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Spinal δ-opioid receptors mediate suppression of systemic SNC80 on excitability of the flexor reflex in normal and inflamed rat

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Abstract

Due to low central nervous system (CNS) bioavailability of δ-opioid peptides, little is known about the effect of systemic administration of δ-opioid receptor ligands. The present study examined the effect of non-peptidergic δ-opioid receptor agonists, (+)-4-[(α*R*)-α-((2*R*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC80) and (−)dibenzoyl-L-tartaric acid salt (SNC86), on the activity of α − motoneurons in decerebrate-spinal rats. The flexor reflex was facilitated by C-afferent conditioning inputs, shown by a decrease in mechanical threshold and increase in touch- and pinch-evoked responses. Systemic administration of SNC80 (10 μmol/kg) prevented and reversed the neuronal hyperactivity. We further examined the effect of this agonist on the hypersensitivity of the flexor reflex induced by intraplantar injection of Freund's adjuvant. SNC80 dose-dependently (1, 3, 5 and 10 μmol/kg) increased the mechanical threshold and decreased touch-, pinch- and Aβ-afferent inputs-evoked responses. Similar effects were seen with SNC86 (5 μmol/kg). Pretreatment with either naloxone (20 μmol/kg, i.p.) or (Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-14β-ethoxy-5β-methylindolo [2′,3′:6′,7′]morphinan-3-ol hydrochloride (SH378; 5 μmol/kg, intraarterially (i.a.)), a novel selective δ-opioid receptor antagonist, completely abolished the anti-hypersensitivity effect of SNC80. The effect of SNC80 remained following intrathecal administration of μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP; 1.5 nmol). These results indicate that systemic injection of SNC80 exerted antihypersensitivity in models of both acute and tonic nociception and these effects are mediated mainly through a spinal δ-opioid mechanism. © 2001 Published by Elsevier Science B.V.

Keywords: Hypersensitivity; Antinociception; Flexor reflex; δ-Opioid receptor; SNC80; SH378

1. Introduction

Opioid drugs are widely used in the treatment of pain. However, the clinical utility of opioids is greatly compromised by their side effects, such as tolerance, physical dependence and respiratory depression (Twycross, 1994; Franklin, 1998). As both the analgesic effect and side effects are primarily mediated via μ-opioid receptors (Twycross, 1994; Franklin, 1998; Mason, 1999), there has been increasing interest in alternative therapeutic drugs that would target other opioid receptors.

In contrast to morphine-like opioids, δ-opioid receptor agonists produce little adverse effects on respiration (Kiritisy-Roy et al., 1989; May et al., 1989; Szeto et al., 1999) and gastrointestinal motility (Porreca et al., 1984; Sheldon et al., 1990) with minimal development of physical dependence (Cowan et al., 1988). It is well established that δ-opioid receptors in both spinal and supraspinal structures are involved in modulating nociceptive processing. Intracerebroventricular (i.c.v.; Mosberg et al., 1991; Erspamer et al., 1989; Mattia et al., 1991; Sanchez-Blazquez and Garzon, 1993; Buzas et al., 1994) and intrathecal (i.t.; Porreca et al., 1984; Dickenson et al., 1987; Hope et al., 1990; Collin et al., 1991; Kalso et al., 1992; Malmberg and Yaksh, 1992; Mattia et al., 1992; Stewart and Hammond, 1993, 1994; Hammond et al., 1998) administration of δ-opioid receptor agonists, [D-Pen^{2,5}]enkephalin and deltorphin, produces analgesic effects which are blocked by δ-, but not by μ-opioid receptor antagonists (Jiang et al., 1990; Suh and Tseng, 1990). Knock down of δ-opioid receptors by antisense oligonucleotide attenuates antinociception induced by i.t. administration of δ-, but not μ- or κ-, opioid receptor agonists (Standifer et al., 1994; Tseng et al., 1994). In addition, δ-opioid receptor activation potentiates analgesia

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produced by morphine-like opioids (Heyman et al., 1989; Malmberg and Yaksh, 1992; Noble et al., 1994; Ossipov et al., 1995).

