Central muscarinic antinociception induced by ET-142 and SS-20 in rodents

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Research Article

Central Muscarinic Antinociception Induced by ET-142 and SS-20 in Rodents

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

The antinociceptive effects of ET-142 (10–50 mg kg^{-1} sc; 10–30 μg per mouse icv) and SS-20 (10–50 mg kg^{-1} sc; 5–30 μg per mouse icv) were examined in mice by using the hot-plate and abdominal constriction tests. A similar antinociceptive profile for both compounds (20–40 mg kg^{-1} ip) was also observed in rats using the paw pressure test. In the antinociceptive dose-range, ET-142 and SS-20 did not impair mouse gross behavior and motor coordination evaluated, respectively, by the Irwin and rotarod tests. The increase in the pain threshold produced by ET-142 and SS-20 was prevented by atropine, dicyclomine, pirenzepine, and hemicholinium-3, but not by naloxone, atropine methyl bromide, and CGP 35348. In vitro experiments showed that the two investigated compounds amplified electrically evoked guinea pig ileum contractions. On the basis of the above data, it can be postulated that ET-142 and SS-20 exert their antinociceptive effect through a potentiation of central cholinergic transmission. Drug Dev. Res. 42:26–34, 1997.

Key words: analgesia; antinociception; cholinergic system

INTRODUCTION

It has long been known that acetylcholine (ACh) [Pedigo et al., 1975], selective M_1 agonists such as McN-A-343 and AF-102B [Bartolini et al., 1992], nonselective muscarinic agonists like tremorine [Lenke, 1958], oxotremorine [George et al., 1962; Bartolini et al., 1987], arecoline [Herz, 1962], pilocarpine [Hendershot and Forsaith, 1959], and cholinesterase inhibitors such as physostigmine [Harris et al., 1969; Ireson, 1970] and diisopropyl fluorophosphate [Lentz et al., 1969] induce antinociception in laboratory animals by activating the cholinergic system. It has also been reported that the antimuscarinic drug atropine, at very low doses, was able to induce a central cholinergic antinociception in laboratory animals regardless of the route of administration and the noxious stimulus applied [Ghelardini et al., 1990]. Interestingly, the typical cholinergic symptomatology did not accompany this antinociceptive activity. The atropine-induced increase in the pain threshold was attributable to the R-(+)-enantiomer of atropine, R-(+)-hyoscyamine, since S-(–)-hyoscyamine was ineffective in all antinociceptive tests used [Ghelardini et al., 1992]. Investigating the antinociceptive effect of atropine using microdialysis techniques, it was demonstrated that R-(+)-hyoscyamine, at effective doses, produced an increase in ACh release from rat cerebral cortex in vivo [Ghelardini et al., 1997].

In order to obtain new cholinergic modulators, the compounds labeled ET-142 (2-(4-chlorophenoxy)-propionic acid, endo 8-methyl-8-azabicyclo[3.2.1]oct-3-

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yl ester) and SS-20 (2-[(4-chlorophenyl)thio]propionic acid, endo 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester) (Fig. 1), structurally related to atropine, have been synthesized [Gualtieri et al., 1994] and their potential antinociceptive properties were investigated in mice and rats.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (23–30 g) and rats (200–300 g) from Morini (San Polo d'Enza, Italy) and guinea pigs (150–200 g) from Rodentia (Bergamo, Italy) breeding farms were used. Fifteen mice, four rats, and four guinea pigs were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were kept at 23 ± 1°C with a 12 h light/dark cycle, light at 7 AM, with food and water ad libitum. All experiments were carried out according to the guidelines of the European Community Council.

Hot Plate Test

The method adopted has been described by O'Callaghan and Holtzman [1975]. Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1°C in a precision water-bath from KW Mechanical Workshop (Siena, Italy). Reaction times (sec), were measured with a stop-watch before and at regular intervals up to a maximum of 45 min after treatment. The endpoint used was the licking of the fore or hind paws. Mice with a licking latency below 12 and over 18 sec in the pretest were rejected (30%). An arbitrary cutoff time of 45 sec was adopted.

