induced mechanical hypersensitivity. Open circle indicates percentage hypersensitivity after intraplantar saline; filled circle after intraplantar carrageenan (plus i.t. vehicle). The effect of 10 nmol i.t. morphine on carrageenan-induced hypersensitivity is shown for comparison. Values are means ± S.E.M. for 8–15 rats per group. *$P<0.05$ compared to vehicle-treated rats. Carra, carrageenan; i.pl., intraplantar.

### 3.3. Rotarod performance

Compound X had no effect on rotarod performance after i.v. (3 mg/kg) or i.t. (3 nmol, 2.4 μg) administration. In contrast, i.v. morphine produced significant motor impairment, reducing time spent on the rotarod by about 40% compared with vehicle (Fig. 3). Like compound X, i.v. administration of indomethacin had no effect on motor performance. Similarly, time spent on the rotarod was unaffected by i.t. morphine (3 nmol, 0.86 μg).

### 3.4. In vivo electrophysiology

Intravenous compound X dose-dependently attenuated the responses of dorsal horn neurones to noxious thermal stimulation, producing a significant reduction of about 40% after 10 mg/kg ($n=5$, see Fig. 4A). Compound X also attenuated neuronal responses to noxious heat when administered directly onto the spinal cord, causing, on average, a highly significant inhibition of about 65% after 100 fmol (81 pg) and 75% after 300 fmol (242 pg, Fig. 5A). In 2 of the 6 cells tested, 100 fmol (81 pg) compound X reduced the nociceptive response to <10% of the pre-drug control response, hence these 2 cells were not further tested with 300 fmol (242 pg). The response of 1 of the 6 neurones was completely unaffected by 300 fmol (242 pg) compound X i.t. or 10 mg/kg i.v. For individual examples see Figs. 4C and 5C. All tested cells were located between L5 and S2 in laminae II to V (primarily III and IV) of the dorsal horn (see Figs. 4B and 5B). Ten of the 11 cells were wide-dynamic range neurones that also responded to prod, tap and pinch stimuli and, in all but one neurone, background activity was low or absent.

Compound X had no effect on blood pressure over that of vehicle when administered i.v. or spinally.

## 4. Discussion

Using behavioural and in vivo electrophysiological paradigms, the present study has shown that a bradykinin $B_1$ receptor antagonist attenuates acute nociceptive responses to noxious heat and that this action is mediated, at least in part, at the level of the spinal cord. The data also confirm that compound X is analgesic under inflammatory conditions when administered spinally. These observations together suggest that spinal bradykinin $B_1$ receptors mediate algesic responses, whether acute or post-inflammatory.

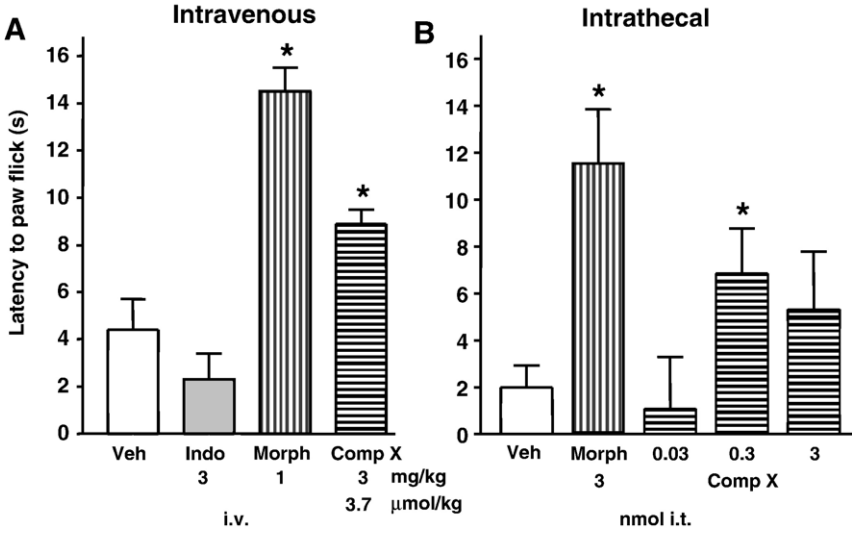


Fig. 2. Intravenous (A) and intrathecal (B) administration of compound X increases paw withdrawal latency to an acute noxious thermal stimulus. The effects of morphine (i.v. and i.t.) and indomethacin (i.v.) are shown for comparison. Values are means ± S.E.M. for 4–5 rats i.v. and 7–9 rats i.t. per treatment and indicate difference in latency from baseline. *$P<0.05$ compared to vehicle-treated rats. Comp X, compound X; indo, indomethacin; morph, morphine; veh, vehicle.