Most nociceptive studies with δ-opioid receptor agonists have relied on local (i.c.v. or i.t.) administration of agonists due, primarily, to the low central nervous system (CNS) bioavailability of δ-opioid peptides. Little is known about the global consequences of δ-opioid receptor activation following systemic administration. (±)-4-((α*R**)-α-((2*S**,5*R**)4-allyl2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N,*N*-diethylbenzamide dihydrochloride (BW373U86), the first systemically active and nonpeptidic δ-opioid receptor agonist (Chang et al., 1993) produces antinociceptive effects in a model of inflammatory pain (Wild et al., 1993; Takasuna et al., 1994; Butelman et al., 1995). However, this compound is less potent in suppressing nociception induced by high intensity stimuli (Wild et al., 1993; Dykstra et al., 1993; Negus et al., 1994). The possibility exists that activation of central δ-opioid receptors by systemic administration may not be sufficient to alleviate pain. Furthermore, the lack of highly specific, non-peptidergic δ-opioid receptor agonists and antagonist makes interpretation of existing studies difficult. The present study was designed to characterise the non-peptide δ-opioid receptor agonist, (+)-4-[(α*R*)-α-((2*R*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC80), a derivative of BW373U86 but with greater selectivity and higher potency for δ-opioid receptors (Calderon et al., 1994; Bilsky et al., 1995; Knapp et al., 1996; Negus et al., 1998) in models of both acute and tonic nociception. Our results showed that the systemic administration of SNC80 dramatically suppressed the spinal excitability and this effect was blocked by a novel and highly selective δ-receptor antagonist, (Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-14β-ethoxy-5β-methylindolo[2′,3′:6′,7′]morphinan-3-ol hydrochloride (SH378); Schmidhammer et al., 1997, 1998; Labarre et al., 1999). A preliminary report of this work has been presented (Cao et al., 1999).

2. Materials and methods

2.1. Surgery

The protocol was approved by the Ethics Committee at AstraZeneca R&D (Montreal) and the animals were handled in strict adherence to the guidelines established by the Canadian Council for Animal Care. Experiments were carried out in adult male Wistar rats (250–320 g). In some rats, unilateral hindpaw inflammation was induced by intraplantar injection of 20 μl complete Freund's adjuvant (Sigma) into the hindpaw (10 μl in the plantar and 10 μl in the dorsal aspect) 24 h prior to the experiment. Animals were anesthetised with halothane in a nitrous oxide–oxygen mixture (2:1) and the trachea and carotid artery cannulated for artificial ventilation and drug administration, respectively. To maintain anesthesia, 1% propofol (Mallinckrodt Veterinary, Ontario, 10 mg/kg) was administered intraarterially (i.a.) until decerebration by aspiration of all the cranial contents rostral to the mesencephalon. The animal was then paralysed by flaxedil (8–10 mg, i.a.), artificially ventilated, and a laminectomy performed to allow transection of the spinal cord at T3–T5. In some animals polyethylene PE-10 tubing was implanted intrathecally with its tip being located at the lumber spinal 4–5 level.

The nerve to the posterior biceps femoris/semitendinousus hamstring muscles was exposed by incision in the popliteal fossa. A very fine filament was split from the nerve and cut, and the central end placed on silver-wire recording electrodes. The sural nerve was dissected free and placed on a pair of silver stimulating electrodes. Both nerves were covered by a pool of warmed mineral oil. At least 1 h was allowed for animal to recover from the anaesthetic and stabilise surgery before the experiment was started. Body temperature was maintained at 36.5°C with a heating blanket and the electrocardiogram was monitored throughout the experiment.

2.2. Electrophysiology

Single unit AC recordings of flexor α-motoneurons (Wall and Woolf, 1984; Woolf and Wall, 1986), obtained from the nerve to the posterior biceps femoris/semitendinousus hamstring muscles, were detected by using a conventional window discriminator. Spike shape was monitored continuously by an analog delay line and spikes were counted with a pulse integrator. To monitor the excitability of the flexor reflex, the test stimuli were applied in a fixed order, once every 5 min. The 5-min cycle of measurements comprised a recording of spontaneous activity of the motoneuron over 10 s, responses to mechanical threshold, standard touch, pinch and Aβ fiber stimulation with each measurement being separated by at least 30 s. The mechanical threshold of the flexor reflex was tested by application of von Frey hair on the plantar surface of the ipsilateral hindpaw toes. The threshold was defined as the value of lowest force of von Frey hair that evoked a consistent discharge on each occasion when applied three times to the plantar skin of any one of the middle three toes. Touch stimuli consisted of eight light touches applied with the flat surface of the experimenter's thumb to the plantar surface of the foot; moving from the mid position of the foot to the distal foot pads. Each touch lasted 2 s and was applied every 4 s. The standard test pinch was applied with a calibrated forceps with a 5×24 mm rectangular surface attached to each blade, sprung at 210 g, on the three middle toes of the ipsilateral hindpaw for 2 s. Responses to low-threshold sural afferents were tested by applying a train of Aβ fiber intensity stimuli (100 μA, 50 μs, 10 Hz and 10 s). Spike counts were integrated over the stimulation period for the sural Aβ