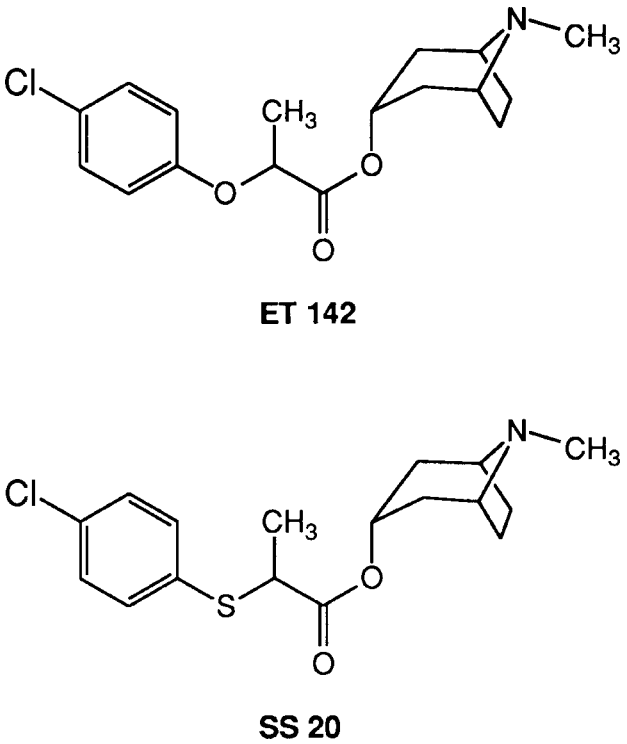

Fig.1. Chemical structure of ET-142 (2-(4-chlorophenoxy)propionic acid, endo 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester) and SS-20 (2-[4-chlorophenylthio]propionic acid, endo 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester).

Abdominal Constriction Test

Mice were injected ip with a 0.6% solution of acetic acid (10 ml kg^{-1}), according to Koster et al. [1959]. The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Paw Pressure Test

The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton et al. [1988]. Threshold pressure was measured before treatment and 15, 30, and 45 min after treatment. Rats scoring below 30 g or over 85 g in the test prior to drug administration were rejected (25%). An arbitrary cutoff value of 250 g was adopted.

Rotarod Test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-slippery surface. This rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of endurance time of the animals on the rotating rod, expressed in seconds, according to Kuribara et al. [1977]. One day before the test the animals were trained twice. On the day of the test only the mice that were able to stay balanced on the rotating rod between 70 and 120 sec (cutoff time) were selected for testing. The performance time was measured before and at various times after treatment.

Irwin Test

The test was performed according to the method described by Irwin [1966].

Spontaneous Activity Meter (Animex)

Locomotor activity in mice was quantified using an Animex activity meter Type S (LKB, Farad, Sweden) set to maximum sensitivity. Every movement of mice, which were placed on the top of the Animex activity meter, produced a signal due to variation in inductance and capacity of the apparatus resonance circuit. Signals were then automatically converted to numbers. On the day of the experiment the mice were treated and the cage, contain-

ing five mice, was put on the measuring platform. Activity counts were made every 15 min for 45 min starting immediately after injection of the drug. Because of the arbitrary scale adopted to quantify movements, drug-treated mice were always compared with saline-treated ones.

Isolated Guinea Pig Ileum

The myenteric plexus longitudinal muscle was prepared according to Paton and Vizi [1969]. The strip was suspended in a 12.5 thermoregulated bath (36–37°C) and, after stabilization, the strip was stimulated electrically (0.1 Hz, 0.5 ms; double threshold voltage). The Krebs-Henseleit solution, bubbled with 95% O_2 and CO_2, had the following composition (mM): NaCl 118.0, KCl 4.7, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 11.0.

Acetylcholinesterase Activity

Acetylcholinesterase (AChE) activity was assayed according to Ellman et al. (1961), using 0.5 mM acetylthiocholine iodide as substrate. The inhibitory effects of ET-142 and SS-20 were tested at various concentrations on a purified preparation of AChE from the electric eel.

