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Antinociceptive and other behavioral effects of the steroid SC17599 are mediated by the μ-opioid receptor

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Abstract

The objective of the present investigation was to evaluate the behavioral effects of SC17599 (17α-acetoxy-6-dimethylaminomethyl-21-fluoro-3-ethoxypregna-3, 5-dien-20-one) in mice and to determine if these effects are selectively mediated by opioid receptors. Although less potent than morphine, SC17599 produced dose-dependent antinociception in both the acetic acid-induced writhing and warm water tail-withdrawal assays. Pretreatment with the opioid antagonist naltrexone and the noncompetitive μ-opioid receptor-selective antagonist methocinnamox, but not the δ-opioid receptor-selective antagonist naltrindole or the κ-opioid receptor-selective antagonist nor-binaltorphimine, antagonized the antinociceptive effects of both SC17599 and morphine. Similarly to morphine, administration of SC17599 induced the Straub tail response in a dose-dependent and naltrexone-sensitive manner. At the highest doses studied, unlike morphine, SC17599 did not alter locomotor activity. The steroid SC17599 is structurally a very unusually selective μ-opioid agonist that produces behavioral effects, which are similar, but not identical, to those of morphine.

Keywords: Antinociception; Locomotor activity; Straub tail; Morphine; SC17599; (Mouse)

1. Introduction

The ''classical'' theory of steroid action indicates that steroids produce their hormonal effects by binding to intracellular receptors and modulating gene transcription (Yamamoto, 1985; Beato, 1991). While many steroid effects have delayed onset of action, occurring slowly over hours or even days, there is also accumulating evidence for rapid nongenomic steroid effects, which occur within seconds to minutes (Schumacher, 1990; McEwen, 1991). In addition, nongenomic steroid effects occur if the steroid is covalently conjugated to a large polymer and thereby prevented from entering the cell (Brann et al., 1995). Rapid steroid effects are not blocked by either protein synthesis inhibitors or classic steroid receptor antagonists, thereby precluding interaction of the steroid with classical steroid receptors (Brann et al., 1995).

Many classes of steroids have been shown to exert rapid effects, including gonadal steroids, glucocorticoids, mineralocorticoids, neurosteroids and Vitamin D3 (Wheling, 1997; Christ et al., 1999). These effects include altered levels of second messengers, neurohormones and neurotransmitters, as well as changes in intracellular pH and ion permeability. One of the most extensively studied interactions between steroids and membrane-bound receptors is the modulation of $GABA_A$ receptors by the A-ring-reduced steroids (Majewska, 1992; Paul and Purdy, 1992; Mellon, 1994; Rupprecht, 1997). 3α-Hydroxy-steroids bind stereoselectively and with high affinity to $GABA_A$ receptors, resulting in increased GABA-ergic effects through increases in both the frequency and the duration of Cl-channel openings (Majewska, 1992; Lambert et al., 1995). While there is great deal of evidence pertaining to the ''nonclassical'' interactions between steroids and $GABA_A$ receptors, there is very little evidence indicating that steroids can interact with opioid receptors.

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Indeed, a detailed study of steroid binding to opioid receptors revealed that opioid receptors are unlikely to be targets for steroid hormones (Schwarz and Pohl, 1994). None of the androgens, gestanes, glucococorticoids, or mineralocorticoids evaluated in this study displayed affinity for any of the three opioid receptor subtypes (μ, δ or κ). Certain estrogens were able to bind to the μ-opioid receptor, but only at very high concentrations. By comparison, the affinity of estrogens for the α-opioid receptor was approximately 1000 times less than that of progestins for $GABA_A$ receptors. The lack of affinity is to be expected, since steroids do not typically possess the structural features associated with interactions with opioid receptors, namely a basic nitrogen and an aromatic ring with a hydroxy substituent (Casy and Parfitt, 1986; Archer, 1993).

Unlike the endogenous steroids studied thus far, there is evidence indicating that the amino-steroid 17α-acetoxy-6-dimethylaminomethyl-21-fluoro-3-ethoxypregna-3,5-dien-20-one (SC17599, Fig. 1) may interact with opioid receptors (Craig, 1968). SC17599 was shown to have antinociceptive activity in the mouse writhing and hot plate assays, as well as the rat tail-flick assay. In addition, SC17599 induced respiratory depression in rabbits, reduced gastrointestinal propulsion, and produced the Straub tail response in mice. Nalorphine did not alter the antinociceptive potency of SC17599, but did antagonize the effects of this steroid on respiration. Thus, it was unclear if the antinociceptive effects of SC17599 are due to opioid actions of the compound.