stimuli and over the stimulation period plus 2 s for touch and pinch stimuli to include any afterdischarge.

After a stable baseline was obtained over 20–30 min, the animal received either a conditioning stimulus (in the uninflamed rat) or drug or vehicle administration. The conditioning stimulus consisted of 30-s 1-Hz train pulse (5 mA and 0.5 ms) applied to the sural nerve. The drugs were administered intraarterially at a volume of 1 ml/kg or intrathecally in a volume of 10 μl followed by 0.1 ml (for i.a.) or 10 μl (for i.t.) of saline to flush the catheter. The location of the intrathecal catheter was verified by a direct visualization of the tip of the catheter after completion of experiment. If the catheter was not located correctly or found to cause any damage (e.g., bleeding), the data were excluded.

2.3. Drugs

SNC80 (Tocris, Ballwin) was dissolved in dimethyl sulfoxide (DMSO), (−)dibenzoyl-L-tartaric acid salt (SNC86) and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP; Sigma) in 10% DMSO and naloxone hydrochloride and SH378 (AstraZeneca R&D Montreal synthesized by Dr. H. Schmidhammer) in saline.

2.4. Data analysis

The values for the responses evoked by touch, pinch and Aβ-afferent were corrected by subtracting the extrapo-

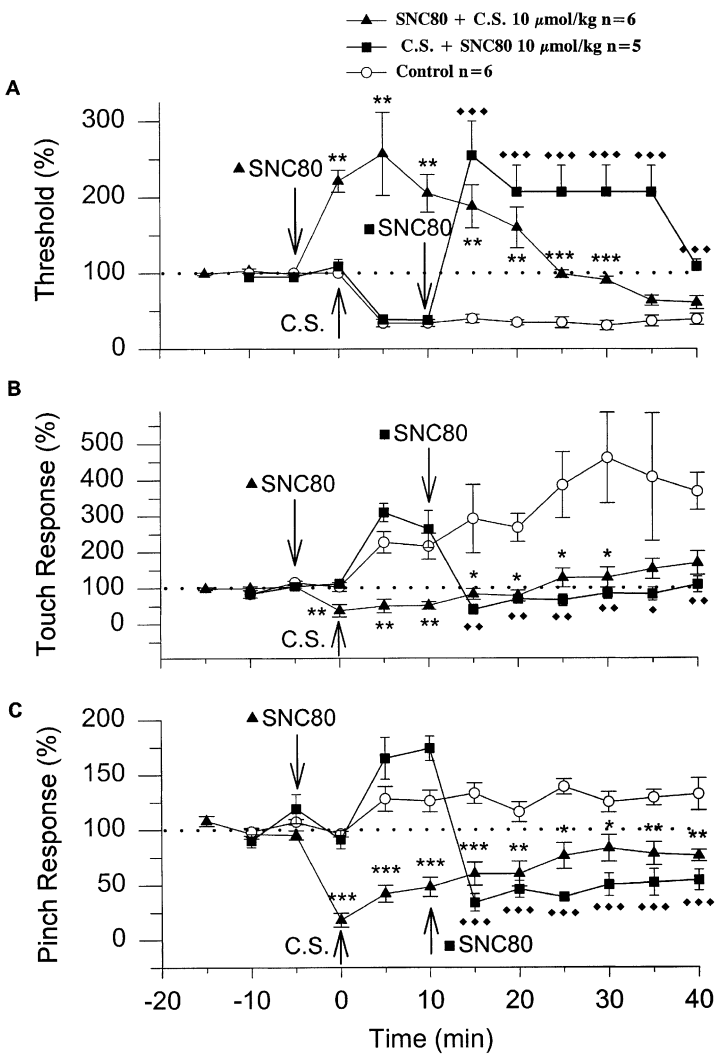

Fig. 1. Effects of pretreatment and posttreatment of SNC80 on the hypersensitivity of flexor reflex induced by conditioning stimulation in normal rats. Conditioning stimuli (c.s.) were delivered to the sural nerve at C-fiber intensity (5 mA, 500 μs, 1 Hz and 30 s). SNC80 was intraarterially injected at a dose of 10 μmol/kg 5 min before c.s. (indicated by arrow plus ▲ SNC80) or 10 min after c.s. (indicated by arrow plus ■ SNC80). Control group was conditional stimulation alone without drug administration (open circle ○). The changes in mechanical threshold (A) and responses of flexor α-motoneuron activity evoked by touch (B) and pinch (C) are shown. The first three measurements were averaged to build a baseline. Values are normalized to a percentage of the baseline, expressed as mean ± S.E.M. The statistic analysis was applied to SNC80 + c.s. vs. c.s. groups (symbol *) or c.s. + SNC80 vs. c.s. (symbol ◆) groups. * or ◆ represent $P < 0.05$, ** or ◆◆ $P < 0.01$ and *** or ◆◆◆ $P < 0.001$.

lated spontaneous activity in the same period. Baseline activity was the mean of the last three readings taken immediately before drug or conditioning stimuli were applied. To avoid individual variation, the data were normalized as percentage of baseline (100%). The maximal changes produced by drug administrations within 60 min were analysed to indicate the effects of the drugs in some groups. Statistical significance was tested on the data with non-parametric ANOVA followed by Mann–Whitney's *U*-test. All data are expressed as mean ± S.E.% in each group. *P* value of less than 0.05 was considered as significant difference between the groups.

3. Results

3.1. Effects of SNC80 on hypersensitivity of the flexor reflex induced by C-fiber input in normal rats

Following conditioning stimulation at C-fiber intensity (5 mA, 0.5 ms, 1 Hz for 30 s) there was an immediate reduction of the mechanical threshold by 66 ± 5% and increases in touch- and pinch-evoked responses by 127 ± 30% and 28 ± 11%, respectively ($n = 6$, Fig. 1). These changes persisted for approximately 40 min and then gradually faded away.