Intracerebroventricular Injection Technique

Intracerebroventricular (icv) administration was performed under ether anesthesia using isotonic saline as solvent, according to the method described by Haley and McCormick [1957]. Briefly, during anesthesia mice were grasped firmly by the loose skin behind the head. A hypodermic needle of 0.4 mm external diameter attached to a 10 μl syringe was inserted perpendicularly through the skull at a depth of no more than 2 mm into the brain of the mouse, where 5 μl was then administered. The injection site was 1.5 mm from either side of the midline on a line drawn through to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice were icv injected with 5 μl of diluted 1:10 Indian ink and their brains examined macroscopically after sectioning.

Reagents and Drugs

The following drugs were used: ET-142 and SS-20 were prepared according to Gualtieri et al. [1994]; hemicholinium-3 hydrobromide (HC-3), pirenzepine dihydrochloride, naloxone hydrochloride (RBI, Natick, MA); morphine hydrochloride (U.S.L. 10/D, Florence); dicyclomine dihydrochloride (Lepetit); clomipramine (Anafranil), CGP 35348 (Novartis, Summit, NJ); oxotremorine (Fluka, Buchs, Switzerland); atropine sulphate, atropine methylbromide, physostigmine emisulphate (Sigma, St. Louis, MO); ketorolac trometamine (Lixidol; Farmitalia Carlo Erba); diphenhydramine hydrochloride (De Angeli). Other chemicals were of the highest quality commercially available. All drugs were dissolved in isotonic (NaCl 0.9%) saline solution. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml kg^{-1} by intraperitoneal (ip) and subcutaneous (sc) route.

Statistical Analysis

Results are given as the mean ± S.E.M.; analysis of variance (ANOVA), followed by Fisher's PLSD procedure for post hoc comparison, was used to verify the significance between two means. P values of less than 0.05

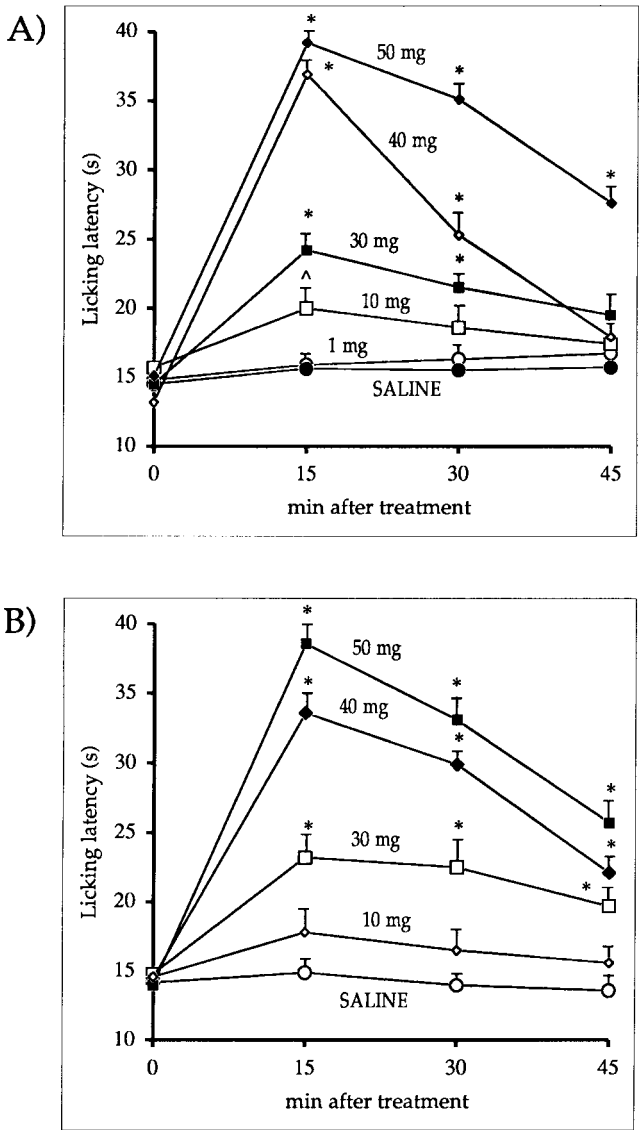

Fig. 2. Dose response curves of ET-142 (**A**) and SS-20 (**B**) administered sc in the mouse hot-plate test. The doses are expressed as mg kg^{-1} sc. Vertical lines show s.e.m. $^{\wedge}P < 0.05$; $^{*}P < 0.01$ in comparison with saline controls. Each point represents the mean of at least 10 mice.