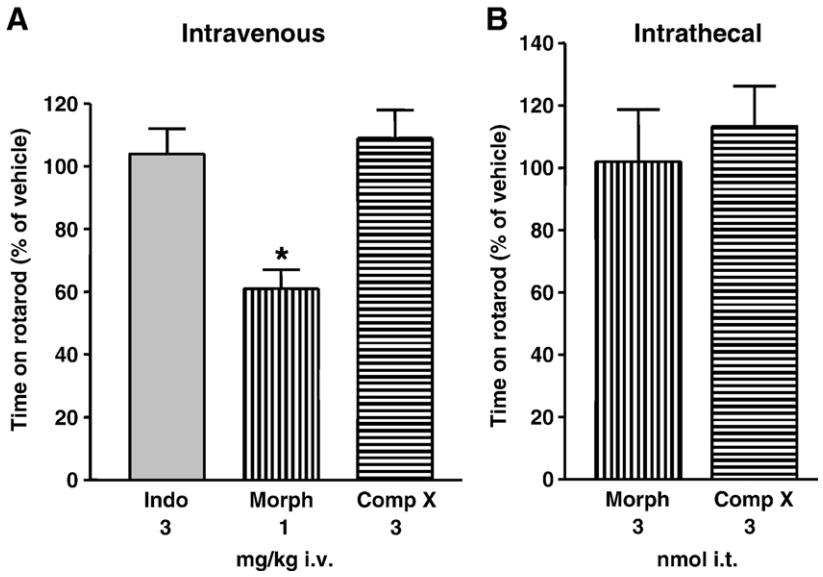


Fig. 3. Intravenous (A) and intrathecal (B) administration of compound X has no detrimental effect on rotarod performance. The effects of morphine (i.v. and i.t.) and indomethacin (i.v.) are shown for comparison. Values are means±S.E.M. for 11–12 rats i.v. and 8 rats i.t. per treatment. *$P<0.05$ compared to vehicle-treated rats. Comp X, compound X; indo, indomethacin; morph, morphine; veh, vehicle.

Compound X is a bradykinin $B_1$ receptor antagonist (Ferrari et al., 1997) with high affinity for the rat bradykinin $B_1$ receptor and selectivity for $B_1$ over $B_2$ receptors. In addition, compound X shows very high bradykinin $B_1$ receptor selectivity (<1000 fold) versus a battery of 120 miscellaneous G-protein coupled receptors and enzyme targets (data not shown, Panlabs Inc.). In order to determine whether compound X, like peptide bradykinin $B_1$ receptor antagonists, is analgesic under inflammatory conditions, the compound was first assessed against carrageenan-induced mechanical hypersensitivity. Systemic administration of compound X completely reversed the algesic response, an observation that is consistent with previous reports that des-Arg$^9$[Leu$^8$]bradykinin reduces mechanical hyperalgesia induced by intraplantar carrageenan or intra-articular complete Freunds adjuvant (Davis and Perkins, 1994a; Rupniak et al., 1997) and thermal hyperalgesia induced by ultraviolet irradiation (Perkins et al., 1993). However, intrathecal administration of compound X only attenuated the hypersensitivity by about 60%, suggesting that compound X may have a peripheral effect at the site of inflammation as well as an antinociceptive action at the spinal level. Previous work supports this view: for example, local administration of a bradykinin $B_1$ receptor antagonist at the site of algogen injection has been shown to reduce hyperalgesia induced by intraplantar carrageenan or intra-articular complete Freunds adjuvant or interleukin-1 β (Davis and Perkins, 1994a,b; Poole et al., 1999).