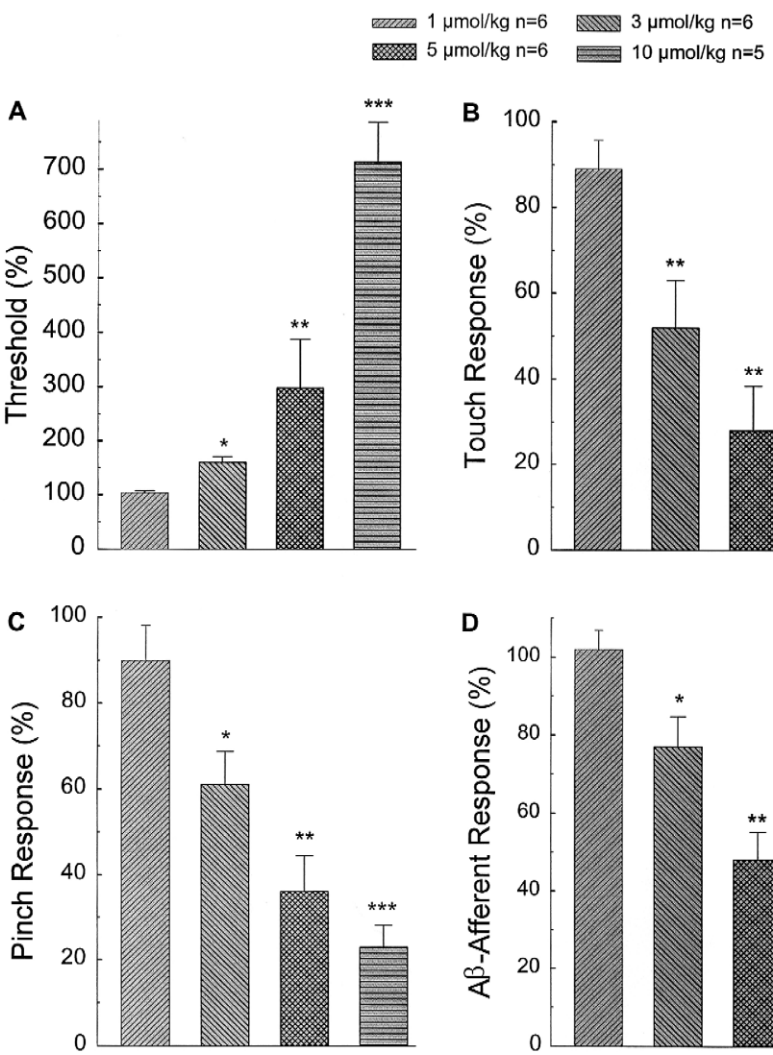

Fig. 2. Dose-related effects of SNC80 on the excitability of the flexor reflex in the inflamed rats. SNC80 was intraarterially injected at doses of 1, 3, 5 and 10 μmol/kg in the rats with unilateral hindpaw inflammation induced by complete Freund's adjuvant. Changes in mechanical threshold and responses of flexor α-motoneuron activity evoked by touch, pinch and Aβ-fiber intensity stimuli applied to the sural nerve within 5 min following the drug administration are shown. The drug was administered cumulatively at 1, 3 and 5 μmol/kg in six rats with 30-min intervals between each dose and 10 μmol/kg in another five rats. Values are normalized to a percentage of pretreatment baseline, expressed as mean ± S.E.M. The control values (open bars) were mean ± S.E.M. of the various measurements at the corresponding time in the vehicle-treated group. Asterisks represent the level of significance, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

SNC80 at a dose of 10 μmol/kg (i.a.) was given in a separate group. SNC80 immediately increased mechanical threshold two-fold and decreased touch- and pinch-evoked responses by 60–80%. Five minutes after administration of SNC80, C-fiber conditioning stimulation was ineffective and also failed to reverse the effect of SNC80 on any parameter measured ($n = 6$; Fig. 1).

In the third group, SNC80 (10 μmol/kg i.a., $n = 5$) injected 10 min after the conditioning stimulus reversed the hypersensitivity induced by C-fiber stimulation. Fig. 1 shows that after the hyper-excitability of the flexor reflex was established by C-fiber conditioning stimulation, SNC80 still increased mechanical threshold by two-fold and decreased responses evoked by touch and pinch by approximately 60–80% and this effect persisted for approximately 30 min.

