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Central effect of SNC 80, a selective and systemically active δ-opioid receptor agonist, on gastrointestinal propulsion in the mouse

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Abstract

We investigated the effects of SNC 80 ((+)-4-[αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide), a new highly selective, non-peptidic and systemically active δ-opioid receptor agonist, on gastrointestinal and colonic propulsion in mice. Intraperitoneally (i.p.) SNC 80 (1, 10 and 30 mg/kg) significantly decreased gastrointestinal propulsion measured as transit of an orally administered charcoal meal. Pretreatment with the δ-opioid receptor antagonist, naltrindole (1 mg/kg) subcutaneously (s.c.), with the non-selective opioid antagonist, naloxone (5 mg/kg, s.c.) or the μ_1-opioid receptor antagonist, naloxonazine (10 mg/kg, i.p.), significantly decreased the antitransit effect of SNC 80 but pretreatment with the non-selective opioid antagonist, naloxone methiodide (5 mg/kg, s.c.), a quaternary salt of naloxone that does not cross the blood–brain barrier, did not. SNC 80 (1, 5 and 10 mg/kg, i.p.), produced dose-related inhibition of colonic propulsion measured as the increase in mean expulsion time of a 3 mm glass bead placed in the distal colon. Naloxone (5 mg/kg, s.c.) and naltrindole (1 mg/kg, s.c.), completely antagonized the colonic antipropulsive effect of SNC 80. In contrast, naloxone methiodide (5 mg/kg, s.c.), left the inhibitory effect of i.p. SNC 80 on colonic function unchanged. These results suggest that peripherally injected SNC 80 inhibits gastrointestinal transit and colonic propulsion. It does so mainly through a central mechanism. Although the gastrointestinal antitransit effect of SNC 80 is naltrindole- and naloxonazine-sensitive, we cannot exclude an opioid-independent mechanism. The colonic antipropulsive effect of SNC 80 confirms the inhibitory role of the central δ-opioid receptor system on colonic motility. © 1998 Elsevier Science B.V.

Keywords: δ-Opioid receptor agonist; Peripheral administration; Gastrointestinal transit; Colonic propulsion; (Mouse)

1. Introduction

Despite the long known link between opioids and gastrointestinal function, the gastrointestinal effects of opioids remain ill-defined. Research needs to especially address multiple sites of actions, multiple types of opioid receptors and multiple pharmacological endpoints. The advent of highly receptor-selective agonist and antagonist drugs has considerably increased our understanding of how the various opioid receptor types mediate gut functions.

In rats and mice, intracerebroventricular (i.c.v.), intrathecal (i.t.) or subcutaneous (s.c.) injection of μ-opioid receptor agonists inhibits both gastrointestinal and colonic propulsion, indicating that opioid-induced inhibition of intestinal transit involves brain, spinal and peripheral μ-opioid receptors (Broccardo et al., 1982; Galligan and Burks, 1983; Porreca et al., 1984; Manara and Bianchetti, 1985; Porreca et al., 1986; Improta and Broccardo, 1994).

Only recently, thanks to the availability of the highly-selective δ-opioid peptides, DPDPE (D-Pen^2, D-Pen^{5}enkephalin) and deltorphin II (D-Ala^2, Glu^{4}deltorphin), has it become possible to study the role of the δ-opioid receptors in gut propulsion. In particular, DPDPE and deltorphin II, when supraspinally and peripherally injected in mice and in rats, have no effect on gastric emptying and gastrointestinal transit (Galligan et al., 1984; Porreca et al., 1984; Burks et al., 1988; Broccardo and Improta, 1992a,b; Improta and Broccardo, 1994); only when i.t. injected in mice does DPDPE decrease gastrointestinal transit, probably by acting at spinal, naloxonazine-sensitive sites (Heyman et al., 1988). Despite having little or no effect on gastrointestinal transit, δ-opioid receptor agonists after i.c.v., i.t. or s.c. administration significantly delay colonic propulsive activity in rats, mice and cats (Kaufman et al., 1988; Krevsky et al., 1991; Broccardo and Improta, 1992a,b)

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indicating that central and peripheral δ-opioid receptors play a fundamental role in modulating colonic motility.

The low bioavailability of the δ-opioid peptides at central nervous system sites of action has, however, made it difficult to assess the consequences of systemic δ-opioid receptor activation. This problem has now been solved by the recent synthesis of SNC 80 ((+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) (Calderon et al., 1994), a new highly selective, nonpeptidic and systemically active δ-opioid receptor agonist which possesses analgesic activity (Bilsky et al., 1995; Knapp et al., 1996). In the mouse vas deferens and guinea-pig ileum preparations (bioassays generally considered to reveal δ- and μ-opioid receptor activity, respectively) SNC 80 shows an approximately 2000-fold selectivity for δ receptors (rather than μ), similar to that of DPDPE. Inhibition studies using radioligands selective for μ-, δ- and κ-opioid receptors further confirm the selectivity of SNC 80 for δ-opioid receptors. The binding selectivity profile of SNC 80 for δ-opioid receptors was of an order of magnitude comparable to that seen with DPDPE (Bilsky et al., 1995).