Recently, Houshyar et al. (1998) characterized the in vitro pharmacological properties of SC17599 using ligand binding and [^{35}S]GTPγS-binding assays. SC17599 was shown to bind with high affinity ($K_i = 19.3$ nM) to μ-opioid receptors. SC17599 bound with low affinity to δ-and κ-receptors, displaying 120- and 100-fold selectivity for the μ-opioid receptor, respectively. In addition, SC17599 was fully efficacious in stimulating G-proteins in the GTPγS assay, and this effect was naloxone-sensitive. Taken together with previous in vivo findings, these data implicate interactions between SC17599 and the μ-opioid receptor.

The purpose of the present investigation was to evaluate the in vivo effects of SC17599 and to determine using selective opioid antagonists whether these effects are mediated through opioid receptors. The results show that SC17599 is a highly efficacious long-lasting μ-selective opioid agonist, producing antinociception in both the acetic acid-induced writhing and warm water tail-withdrawal assays, as well as inducing the Straub tail response in mice. These data are the first to illustrate in vivo functional interactions between a steroidal structure and the μ-opioid receptor.

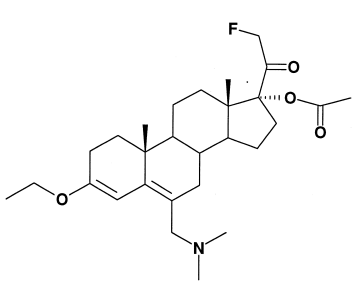

Fig. 1. Structure of SC17599.

2. Materials and methods

2.1. Materials

Methocinnamox was a kind gift from John Lewis, and has recently been described in detail (Lewis et al., 1988). Morphine sulfate was purchased from Mallinickrodt (St. Louis, MO). Naltrexone hydrochloride was a generous gift from the National Institute on Drug Abuse (Rockville, MD). Naltrindole and nor-binaltorphimine were kind gifts from K.C. Rice of the National Institute of Health (Bethesda, MD) and SC17599 was a generous gift from Searle (Skokie, IL). All drugs with the exception of SC17599 were dissolved in sterile water. SC17599, being water insoluble, was dissolved in a 20% solution of dimethyl sulfoxide (DMSO).

2.2. Subjects

Male NIH mice weighing 20–30 g were used. Subjects were housed in groups in a colony maintained at 20°C, 40–50% humidity with a 12 h light/dark cycle. Food and water were available ad libitum until the time of experiment. Each subject was tested only once and all experiments were performed between 0800 and 1800 h. Animals used in these studies were maintained in accordance with the University Committee on the Use and Care of Animals, University of Michigan, and Guidelines of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Health Council (Department of Health, Education and Welfare, Publication ISBN 0-309-05377-3, revised 1996).

2.3. Acetic acid-induced writhing assay

The acetic acid-induced writhing assay adapted from Koster et al. (1959) was used. Mice received s.c. injection of drug prior to i.p. injection of 0.6% acetic acid (0.4 ml per animal). After acetic acid injection, mice were placed in individual Plexiglass boxes (18 × 18 × 13 cm) for observation. Five minutes after injection of acetic acid, the number of writhes occurring during a 5-min period were recorded. For the purposes of this study, a writhe was defined as the extension of an animal's abdomen and hind legs. For each new batch of mice that arrived in the animal

colony, control values for writhing were determined and averaged across all batches (Zernig et al., 1995). Inhibition of writhing was expressed as percent writhes observed in control animals:

$$\%\,\text{Control Writhes} = \frac{(\#\,\text{writhes with sterile water} - \#\,\text{writhes with drug})}{(\#\,\text{writhes with sterile water})} \times 100$$