3.2. Effects of SNC80 and SNC86 on the excitability of the flexor reflex in the inflamed rat

Rats with unilateral hindpaw inflammation had a lower baseline mechanical threshold (43.1 ± 7.7 vs. 12.3 ± 3.0 g) and enhanced responses to touch, pinch and Aβ-fiber stimuli compared to normal rats, indicating a tonic hypersensitivity (Cao et al., 1999). This was consistent with previous study (Ma and Woolf, 1996). SNC80 was given cumulatively at 1, 3 and 5 μmol/kg (i.a.) with 30-min intervals between each dose ($n = 6$). SNC80 was also administered in a separate group of rats at one dose of 10 μmol/kg. SNC80 significantly depressed excitability of the flexor reflex in a dose-related manner (3, 5 and 10 μmol/kg i.a.; Fig. 2). The greatest effect was seen at 10 μmol/kg with an increase in mechanical threshold of $715 \pm 73\%$ and decreases in the responses evoked by touch, pinch and Aβ-fiber stimuli to $24 \pm 6\%$, $23 \pm 5\%$ and $62 \pm 6\%$ of control, respectively ($P < 0.01$–0.001). These effects lasted for approximately 30 min. The time course of SNC80 at a dose of 10 μmol/kg is illustrated in Fig. 3.

To further confirm the consequences of systemic δ-opioid receptor activation, another non-peptide δ-opioid receptor agonist, SNC86, was administered at a dose of 5 μmol/kg (i.a., $n = 5$) in animals with unilateral hindpaw inflammation. SNC86 immediately increased mechanical threshold to $466 \pm 59\%$ and decreased responses evoked by touch, pinch and Aβ-fiber stimuli to $35 \pm 8\%$, $42 \pm 8\%$ and $66 \pm 13\%$, respectively ($P < 0.05$–0.01, Fig. 3) and these persisted for 20–40 min.