In this study we investigated the effects of peripherally injected SNC 80 on gastrointestinal and colonic transit in mice.

2. Materials and methods

2.1. Animals

All animal experiments complied with the Italian D.L. No. 116 of 27 January 1992 and associated guidelines, in the European Communities Council Directive of 24 November, 1986 (86/609/EEC). Male Swiss mice weighing 25–30 g were used for the experiments and all mice were examined at 09.00 h. Mice were placed individually in plastic cages under standard lighting (a 12-h light/dark cycle) and temperature (22°C) conditions. For the gastrointestinal transit test, the mice were fasted, but had free access to water for 24 h. Different groups of mice were used for each dose of drugs tested.

2.2. Upper gastrointestinal transit test

Upper gastrointestinal transit was measured with the charcoal meal test (Schulz et al., 1979). Mice received 0.3 ml of a 20% (w/v) charcoal suspension in a 5% (w/v) gum arabic solution via a stomach tube. Immediately afterwards, SNC 80 was injected i.p. at doses of 1, 10 and 30 mg/kg. 25 min after receiving the charcoal meal, the mice were killed (CO_2, 70%) and the small intestine was removed en bloc. Small bowel propulsion was determined by calculating the ratio between the distance travelled by the charcoal meal and the total length of the small bowel for each mouse. The data are presented as the percent of gastrointestinal transit measured as a quotient of the propulsion value in drug-treated mice and that in vehicle-treated mice (64.1 = 100%).

2.3. Colonic propulsion test

Distal colonic propulsion was measured according to the method of Raffa et al. (1987) previously described by Jacoby and Lopez (1984). Immediately after i.p. administration of SNC 80 (1, 5 and 10 mg/kg) a single 3 mm glass bead was inserted 2–3 cm into the distal colon of each mouse. The time required for expulsion of the glass bead was determined (to the nearest 0.1 min) for each animal. Inhibition of colonic propulsion was measured as the increase in mean expulsion time of the glass bead compared to that for vehicle-treated mice (controls). The higher the mean expulsion time value, the stronger the inhibition of colonic propulsion.

2.4. Drugs

SNC 80 ((+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) (MW 449,63), was purchased from Tocris Cookson (Bristol, England) and was dissolved in 10% DMSO (dimethyl sulphoxide) and then diluted to the final concentration with distilled water. Naltrindole HCl, a selective δ-opioid receptor antagonist (Portoghese et al., 1988), was purchased from Research Biochemicals Incorporated (Natick, MA). Naloxone, a non-selective opioid receptor antagonist, was purchased from Endo Laboratories. Naloxone methiodide, a quaternary salt of naloxone that does not cross the blood–brain barrier (Milne et al., 1990), was purchased from Research Biochemicals Incorporated (Natick, MA). Antagonists were administered s.c. 15 min before agonist (or vehicle) injections and were dissolved in distilled water. Naloxonazine, a selective μ_1-opioid receptor antagonist (Ling et al., 1986) purchased from Nova Pharmaceutical Co. (Baltimore, USA), was dissolved in 0.1% acetic acid and administered 24 h before agonist or vehicle, as only its irreversible actions are μ_1-opioid receptor-selective.

2.5. Statistical analysis

All results are expressed as means ± S.E. The data were evaluated with a one-way analysis of variance (ANOVA) and Duncan's multiple range test on an Apple II Computer.

3. Results

3.1. Upper gastrointestinal transit test

At doses of 1, 10 and 30 mg/kg, i.p.-injected SNC 80 inhibited upper gastrointestinal transit of a charcoal meal

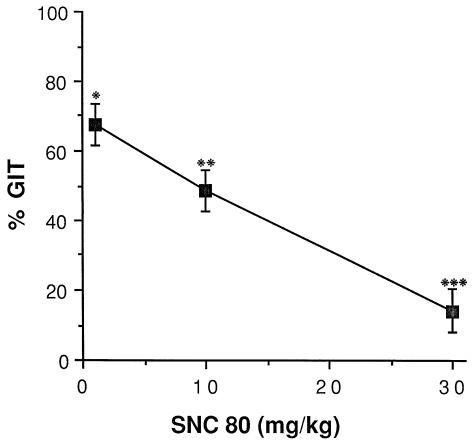

Fig. 1. Effects of SNC 80 (1, 10 and 30 mg/kg, i.p.), on upper gastrointestinal transit (GIT) of a charcoal meal in mice. Each point represents the mean ± S.E. of values for at least 8 mice, expressed as per cent change in gastrointestinal transit versus vehicle-treated mice (= 100). The lower the value, the stronger the inhibition of propulsion. Difference from vehicle-treated mice $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