2.4. Warm water tail-withdrawal assay

The warm water tail-withdrawal assay adapted from Janssen et al. (1963) was used. Animals were placed in clear, well-ventilated plastic cylinders (Harvard Apparatus, South Natick, MA) with their tails protruding. The lower third of the tail was immersed into a 50°C water bath and the latency to tail-withdrawal was measured using a stop watch. Only mice displaying baseline latencies of less than 4 s were used. The cutoff latency was 20 s, and if the mouse did not remove its tail at this time the tail was manually removed from water and was scored as being fully antinociceptive. A cumulative dosing procedure as described previously was utilized (Zernig et al., 1996). Briefly, mice were injected i.p. at 30-min intervals for the cumulative dose–response experiments, and tail-withdrawal latencies were recorded 25 min after each injection. For the time-course studies, bolus injection of agonist was administered i.p., followed 30 min later by bolus injection of antagonist. Antinociception was recorded as percent maximum possible effect (%MPE):

$$\%\,\text{MPE} = \frac{(\text{test time} - \text{control time})}{(\text{cutoff time} - \text{control time})} \times 100$$

2.5. Locomotor activity assay

Mice were tested in Omnitech activity monitors kindly provided by E. F. Domino (University of Michigan). Each activity unit was composed of two rectangular clear Plexiglas chambers (46 × 24 × 18.5 cm), separated from each other by opaque white paper (Gwynn and Domino, 1984). A Plexiglas plate with ventilation holes covered each chamber. Each box contained a single set of sensors that monitor the animals activity via 16 infrared light beams spaced 2.5 cm apart and 2.4 cm above the cage floor. Individual mice were placed in each chamber for 1 h immediately prior to the initiation of the experiment to habituate mice to the novel environment. After 1 h, the animals received an i.p. injection of agonist and were immediately placed back into the activity chambers. Total locomotor activity was automatically recorded every 5 min for a total duration of 4 h and processed by a Digiscan Micro Analyzer.

2.6. Straub tail assay

The presence of a Straub tail was scored according to methods adopted from Aceto et al. (1969). Forty-five minutes after i.p. administration of agonist, mice were observed during a 5-min period. The Straub tail effect, defined as a tail curvature of 45° above the horizontal plane, was expressed as the percent of mice displaying a Straub tail.

2.7. Data analysis

All data are presented as means ± S.E.M. For the data obtained using the tail-withdrawal assay, ED_{50} values and 95% confidence limits were determined according to the method of Ko et al. (1999). A significant difference was described as a lack of overlap of values contained within the 95% confidence limits. For the data obtained using the acetic acid-induced writhing and locomotor activity assay, one- or two-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Statistica (v. 5.0: Statsoft, Tulsa, OK). Tukey Honest Significant Different tests ($P < 0.05$) were used to determine significance.

3. Results

3.1. Acetic acid-induced writhing assay

Antinociceptive effects of SC17599 and morphine were evaluated in the acetic acid-induced writhing assay. Injection of SC17599 vehicle (20% DMSO) did not significantly alter acetic acid-induced writhing ($P > 0.05$, data not shown). SC17599 dose-dependently and significantly suppressed writhing with a potency approximately 10-fold less than morphine (Fig. 2A and B). Pretreatment with the μ-opioid receptor-selective antagonist naltrexone (1 mg/kg, 15 min) significantly inhibited the antinociceptive effects of both SC17599 and morphine in the writhing assay. Similarly, 1 h pretreatment with the long-lasting μ-opioid receptor-selective antagonist methocinnamox blocked SC17599- and morphine-induced antinociception. In contrast, pretreatment with the δ-opioid receptor-selective antagonist naltrindole (10 mg/kg, 15 min) or the κ-opioid receptor-selective antagonist nor-binaltorphimine (32 mg/kg, 24 h) did not significantly affect SC17599-induced antinociception ($P > 0.05$, data not shown).

3.2. Warm water tail-withdrawal assay

Antinociceptive effects of SC17599 and morphine were also evaluated in the mouse warm (50°C) water tail-withdrawal assay (Fig. 3A and B). Injection of SC17599 vehicle (20% DMSO) did not significantly alter latency for tail removal from warm water ($P > 0.05$, data not shown). SC17599 and morphine dose-dependently and significantly