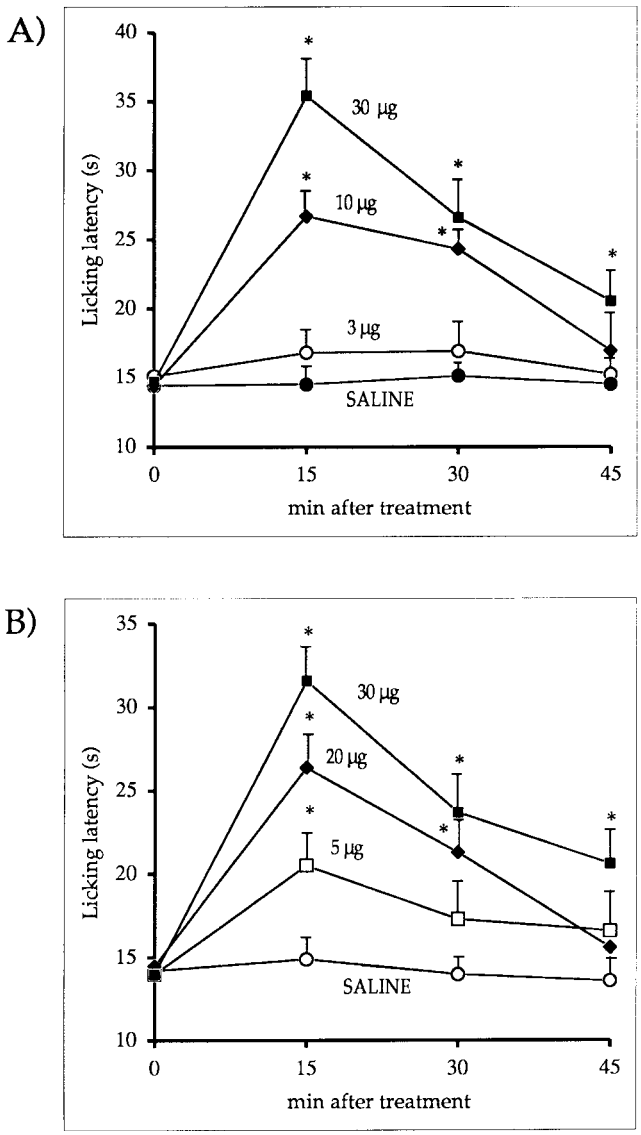

Fig. 3. Dose response curves of ET-142 (**A**) and SS-20 (**B**) administered icv in the mouse hot-plate test. The doses are expressed as µg per mouse icv. Vertical lines show s.e.m. $*P < 0.01$ in comparison with saline controls. Each point represents the mean of at least 15 mice.