The next series of tests used behaviour and electrophysiology to assess the effects of compound X against acute thermal nociception. The data from both studies clearly show that compound X significantly reduced acute nociceptive responses after systemic and i.t./direct spinal administration. This is consistent with the observation that $BK_1R^{-/-}$ mice are hypoalgesic to acute noxious thermal, mechanical and chemical stimuli (Pesquero et al., 2000; Boyce et al., 2001) but contrasts with previous reports that peptide bradykinin $B_1$ receptor antagonists are ineffective against acute noxious stimuli (Dray et al., 1988). However, peptidergic compounds are characterised, in general, by poor central nervous system penetration, metabolic instability and rapid elimination and these properties may explain the lack of antinociceptive efficacy of peptide bradykinin $B_1$ receptor antagonists. This explanation is further supported by data from the present study and Pesquero et al. (2000) which suggests that the bradykinin $B_1$ receptors that mediate nociceptive responses are located in the spinal cord. The present study is the first to use a small molecule bradykinin $B_1$ receptor antagonist to examine the role of bradykinin $B_1$ receptors at the spinal level in acute nociception and consequently the first to demonstrate unequivocally that blockade of spinal bradykinin $B_1$ receptors is antinociceptive.

The antinociceptive effects of compound X are also consistent with recent reports that i.t. administration of a bradykinin $B_1$ receptor agonist in naïve animals evokes hyperalgesia (Ferreira et al., 2002; Fox et al., 2003). While these findings suggest that bradykinin $B_1$ receptors are constitutively-expressed in the spinal cord, evidence shows that inflammation, disease or injury upregulate bradykinin $B_1$ receptor expression (see Hall and Morton, 1997; Marceau, 1997). The rats used in the present study were not ‘specific pathogen free’ and, as a result, may have had higher levels of bradykinin $B_1$ receptors than expected in naïve animals. However, this seems unlikely to account for the antinociceptive efficacy of compound X as intracerebroventricular administration had no effect on acute thermal nociception assessed behaviourally (data not shown). In addition, it is possible that the surgical preparation in the electrophysiological experiments caused some induction of bradykinin $B_1$ receptor expression in the spinal cord. While further investigation is required to rule out this possibility, the antinociceptive effects of compound X administered i.t. in the behavioural studies