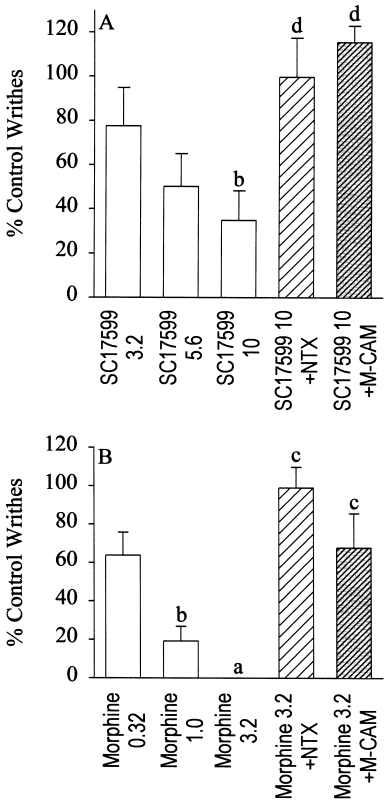

Fig. 2. Antinociceptive effects of s.c. administered SC17599 (A) and morphine (B), and their antagonism by naltrexone (NTX; 1.0 mg/kg, 15 min) and methocinnamox (M-CAM; 1.8 mg/kg, 1 h) in the mouse acetic acid induced writhing assay. Each value represents the mean ± S.E.M. ($n = 6–12$). Letters represent a significant difference (a, $P < 0.001$; b, $P < 0.01$) from control as well as significant difference from agonist treatment (c, $P < 0.001$; d, $P < 0.01$). Abscissae, agonist doses in mg/kg alone and in combination with antagonist doses in mg/kg. Ordinates, percent of control number of writhes per mouse.

produced antinociception in the tail-withdrawal assay. SC17599 was approximately twofold less potent than morphine, affording an ED_{50} value of 25.11 mg/kg (95%CI = 12.12–52.05 mg/kg) as compared to 10.48 mg/kg (95%CI = 8.91–12.32 mg/kg) for morphine. Pretreatment with naltrexone 1 and 10 mg/kg (15 min) produced 5- and 12-fold parallel rightward shifts in the dose–response curve for morphine, significantly increasing the ED_{50} for morphine to 52.16 mg/kg (CI = 30.27–89.89) and 127.28 mg/kg (CI = 64.15–252.54), respectively. Pretreatment with naltrexone 1 and 10 mg/kg (15 min) produced nonsignificant parallel rightward shifts in the SC17599 dose–response curve, increasing the ED_{50} for SC17599 approximately twofold and threefold, respectively, giving ED_{50} values for SC17599 of 54.00 (CI = 51.69–56.40) and 79.52 (CI = 40.97–154.34), respectively. However, pretreatment with naltrexone (1 and 10 mg/kg, 15 min) did produce a significant loss of antinociception seen at 32 mg/kg dose of SC17599 (Fig. 3A). Pretreatment with the irreversible μ-selective antagonist methocinnamox (1.8 mg/kg, 1 h), produced a significant downward shift in the dose response curves for both SC17599 and morphine, thereby almost completely inhibiting production of antinociception by these compounds (Fig. 3A and B).

The reduced effectiveness of naltrexone in antagonizing SC17599-induced antinociception was investigated by studying the effect of the SC17599 vehicle, namely 20% DMSO (Fig. 4). Dissolving morphine in the SC17599 vehicle (20% DMSO) did not significantly affect the morphine dose–response curve, such that the ED_{50} for morphine dissolved in 20% DMSO was 10.05 mg/kg (CI = 5.91–17.12). However, the magnitude of shift of the morphine dose–response curve by naltrexone was significantly reduced when morphine was dissolved in 20% DMSO. Naltrexone (1 mg/kg, 15 min) pretreatment produced a fivefold increase in the ED_{50} value for morphine dissolved in sterile water, but only a twofold increase in ED_{50} of morphine dissolved in 20% DMSO. Thus, naltrexone nonsignificantly increased the ED_{50} for morphine dissolved in 20% DMSO from a control value of 10.05 mg/kg (CI = 5.91–17.12) to 23.83 mg/kg (CI = 15.09–37.64).