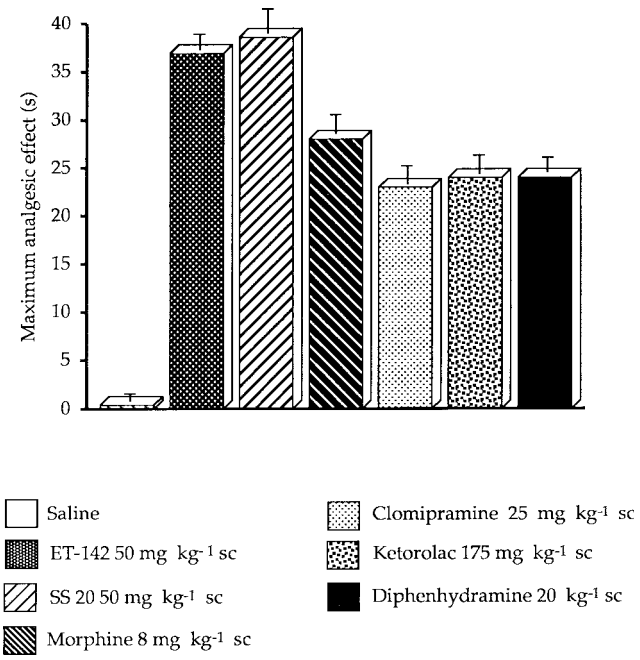

Fig. 4. Maximum antinociceptive effect of both ET-142 and SS-20 in comparison with morphine, clomipramine, ketorolac, and diphenhydramine evaluated in mouse hot-plate test. The nociceptive responses were recorded 15 min after administration of clomipramine, ketorolac, and diphenhydramine and 30 min after morphine injection. Each column represents the mean of at least 10 mice. Vertical lines show s.e.m.

were considered significant. Data were analyzed with StatView for the Macintosh computer program (1992).

RESULTS

Antinociceptive Activity of ET-142 and SS-20

ET-142 (10–50 mg kg^{-1} sc; Fig. 2, panel A) and SS-20 (30–50 mg kg^{-1} sc; Fig. 2, panel B) produced a dose-dependent increase in the pain threshold in the mouse hot-plate test. A similar antinociceptive profile was obtained after icv of ET-142 (10–30 µg per mouse; Fig. 3, panel A) and SS-20 (5–30 µg per mouse; Fig. 3, panel B) administration. The antinociceptive effect of both compounds peaked 15 min after injection and then slowly diminished. The maximum antinociceptive effects of ET-142 and SS-20 were greater than that produced by morphine (8 mg kg^{-1} sc), clomipramine (25 mg kg^{-1} sc), ketorolac (175 mg kg^{-1} sc), and diphenhydramine (20 mg kg^{-1} sc), used as reference drugs (Fig. 4). Figure 5 illustrates the analgesic effect of ET-142 and SS-20 in the mouse acetic acid abdominal constriction test where they increased the pain threshold at the dose of 10 and 20 mg kg^{-1} sc.

ET-142 and SS-20 were able to produce an increase in the pain threshold not only in mice but also in rats. In the paw pressure test, ET-142 and SS-20 administered ip at the dose of 20–40 mg kg^{-1} reached a maximum antinociception 15 min after injection and then slowly diminished (Table 1).

Antagonism of ET-142 and SS-20-Induced Antinociception

In the mouse hot-plate test, the antinociceptive effects of ET-142 (40 mg kg^{-1} sc) and SS-20 (40 mg kg^{-1} sc) were not antagonized by the opiate antagonist naloxone (1 mg kg^{-1} ip), the peripherally acute cholinergic antagonist atropine methylbromide (5.5 mg kg^{-1} ip) or the $GABA_B$ antagonist CGP-35348 (100 mg kg^{-1} ip) (Table 2). Conversely, atropine (5 mg kg^{-1} ip), dicyclomine (10 mg kg^{-1} sc),