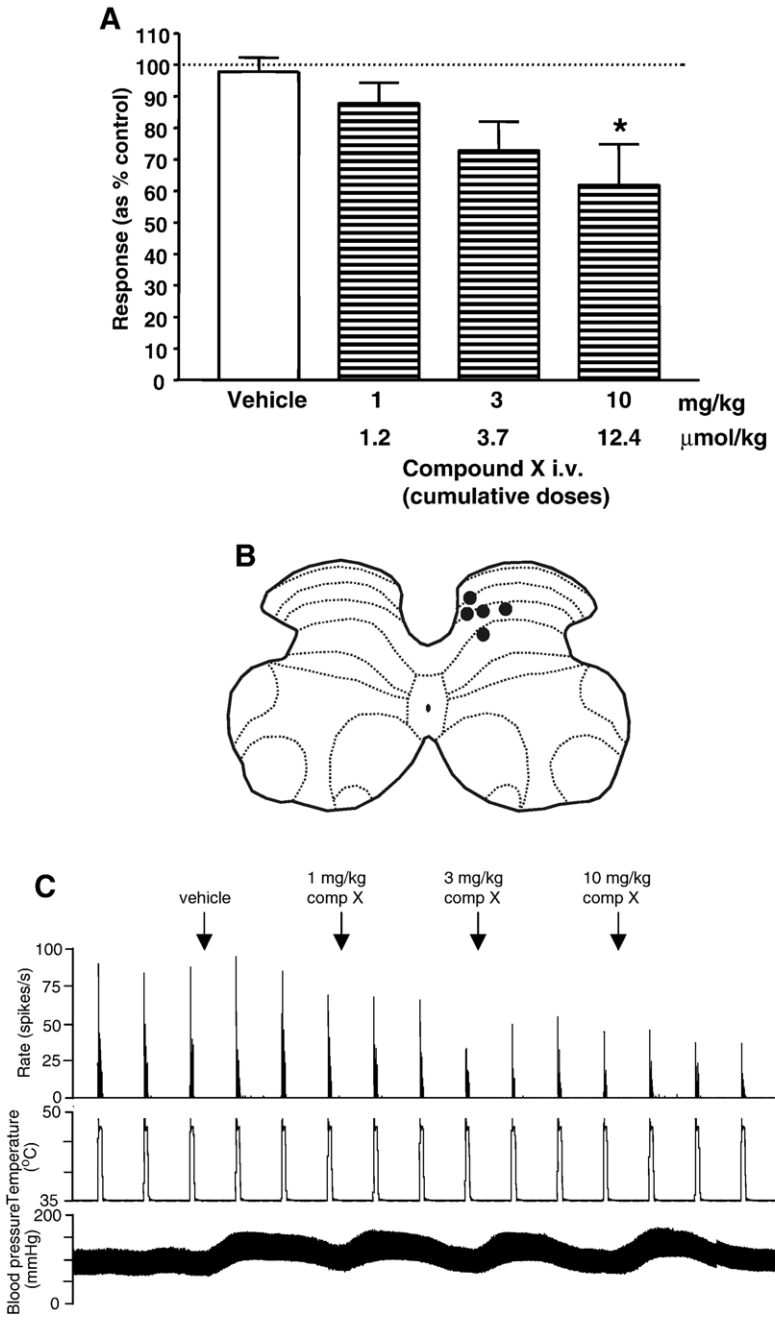


Fig. 4. (A) Intravenous compound X attenuates responses of neurones in the spinal dorsal horn to noxious thermal stimulation. Values are means±S.E.M. for 5 rats. Doses are expressed in mg/kg and μmol/kg to aid comparison with data in Fig. 5. (B) Locations of tested neurones within the dorsal horn. Dotted lines delineate laminae. (C) Example of the effects of compound X on the responses of an individual neurone to noxious thermal stimulation. *$P<0.05$ compared to vehicle treatment. Comp X, compound X.

suggests that the compound was indeed acting at constitutive bradykinin $B_1$ receptors.

It is also possible in the behavioural experiments that, when administered i.t., compound X acted on the dorsal root ganglia rather than on the spinal cord. Previous studies have identified constitutively-expressed bradykinin $B_1$ receptors in the rat dorsal root ganglia (Wotherspoon and Winter, 2000; Ma, 2001); however, Pesquero et al. (2000) have reported that bradykinin $B_1$ receptor deletion does not affect the noxious heat sensitivity of isolated dorsal root ganglia neurones. Furthermore, bradykinin $B_1$ receptor blockade in the dorsal root ganglia seems an unlikely explanation for the present electrophysiological data as the inhibitory effects of compound X on cell firing were instant and diffusion of compound X from the site of administration was limited by a pool of mineral oil.

The behavioural tests compared the effects of compound X with those of indomethacin and morphine. While indomethacin, a non-steroidal anti-inflammatory drug, was predictably inactive in the acute nociception test, morphine was more effective than the bradykinin $B_1$ receptor antagonist. However, unlike