The antinociceptive time course of SC17599 relative to morphine was evaluated in the mouse warm water tail-withdrawal assay using equivalent antinociceptive doses (Fig. 5A and B). Similarly to morphine, significant

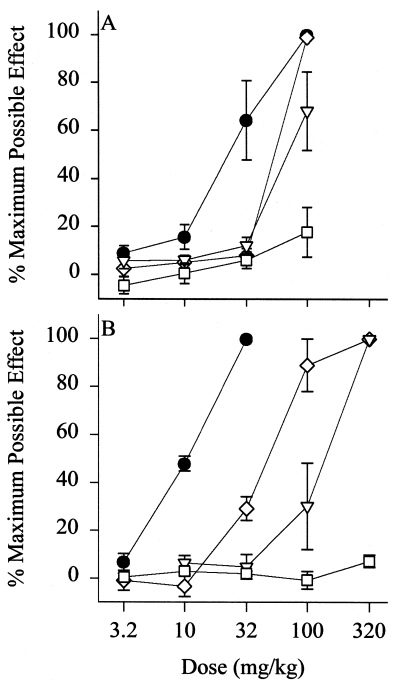

Fig. 3. Antinociceptive effects of cumulative doses of i.p. SC17599 and morphine, and antagonism by naltrexone (1 and 10 mg/kg, 15 min) and methocinnamox (1.8 mg/kg, 1 h) in the mouse warm (50°C) water tail-withdrawal assay. (A) Mice treated with (●) SC17599, (◇) SC17599 + naltrexone (1 mg/kg), (▽) SC17599 + naltrexone (10 mg/kg), or (□) SC1799 + methocinnamox (1.8 mg/kg). (B) Mice treated with (●) morphine, (◇) morphine + naltrexone (1 mg/kg), (▽) morphine + naltrexone (10 mg/kg), or (□) morphine + methocinnamox (1.8 mg/kg). Each value represents the mean ± S.E.M. ($n = 5$). Abscissae, agonist doses in mg/kg. Ordinates, percent of maximum possible effect (%MPE).

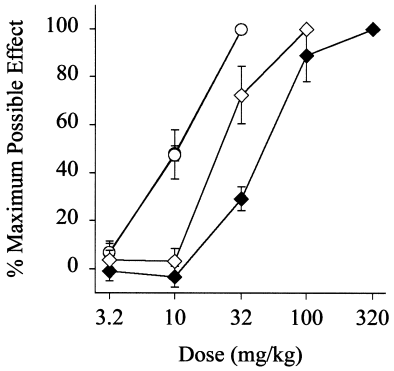

Fig. 4. Antagonism of antinociceptive effects of i.p. morphine dissolved in water and morphine dissolved in 20% DMSO by naltrexone (1 mg/kg, 15 min) in the mouse warm (50°C) water tail-withdrawal assay. Mice treated with (●) morphine, (♦) morphine + naltrexone (1 mg/kg), (○) morphine dissolved in 20% DMSO, or (◇) morphine dissolved in 20% DMSO + naltrexone (1 mg/kg). The dose–response curves for morphine dissolved in water and morphine dissolved in 20% DMSO are identical. Other details are as in Fig. 3.

antinociceptive effects of SC17599 were apparent within 15 min after administration. However, as compared to morphine, SC17599 displayed a significantly longer duration of action. Continued antinociceptive effects of SC17599 were observed 240 min after administration, whereas antinociceptive effects of morphine were no longer apparent 180 min after administration. The ability of naltrexone to reverse the antinociceptive effects of SC17599 and morphine was evaluated. Thirty min after administration of SC17599 or morphine, naltrexone (1 mg/kg) was administered and 15 min after administration of naltrexone, the antinociceptive effects of both SC17599 and morphine were fully reversed.

3.3. Locomotor activity assay

The effects of SC17599 and morphine on locomotor activity were determined (Fig. 6A and B). Placement of mice in the novel environment of the locomotor activity chambers produced an increase in total locomotor activity, which significantly decreased during the first h. Thus, mice were allowed to habituate to this environment for one h, after which time the effects of SC17599 and morphine on locomotor activity were evaluated. Injection of sterile water or SC17599 vehicle (20% DMSO) did not significantly alter locomotor activity in mice ($P > 0.05$). Morphine dose-dependently and significantly increased locomotor activity in mice. Significant increases in locomotor activity were observed approximately 45 min after injection of morphine (32 and 100 mg/kg). Enhanced locomo-