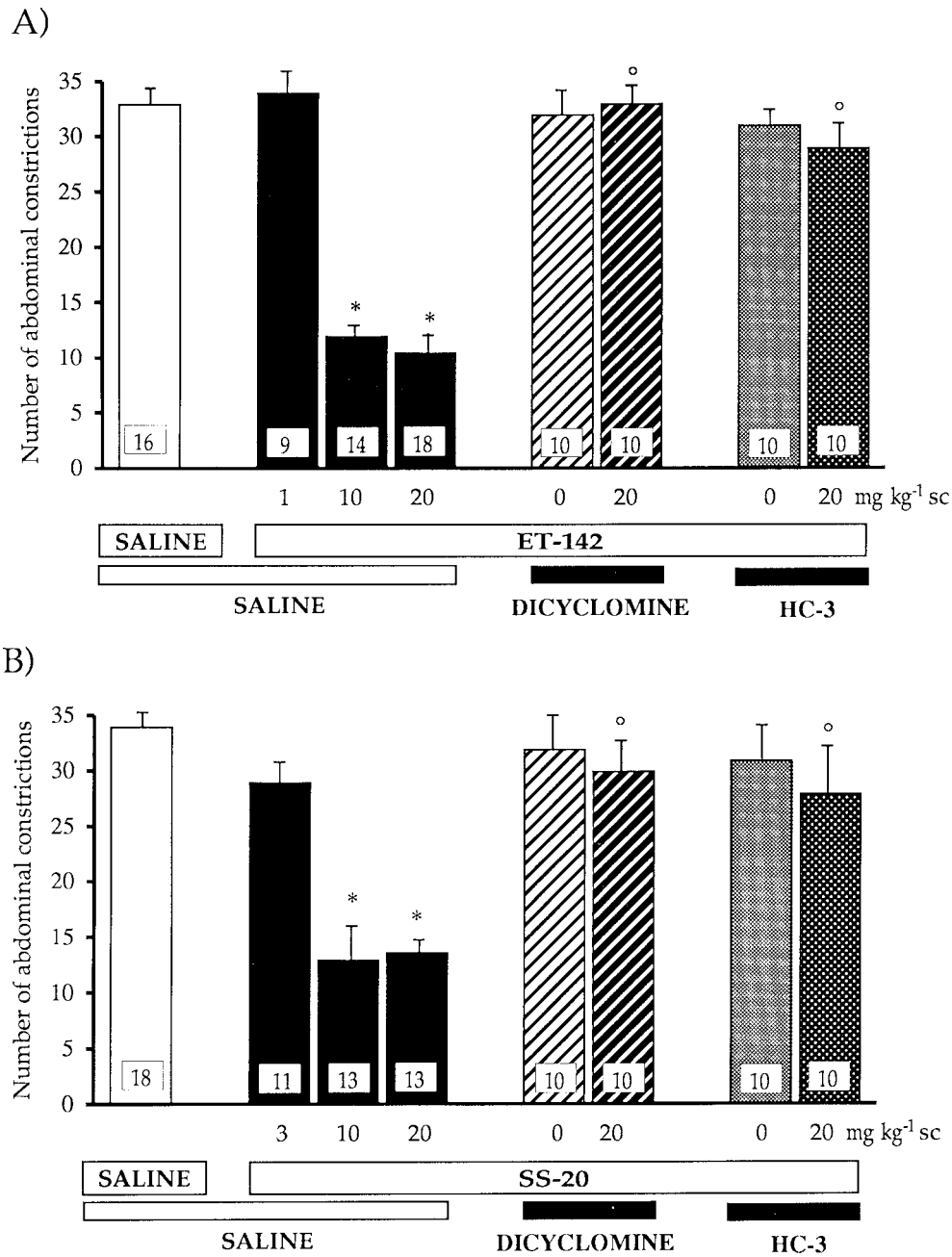

Fig. 5. Dose-response curves of both ET-142 (**A**) and SS-20 (**B**) administered sc and antagonism exerted by dicyclomine (10 mg kg^{-1} i.p.) and hemicholium-3 (1 μg per mouse i.c.v.) on antinociception induced by both compounds administered at the dose of 20 mg kg^{-1} ip in the mouse abdominal constriction test induced by acetic 0.6% acid. The nociceptive responses were recorded 15 min after ET-142 and SS-20 administration. Dicyclomine and hemicholinium-3 were injected 5 min and 5 h respectively before ET-142 and SS-20. Vertical lines show s.e.m. $*P < 0.01$ in comparison with saline controls. $°P < 0.01$ in comparison with ET-142 or SS-20. Numbers inside the columns indicate the number of mice.

pirenzepine (0.1 μg per mouse icv), and hemicholinium-3 (1 μg per mouse icv) were able to completely prevent ET-142 and SS-20 antinociception in the mouse hot-plate and abdominal constriction tests (Table 2, Fig. 5). All antagonists were injected 15 min before ET-142 and SS-20, with the exception of CGP 35348 and HC-3 which were injected 5 min and 5 h respectively before the two compounds.