3.3. Antagonism of SNC80-induced responses by naloxone and HS378 but not by CTOP in inflamed rats

Naloxone (20 μmol/kg, i.p.) had no effect on the excitability of the flexor reflex by itself. Ten minutes after

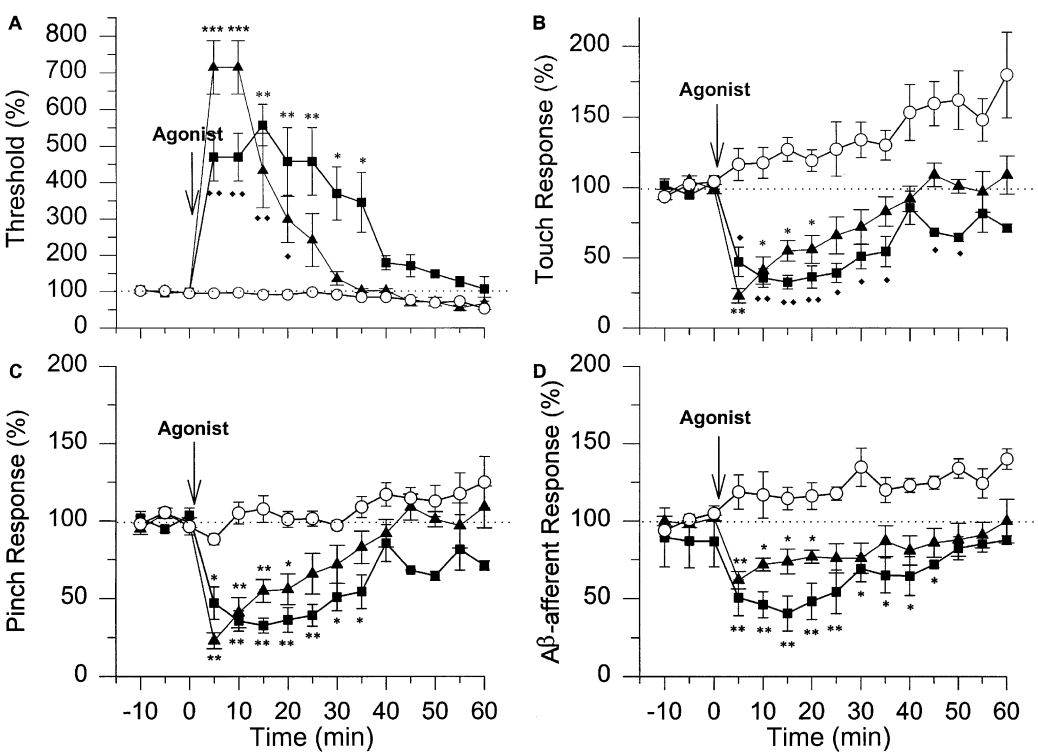

Fig. 3. Time course of the effects of SNC80 and SNC86 on hypersensitivity of the flexor reflex in inflamed rats. SNC80 (10 μmol/kg; ▲), SNC86 (5 μmol/kg; ■) or vehicle (1 ml/kg; ○) was intraarterially injected in the rats with unilateral hindpaw inflammation. The changes of the mechanical threshold and responses of flexor α-motoneuron activity evoked by touch, pinch and Aβ-fiber intensity stimuli are shown in panels A, B, C and D, respectively. The statistical analysis was applied to SNC80 vs. vehicle groups (symbol *) or SNC86 vs. vehicle (symbol ◆) groups.

naloxone injection, administration of SNC80 (10 μmol/kg, i.a.) failed to increase mechanical threshold or decrease responses evoked by touch, pinch and Aβ-fiber stimuli ($n = 4$; Fig. 4).

The novel and highly selective δ-opioid receptor antagonist, HS378 (5 μmol/kg, i.a.), injected 10 min prior to SNC80 (10 μmol/kg, i.a.) prevented the reduction of flexor-reflex excitability induced by SNC80 ($n = 6$; Fig. 4). HS378 by itself did not affect excitability of the flexor reflex.

The highly specific μ-opioid receptor antagonist, CTOP (1.5 nmol, i.t.), given 10 min before SNC80 (10 μmol/kg, i.a.), did not alter the baseline excitability of the flexor reflex, nor the SNC80-induced increase in mechanical threshold and decreases in responses evoked by touch, pinch and Aβ-fiber stimuli ($n = 4$; Fig. 4).