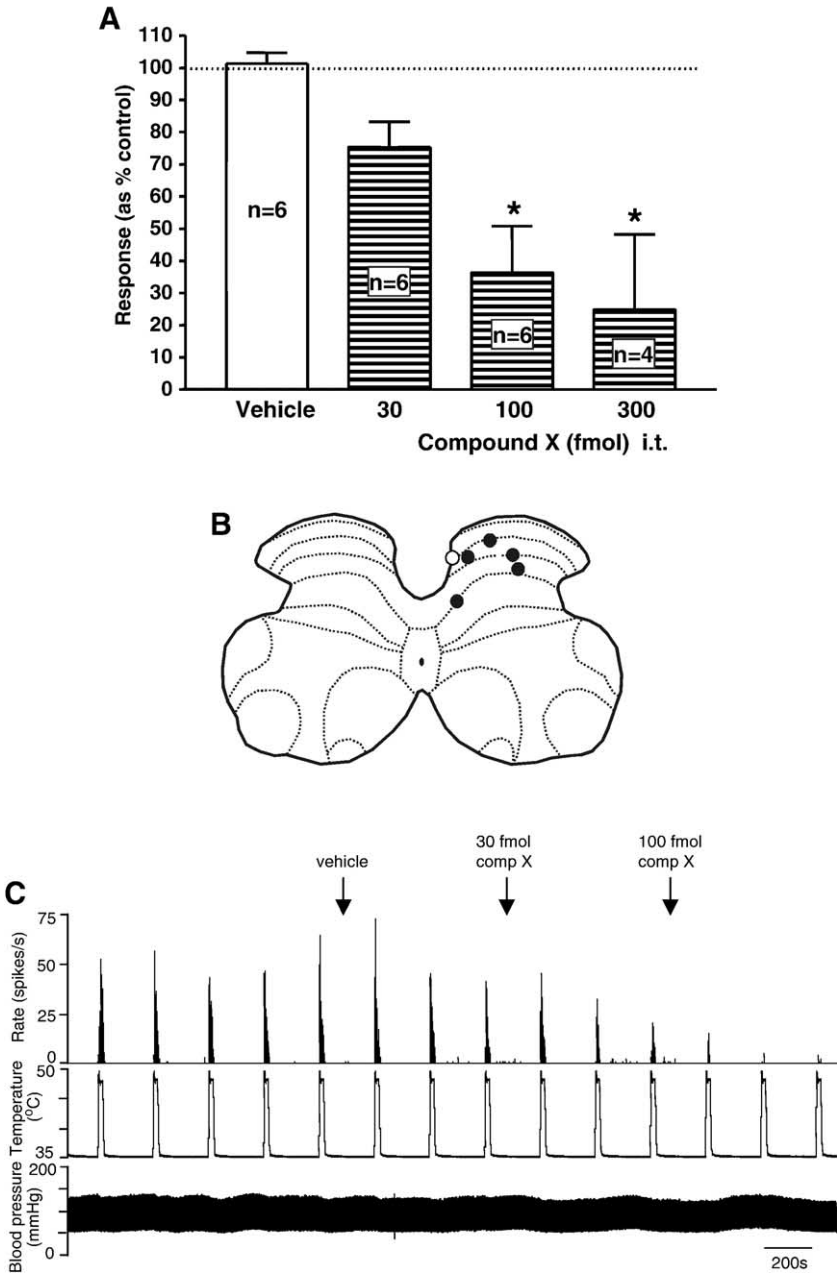


Fig. 5. (A) Direct spinal administration of compound X significantly reduces responses of dorsal horn neurones to noxious thermal stimulation. Values are means± S.E.M. for 4–6 rats. (B) Locations of tested neurones within the dorsal horn. Dotted lines delineate laminae. Open circle indicates neurone which was not inhibited by compound X. (C) Example of the effects of compound X on the responses of an individual neurone to noxious thermal stimulation. *$P<0.05$ compared to vehicle treatment. Comp X, compound X.

compound X, i.v. morphine produced motor impairment in the rotarod test at the antinociceptive dose, suggesting that sedation or reduced motor performance contributed to the presumed antinociceptive effect of morphine. In contrast, the antinociception produced by compound X had no motor component.

Intrathecal (or direct spinal) administration of compound X attenuated responses to noxious heat in both electrophysiological and behavioural tests; however, compound X was considerably more potent in the electrophysiological assay (100 fmol v. 300 pmol). The most likely explanation for this difference is that, while in the electrophysiological experiments compound X was applied directly onto the spinal surface immediately above the recording site, in the behavioural studies the drug was injected at the level of the L5 vertebra from where diffusion to the spinal site of action resulted in dilution of compound X in the cerebrospinal fluid. In addition, the delay between administration of compound X and test in the behavioural assay was 30 min compared with only 4 min in the electrophysiological experiments so that local availability of compound X may have been relatively lower prior to the behavioural test. At the established fmol doses that were antinociceptive in the electrophysiological tests, it is unlikely that compound X caused off-target effects and this further supports a selective action of compound X at bradykinin $B_1$ receptors.

In conclusion, the present study used behavioural and in vivo electrophysiological assays to show that a bradykinin $B_1$ receptor antagonist acts at the level of the spinal cord to reduce acute nociceptive responses to noxious thermal stimuli. Behavioural experiments also confirmed that intrathecal administration of compound X is analgesic under inflammatory conditions. These findings suggest that bradykinin $B_1$ receptor antagonists may have therapeutic utility as broad spectrum analgesics.

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