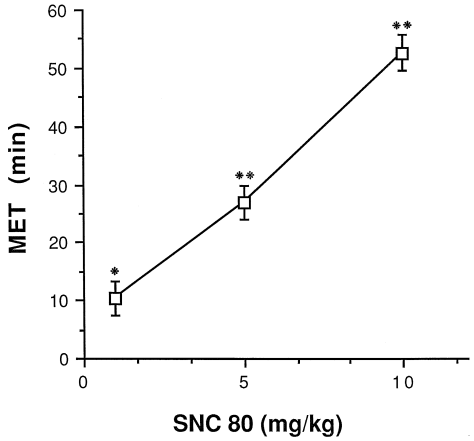

Fig. 3. Effects of SNC 80 (1, 5 and 10 mg/kg, i.p.) on colonic propulsion in mice. Each point is the mean ± S.E. of values for 8 or more mice, expressed as mean expulsion time (MET) of a glass bead. The higher the value, the stronger the inhibition of colonic propulsion. The MET for vehicle-treated mice was 4.7 ± 0.5 min. Difference from vehicle-treated mice $^{*}P < 0.05$, $^{**}P < 0.001$.

in a dose-related manner. The highest dose used reduced gastrointestinal transit by 86% (Fig. 1). The non-selective opioid receptor antagonist, naloxone (5 mg/kg s.c.), the selective δ-opioid receptor antagonist, naltrindole (1 mg/kg, s.c.) and the selective μ_1-opioid receptor antagonist, naloxonazine (10 mg/kg i.p.), which injected alone did not modify gastrointestinal transit, partially decreased (by 50%) SNC 80-induced antitransit (Fig. 2). Naloxone methiodide (5 mg/kg, s.c.), a quaternary salt of naloxone that does not cross the blood–brain barrier, failed to antagonize SNC 80-induced inhibition of upper gastrointestinal transit (Fig. 2).

3.2. Colonic propulsion test

SNC 80, i.p. injected, induced a dose-related inhibition of colonic propulsion: the mean expulsion time for vehicle-treated mice was 4.7 ± 0.5 min. SNC 80 significantly increased the mean expulsion time, reaching 52.7 ± 8.3 min at the dose of 10 mg/kg (Fig. 3). No significant difference was observed between the mean expulsion time of vehicle-treated mice and of naltrindole-, naloxone- and naloxone methiodide-treated mice (Fig. 4). Naloxone (5 mg/kg, s.c.), and naltrindole (1 mg/kg), s.c., completely antagonized the increase in mean expulsion time produced by SNC 80 (10 mg/kg i.p.), whereas naloxone methiodide (5 mg/kg) left the SNC 80-induced inhibition of colonic propulsion unchanged (Fig. 4).

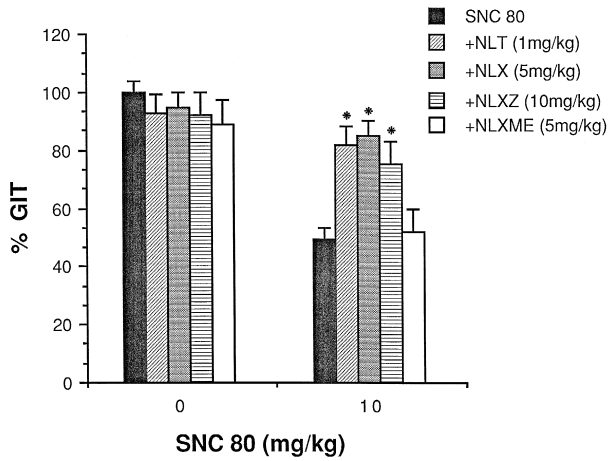

Fig. 2. GI antipropulsive effects of a single dose of SNC 80 (10 mg/kg, i.p.) in vehicle-pretreated or naltrindole (NLT)-, naloxone (NLX)-, naloxonazine (NLXZ)- and naloxone methiodide (NLXME)-pretreated mice. Each point represents the mean ± S.E. for at least 8 mice. Difference from SNC 80-treated group, $^{*}P < 0.01$.