Evaluation of the ET-142 and SS-20 Effect on Gross Behavior and Motor Coordination

ET-142 and SS-20, unlike oxotremorine and physostigmine, increased the pain threshold without causing the typical cholinergic symptomatology (Table 3). Furthermore, both compounds elicited their antinociceptive effects without changing motor coordination, evaluated

TABLE 1. Antinociceptive Effect of ET-142 and SS-20 in the Rat Paw-Pressure Test

Treatment ip	Dose per kg^{-1}	Paw-pressure (g) Before treatment	After treatment 15 min	30 min	45 min
Saline		60.2 ± 3.8 (10)	63.7 ± 4.2 (10)	60.5 ± 3.4 (10)	64.2 ± 4.6 (10)
ET-142	20 mg	58.5 ± 3.8 (5)	90.6 ± 4.2** (5)	78.4 ± 4.4* (5)	64.8 ± 6.0 (5)
ET-142	40 mg	60.4 ± 4.0 (7)	134.6 ± 6.8** (7)	123.6 ± 4.5** (7)	84.6 ± 6.4* (7)
SS-20	20 mg	56.4 ± 3.9 (5)	93.4 ± 5.2** (5)	64.6 ± 5.0 (5)	59.5 ± 4.3 (5)
SS-20	40 mg	60.6 ± 4.2 (6)	121.6 ± 5.5** (6)	98.6 ± 5.2** (6)	74.2 ± 4.0 (6)

*$P < 0.05$; **$P < 0.01$ in comparison with controls. The number of rats is shown in parentheses.

by using the rotarod test (Table 3). The rotarod performance of mice treated with ET-142 and SS-20 at the dose of 50 mg kg^{-1} sc was not impaired in comparison with controls (Table 3). On the contrary, ET-142 and SS-20 administered at the dose of 60 mg kg^{-1} sc significantly impaired rotarod performance (Table 3).

In Vitro Functional Studies

As shown in Figure 6, ET-142 and SS-20 added to the organ bath at concentrations ranging from 10^{-12}–10^{-9} M potentiated the contractions evoked by electrical stimulation. The potentiation was no longer observed when the concentration of ET-142 and SS-20 in the me-

TABLE 2. Effects of Atropine, Pirenzepine, Hemicholinium-3 (HC-3), Atropine Methylbromide, Naloxone, and CGP-35348 on Antinociception Induced by ET-142 (40 mg/kg^{-1} sc) and SS-20 (40 mg kg^{-1} sc) in the Mouse Hot-plate Test