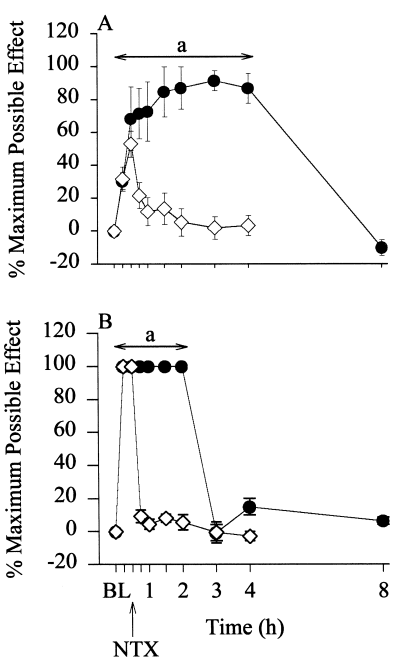

Fig. 5. Time course of antinociceptive effects of i.p. morphine (32 mg/kg) and i.p. SC17599 (32 mg/kg) alone and reversal of these effects with naltrexone (1 mg/kg) injected 30 min after agonist administration. Arrow indicates time of naltrexone (NTX) injection. (A) Mice treated with (●) SC17599 or (◇) SC17599 followed by naltrexone (1 mg/kg). (B) Mice treated with (●) morphine or (◇) morphine followed by naltrexone (1 mg/kg). Letters represent a significant difference (a, $P < 0.001$) from control. Abscissae, time in min. Ordinates, percent of maximum possible effect (%MPE). Other details are as in Fig. 3.

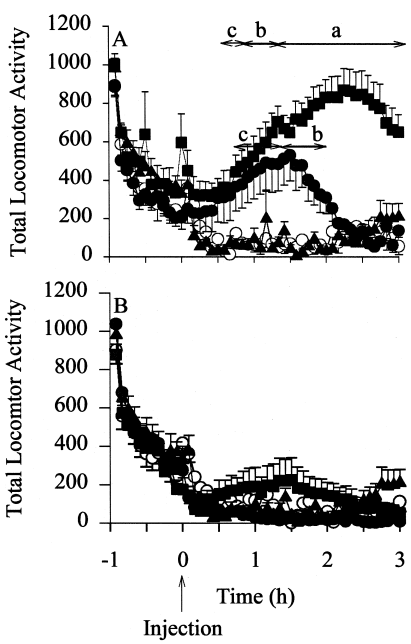

Fig. 6. Time course of the locomotor effects of i.p. morphine (10, 32, and 100 mg/kg) and SC17599 (10, 32 and 100 mg/kg). (A) Mice treated with (○) 20% DMSO, (△) SC17599 (10 mg/kg), (●) SC17599 (32 mg/kg) or (■) SC17599 (100 mg/kg). (B) Mice treated with (○) sterile water, (△) morphine (10 mg/kg), (●) morphine (32 mg/kg) or (■) morphine (100 mg/kg). Arrow indicates time of agonist injection. Each value represents the mean ± S.E.M. ($n = 8$). Letters represent a significant difference (a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$) from control. Abscissae, time in minutes. Ordinates, total locomotor activity counts.

tor activity in response to morphine persisted for about 2 h at a dose of 32 mg/kg and at least 3 h at a dose of 100 mg/kg. SC17599 up to a dose of 100 mg/kg did not significantly alter locomotor activity ($P > 0.05$). Effects of higher doses of SC17599 on locomotor activity could not be investigated due to solubility limitations. Using 20% DMSO as a vehicle for morphine did not significantly affect the ability of morphine to increase locomotor activity in mice ($P > 0.05$, data not shown).

3.4. Straub tail assay

The effectiveness of SC17599 in producing the Straub tail response was compared to morphine (Fig. 7A and B). Administration of SC17599 and morphine dose-dependently and significantly produced the Straub tail response in mice. Morphine (100 mg/kg) produced the Straub tail response in all of the mice studied. On the other hand, SC17599 (100 mg/kg) produced the Straub tail reaction in only 40% of the mice. Higher doses of SC17599 could not be investigated due to solubility limitations. Pretreatment with naltrexone (1 mg/kg, 15 min) completely antagonized both SC17599- and morphine-induced Straub tail reaction. Using 20% DMSO as a vehicle did not alter the ability of morphine to produce the Straub tail reaction (data not shown).