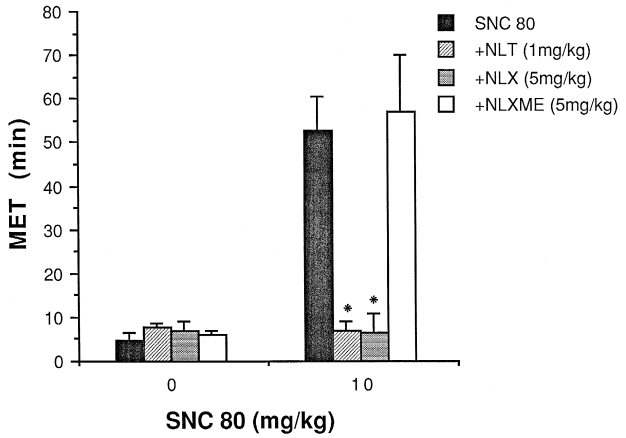

Fig. 4. Colonic antipropulsive effects of a single dose of SNC 80 (10 mg/kg, i.p.) in vehicle-pretreated or naltrindole (NLT)-, naloxone (NLX)- and naloxone methiodide (NLXME)-pretreated mice. Each point represents the mean ± S.E. for at least 8 mice. Difference from SNC 80-treated group, $^{*}P < 0.01$.

4. Discussion

In our experiments i.p.-administered SNC 80 significantly inhibited upper gastrointestinal transit in the mouse. The observation that pretreatment with the selective δ-opioid receptor antagonist, naltrindole, partially reduced SNC 80 antitransit suggests that SNC 80 could act via the δ-opioid receptor system. Pretreatment with naloxonazine partially but significantly reduced SNC 80-induced gastrointestinal antipropulsion, indicating that this effect is also exerted at naloxonazine-sensitive (μ_1) sites. This agrees with a previous observation (Heyman et al., 1988) that DPDPE decreases gastrointestinal transit by acting via a spinal, naloxonazine-sensitive site in mice. Why naltrindole and naloxonazine induced only partial antagonism in our experiments is difficult to explain. SNC 80 might act on a μ–δ-opioid receptor complex, termed the δ_{cx} (Cha et al., 1995), having two sites (μ and δ) both involved in the control of gastrointestinal propulsion. Otherwise, the antitransit effect of SNC 80 might even be controlled partially through an opioid-independent mechanism. Administered in vivo at the doses we used, SNC 80 might also not be a perfectly selective δ-opioid receptor agonist. This agrees with recent data (McIntyre and McHarg, 1996) from radioligand binding studies with the highly selective δ-opioid receptor agonist, [^{3}H]-SNC 80. Using a stable line of Chinese hamster ovary (CHO) cells expressing the human δ opioid receptor, these investigators showed that although most of the [^{3}H]-SNC 80 binding is of the δ subtype, some ligands reduced binding below non-specific binding levels, suggesting that this compound may interact with more than one receptor.

To determine whether SNC 80 acts on upper gastrointestinal transit at a peripheral or central site, we tested naloxone, a non-selective opioid receptor antagonist which crosses the blood–brain barrier when injected peripherally and naloxone methiodide, a non-selective opioid receptor antagonist known to have similar antagonist properties but only peripheral effects (Milne et al., 1990). Our results show that naloxone administered at a no longer selective dose (Lord et al., 1977; Magnam et al., 1982) reduced SNC 80 antipropulsion, whereas the same dose of the peripheral opioid antagonist left this effect unchanged. Hence we conclude that SNC 80 acts mainly via a central mechanism, although our findings do not allow us to specify whether it acts at a spinal or supraspinal site.

Peripherally injected SNC 80 also inhibited colonic propulsion in mice, significantly and in a dose-related manner. Because naltrindole, the selective δ-opioid receptor antagonist, completely blocked SNC 80-induced inhibition, we suggest that the effect of SNC 80 on colonic propulsion is mainly mediated by the δ-opioid receptor system. To determine whether SNC 80 acts on colonic propulsion at a central or peripheral site, as we did for upper gastrointestinal transit, we tested naloxone, the non-selective opioid receptor antagonist which crosses the blood–brain barrier when peripherally injected and naloxone methiodide, the non-selective opioid receptor antagonist which does not cross the blood–brain barrier. Naloxone completely reduced the antipropulsive effect of SNC 80 whereas the peripheral opioid antagonist left it unchanged. We therefore conclude that peripherally injected SNC 80 inhibits colonic propulsion in mice through a central opioid mechanism, confirming the inhibitory role of the central δ-opioid receptor system on this colonic function in the mouse (Krevsky et al., 1991; Broccardo and Improta, 1992b).

Some investigators have reported that peripheral administration of SNC 80 induces a potent analgesic effect (Bilsky et al., 1995). In the development of therapeutically useful analgesics, opioids acting selectively via δ-opioid receptors could have some advantages over the currently available μ-opioid analgesics, for example the lack of adverse gastrointestinal effects (Porreca et al., 1984; Galligan et al., 1984; Sheldon et al., 1990; Rapaka and Porreca, 1991). Nevertheless, our observation that SNC 80 inhibits gastrointestinal propulsion and does so at doses ten times lower than those needed for antinociception, may limit its possible use as an analgesic. These results could, however, be useful in understanding the role of the δ-opioid receptor system in the physiological regulation of gastrointestinal motility and in disorders characterized by altered propulsion.

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