4. Discussion

Little is known about the effect of systemic administration of δ-opioid receptor ligands because of low CNS bioavailability of δ-opioid peptides. The non-peptidergic δ-opioid receptor agonist, SNC80, has been reported to raise the nociceptive threshold in normal mice and primates (Bilsky et al., 1995; Negus et al., 1998). However, the effect of SNC80 on hypersensitivity of pain is not known. The present study showed that systemic injection of SNC80 and SNC86 suppresses hypersensibility of the flexor reflex induced by C-fiber stimulation and peripheral inflammation. This is the first study to demonstrate the potent antihypersensitivity of the δ-opioid receptor agonists administered systemically in acute and chronic nociceptive models in the rat.

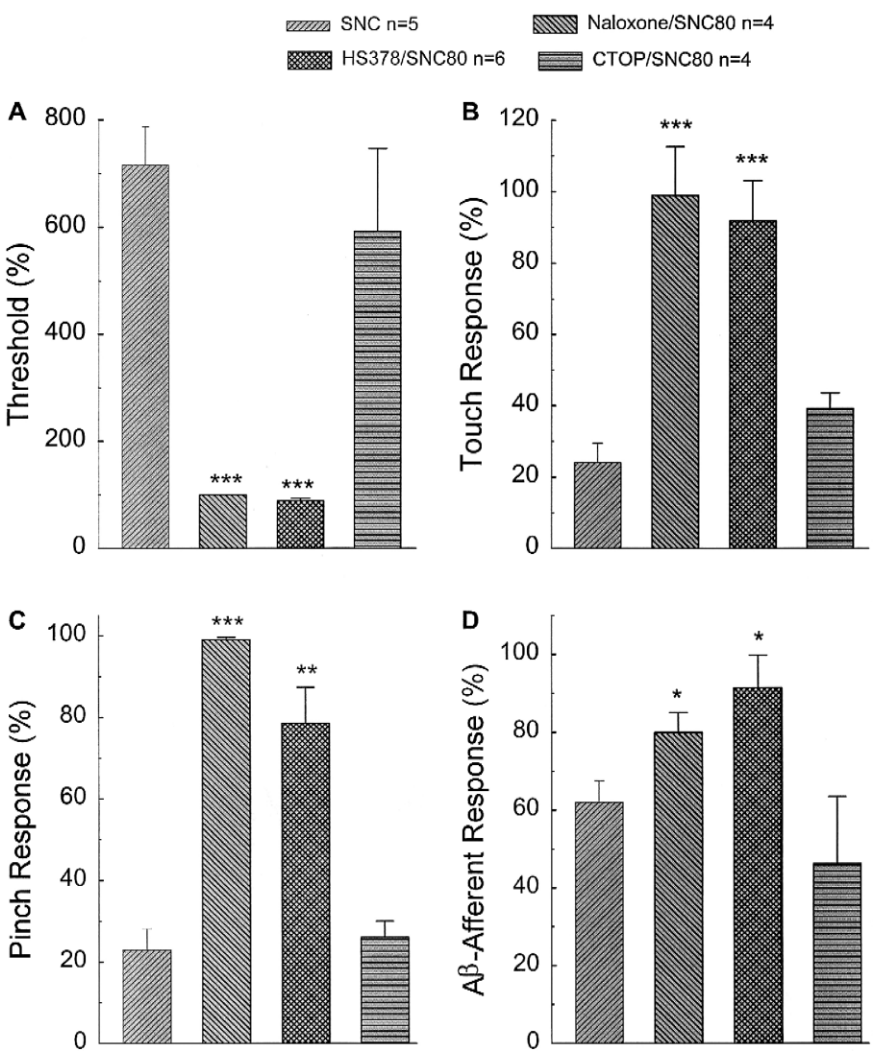

Fig. 4. Effects of SNC80 on the excitability of the flexor reflex in the presence of antagonists in the inflamed rats. SNC80 (10 μmol/kg, i.a.) was injected 20 min after naloxone (20 μmol/kg, i.p.) or 10 min after HS378 (10 μmol/kg, i.a.) or CTOP (1.5 nmol, i.t.) in the rats with unilateral hindpaw inflammation. The maximal changes were illustrated of mechanical threshold and responses of flexor α-motoneuron activity evoked by touch, pinch and Aβ-fiber intensity stimuli during 60 min of experiment. Statistical comparison was conducted between the groups of antagonist/SNC80 and SNC80 alone. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