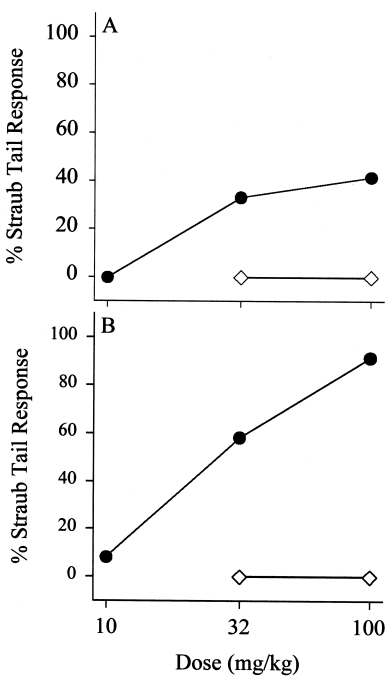

Fig. 7. Percent of mice displaying the Straub tail response following injection of SC17599 and morphine alone and in the presence of naltrexone (1 mg/kg). (A) Mice treated with (●) SC17599 or (◇) SC17599 + naltrexone (1 mg/kg). (B) Mice treated with (●) morphine or (◇) morphine + naltrexone (1 mg/kg). Each value represents the percent of mice displaying the Straub tail response ($n = 6$). Abscissae, dose of agonist in mg/kg. Ordinates, percent Straub tail response.

4. Discussion

SC17599 and morphine were fully effective in the acetic acid-induced writhing assay, each producing a dose-dependent suppression of the writhing response. In the writhing assay, morphine was approximately 10-fold more potent than SC17599, which is in agreement with a previous report that morphine was 8.4-fold more potent than SC17599 in the same assay (Craig, 1968). Pretreatment with the μ-opioid receptor-selective antagonist naltrexone completely blocked both SC17599- and morphine-induced antinociception. Similarly, pretreatment with the irreversible μ-opioid receptor-selective antagonist methocinnamox completely antagonized the antinociception produced by SC17599. On the other hand, neither the δ-opioid receptor-selective antagonist naltrindole nor the κ-opioid receptor-selective antagonist nor-binaltorphimine had any effect on SC17599-induced antinociception. SC17599 and morphine were also fully effective in producing antinociception in the mouse warm water tail-withdrawal assay. In the tail-withdrawal assay, SC17599 was 2.4-fold less potent than morphine, in agreement with the previous report that morphine was approximately four-fold more potent than SC17599 in the rat tail-flick assay (Craig, 1968). Previously, it was reported that in contrast to morphine, pretreatment with the partial agonist nalorphine did not antagonize the antinociceptive activity of SC17599 in the rat tail-flick assay (Craig, 1968). Similarly, in the present study, pretreatment with the opioid antagonist naltrexone resulted in a significant parallel rightward shift in the dose–response curve for morphine at doses which did not cause a significant shift in the dose–effect curve for SC17599. However, pretreatment with methocinnamox resulted in insurmountable antagonism of both SC17599- and morphine-induced antinociception. These findings provide strong evidence that SC17599-induced antinociception in both the mouse writhing and tail-withdrawal assays is selectively mediated by μ-opioid receptors.

Pretreatment with naltrexone (1 mg/kg, 15 min) increased the ED_{50} of morphine fivefold, whereas the ED_{50} for SC17599 was increased only twofold. This discrepancy was further investigated by studying the effect of the SC17599 vehicle, namely 20% DMSO. Dissolving morphine in 20% DMSO did not alter the potency of this opioid in producing antinociception. However, naltrexone was considerably less effective in antagonizing morphine dissolved in 20% DMSO as compared to morphine dissolved in sterile water. Indeed, naltrexone pretreatment resulted in a fivefold increase in the ED_{50} for morphine when water was used as a vehicle, but only a twofold increase in the ED_{50} for morphine when it was dissolved in 20% DMSO. Thus, using 20% DMSO as a vehicle for the agonist reduced the effectiveness of naltrexone. These findings are supported by previous reports that concurrent use of DMSO alters the analgesic and central nervous

system effects of other drugs, likely by altering the permeability of the blood-brain barrier to these drugs (Scheld, 1989; Mori et al., 1992; Dajani et al, 1999). Another potentially contributing factor may be the method of administration of the agonists and the antagonist. In the writhing assay, in which naltrexone, morphine and SC17599 are all administered s.c., naltrexone is equally effective in antagonizing SC17599 and morphine. However, in the tail-withdrawal assay, wherein naltrexone, morphine and SC17599 are all injected i.p., naltrexone is less effective in antagonizing SC17599 as compared to morphine. Thus, the mode of administration of SC17599, as well as the vehicle used to dissolve this steroid, may have affected the magnitude of antagonism of this compound by naltrexone. This may explain why Craig (1968) was unable to antagonize the antinociceptive effects of SC17599 with nalorphine.

While SC17599 was less potent than morphine in the tail-withdrawal assay, it displayed a similar onset of action (15 min) as compared with morphine. Furthermore, SC17599 displayed a much longer duration of action (4 h) as compared to morphine (2 h). Previously, it has been reported that some opioids with long durations of action, such as buprenorphine, may interact pseudo-irreversibly with opioid receptors (France et al., 1984). However, similarly to morphine, the antinociceptive effect of SC17599 was completely reversed upon administration of naltrexone (1 mg/kg). These findings indicate that the long duration of action of SC17599 is not due to long-lasting binding to the μ-opioid receptor, but more likely due to continued presence of this steroid in CNS areas responsible for μ-opioid receptor-mediated antinociception.

Since SC17599 produced antinociception through a μ-opioid receptor mechanism, it was of interest to determine if this steroid produces other behavioral effects typically associated with μ-opioid receptor agonists, such increased locomotor activity and the Straub tail response (Aceto et al., 1969; Rethy et al., 1971). Morphine (32 mg/kg) at a dose that produced complete antinociception in the tail-withdrawal assay resulted in a significant but relatively brief increase in locomotor activity and produced the Straub tail response in 60% of the mice. A higher dose of morphine (100 mg/kg) resulted in a larger and more prolonged locomotor response and produced the Straub tail response in 100% of the mice. A dose of SC17599 (100 mg/kg) that produced complete antinociception in the tail-withdrawal assay did not produce an increase in locomotor activity and afforded the Straub tail reaction in only 40% of the mice. This finding is in agreement with a previous report that SC17599 afforded the Straub tail phenomenon in mice at doses of 40 mg/kg and above (Craig, 1968). Higher doses of SC17599 may be more effective in increasing locomotor activity or producing the Straub tail response; however, due to solubility limitations, these potential effects could not be further investigated. Nevertheless, there does appear to be a difference in the ratio of doses necessary to produce antinociception, increase locomotion, and afford the Straub tail response between morphine and SC17599.

A recent study in our laboratory has provided in vitro data in support of a μ-opioid receptor-mediated mechanism for the actions of SC17599 (Houshyar et al., 1998). SC17599 was shown to bind with good affinity to μ-opioid receptors, but with much lower affinity to δ- and κ-opioid receptors. In addition, in membranes from SH-SY5Y cells transfected with μ-opioid receptors, SC17599 was fully efficacious in stimulating [^{35}S]GTPγS binding in a naloxone-reversible manner. The in vivo data presented in this paper and the in vitro data described previously provide conclusive evidence for interactions between the steroid structure SC17599 and μ-opioid receptors. These findings are novel, considering previous studies, which have shown that endogenous steroids are unlikely to interact with opioid receptors (Schwarz and Pohl, 1994), although a related steroid does have some affinity for [^{3}H]naloxone labeled receptors (LaBella et al., 1978). The presence of a tertiary nitrogen center and the planar system of conjugated bonds in the structure of SC17599 may be sufficient for interaction of this steroid with μ-opioid receptors.

In summary, the fast onset of action of SC17599 precludes a genomic mechanism of action for this steroid (Schumacher, 1990; McEwen, 1991; Brann et al., 1995). It is well established that steroids are capable of producing rapid behavioral effects through nongenomic mechanisms (Majewska, 1992; Lambert et al., 1995; Morrow, et al., 1995; Rupprecht, 1997). The findings of the present study provide the first evidence illustrating behavioral effects of a steroid mediated by opioid receptors. The present study demonstrates that the steroid SC17599 acts as a highly selective agonist at μ-opioid receptors, producing antinociception in the mouse writhing and tail-withdrawal assays, as well as resulting in the Straub tail reaction. SC17599 is the first compound with a steroidal structure to interact selectively with μ-opioid receptors, thereby displaying an in vivo profile similar to, but not identical with, the prototypical opioid morphine.

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