It has been demonstrated that one characteristic of clinical pain is an abnormal sensitivity to noxious stimuli which has been ascribed to central sensitisation (Woolf, 1994). Central sensitisation is believed to play a major role in the generation of pain hypersensitivity in animals and humans (Dubner and Ruda, 1992; Coderre et al., 1993; McMahon et al., 1993; Woolf and Chong, 1993; Koltzenburg et al., 1992; Torebjörk et al., 1992). Stimulation of C-fibers in the sural nerve in animal has been shown to result in a dramatic reduction of mechanical threshold and an increase of touch- and pinch-evoked responses (Woolf, 1983; Woolf and Wall, 1986). In rats with unilateral hindpaw inflammation, the responses of the flexor reflex activity to touch, pinch and Aβ inputs are greatly enhanced (Ma and Woolf, 1996) and this hypersensitivity is more persistent than by only C-fiber stimulation. Therefore, the present findings that SNC80 prevented and/or reversed the development of hypersensitivity of the flexor reflex activity in both acute and chronic nociceptive models are very interesting from the clinical point of view.

Central sensitisation plays a dominant role in the models used in this study and, therefore, the suppression of the hypersensitivity of the flexor reflex produced by SNC80 can be considered a centrally mediated response. Since the spinal cord was transected in the present study, it is probably spinal mechanisms that underlay the SNC80-induced effects. Similar responses have been observed with intrathecal administration of SNC80 and deltorphin II (Hong et al., 1999).

SNC80 is a selective δ-opioid receptor agonist with more than 500 selectivity for δ- vs. μ-opioid receptors (Calderon et al., 1994; Bilsky et al., 1995). In the present study, the prevention of the SNC80-induced suppression by the non-selective opioid receptor antagonist, naloxone, strongly supports that this was an opioid receptor-mediated response. More specifically, the highly selective and potent δ-opioid receptor antagonist, HS378 (Schmidhammer et al., 1997, 1998; Labarre et al., 1999), also prevented and reversed the effects of SNC80. Our study is the first demonstration of a systemic activity of this δ-opioid receptor antagonist which has been shown to have greater selectivity for δ-opioid receptors than either naltrindole or naltriben (Schmidhammer et al., 1997, 1998; Labarre et al., 1999). The systemic activity of HS378 also demonstrates that this novel δ-opioid antagonist is a useful pharmacological tool to help elucidate the role of δ-opioid receptors in vivo.

The lack of μ receptor-mediated actions of SNC80 was confirmed by the failure of intrathecal administration of CTOP, a potent and highly selective μ-opioid receptor antagonist (Pelton et al., 1986; Hawkins et al., 1989), to block the effects of SNC80. The dose of CTOP used here has shown previously to completely block the antinociceptive effect produced by intrathecal administration of [D-Ala^2,NmePhe4,Gly-ol^5]enkephalin (DAMGO; Hong et al., 1999). Therefore, it is highly likely that the potent antinociception induced by systemic injection of SNC80 was mediated via δ-opioid receptors, which was suggested by previous studies (Bilsky et al., 1995; Negus et al., 1998). Our observation is consistent with other studies looking at the antinociceptive efficacy of δ-opioid receptor agonists at the spinal level (Stanfa et al., 1992; Stewart and Hammond, 1994; Hammond et al., 1998).

In conclusion, the present study demonstrated that SNC80 is a potent and effective antinociceptive agent in both acute and persistent inflammatory models of hypersensitivity in the rat and systemic route of administration can achieve a good CNS bioavailability of δ-opioid receptors. In addition, the systemic activity of a novel, highly selective δ-opioid receptor antagonist, HS378, shows that this antagonist is a useful tool for investigating the δ-opioid receptor mechanism in vivo. The efficacy of non-peptidergic δ-opioid receptor ligands and the potent suppression on the hypersensitivity of the flexor reflex in both acute and chronic nociceptive models strongly supports δ-opioid receptors as a potential pharmaceutical analgesic target.

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