Pretreatment	Treatment	No.	Licking latency (sec) Before pretreatment	After treatment 15 min	30 min	45 min
Saline 10 ml kg^{-1} ip	Saline	36	14.4 ± 0.6	14.1 ± 1.1	13.7 ± 1.1	14.0 ± 0.3
Saline 5 µl icv	Saline	16	14.1 ± 0.9	15.2 ± 1.1	14.3 ± 0.8	14.3 ± 0.5
Saline	ET-142	20	14.2 ± 0.4	36.9 ± 2.4[1]	25.3 ± 2.1[1]	17.9 ± 0.8
ip or icv	SS-20	22	14.5 ± 0.8	33.6 ± 2.1[1]	29.8 ± 1.9[1]	22.1 ± 1.3[1]
Atropine	Saline	10	14.2 ± 0.8	13.8 ± 1.2	14.4 ± 1.6	14.8 ± 1.8
5 mg kg^{-1}	ET-142	10	14.3 ± 0.7	17.8 ± 2.5[3]	17.1 ± 2.1[3]	14.6 ± 1.7
ip	SS-20	10	13.9 ± 0.9	18.1 ± 2.2[3]	17.9 ± 1.8[3]	15.2 ± 2.2[3]
Pirenzepine	Saline	10	14.3 ± 1.0	15.1 ± 1.6	15.3 ± 1.9	14.5 ± 1.7
0.1 µg mouse	ET-142	12	14.0 ± 0.7	18.2 ± 2.5[3]	14.7 ± 2.0[3]	14.3 ± 1.4
icv	SS-20	11	13.6 ± 0.9	16.9 ± 2.1[3]	17.7 ± 1.4[3]	13.8 ± 1.8[3]
HC-3	Saline	10	15.1 ± 0.7	14.1 ± 1.5	15.2 ± 1.8	13.9 ± 1.7
1 µg mouse	ET-142	12	13.3 ± 1.1	17.6 ± 2.3[3]	17.5 ± 2.4[3]	14.6 ± 1.9
icv	SS-20	12	14.5 ± 0.8	16.8 ± 2.5[3]	18.3 ± 2.5[3]	16.7 ± 1.5[3]
Atropine CH_3Br	Saline	10	14.5 ± 0.8	13.6 ± 1.6	15.7 ± 1.3	14.2 ± 1.1
5.5 mg kg^{-1}	ET-142	11	13.5 ± 0.9	35.5 ± 2.2[1]	26.4 ± 1.9[1]	19.3 ± 1.9[2]
ip	SS-20	8	15.1 ± 0.7	33.9 ± 2.1[1]	25.2 ± 2.3[1]	18.9 ± 1.6
Naloxone	Saline	10	14.5 ± 0.8	13.5 ± 1.2	13.4 ± 1.3	14.6 ± 1.4
1 mg kg^{-1}	ET-142	9	13.8 ± 1.1	34.7 ± 1.8[1]	27.4 ± 26[1]	18.6 ± 2.1
ip	SS-20	10	14.5 ± 1.1	31.7 ± 2.4[1]	26.2 ± 1.8[1]	20.3 ± 1.7[2]
CGP 35348	Saline	10	13.0 ± 0.9	11.7 ± 1.4[2]	13.5 ± 1.6	14.2 ± 1.3
100 kg^{-1}	ET-142	10	13.9 ± 1.0	30.6 ± 2.0[1]	26.8 ± 2.1[1]	20.4 ± 1.7[2]
ip	SS-20	9	14.6 ± 1.1	32.1 ± 2.3[1]	23.7 ± 2.1[1]	19.7 ± 1.5[2]

[1]$P < 0.01$.
[2]$P < 0.05$ in comparison with saline-saline.
[3]$P < 0.01$ vs. saline-ET-142/SS-20-treated mice.

TABLE 3. Effect of ET-142 and SS-20 in Comparison With Oxotremorine and Physostigmine in: a) the Irwin test; b) Rotarod Test

a)	Tremors	Salivation	Lacrimation	Diarrhea	Abdominal tone	Spontaneous motility
Saline sc	0	0	0	0	4	4
ET-142 50 mg kg^{-1} sc	0	0	0	0	4	4
SS-20 50 mg kg^{-1} sc	0	0	0	0	4	4
Oxotremorine 0.1 mg kg^{-1} sc	4	4	+	+	0	2
Physostigmine 0.2 mg kg^{-1} sc	2	6	+	+	2	0
Tremors:	absent = 0		maximum score = 8			
Salivation:	absent = 0		maximum score = 8			
Lacrimation:	absent = 0		present +			
Diarrhea:	absent = 0		present +			
Abdominal tone:	flaccid abdomen = 0	normal = 4	abdomen board-like = 8			
Spontaneous motility:	absent = 0	normal = 4	maximum score = 8			

Each value represents the mean of five mice. Spontaneous motility was evaluated by Animex test.

b)			Endurance time on rotarod(s)			
	Dose mg kg^{-1} sc	N	Before treatment	After treatment 15 min	30 min	45 min
Saline		12	97.6 ± 5.6	99.6 ± 6.8	102.4 ± 5.2	96.8 ± 8.3
ET-142	50	10	97.7 ± 5.8	95.6 ± 5.8	90.6 ± 4.2	101.5 ± 7.6
ET-142	60	10	103.2 ± 7.2	81.32 ± 5.4*	85.1 ± 7.2*	97.4 ± 7.7
SS-20	50	12	94.6 ± 6.8	98.1 ± 7.3	91.7 ± 5.4	94.9 ± 5.1
SS-20	60	8	97.5 ± 7.8	84.3 ± 7.2*	97.6 ± 6.2	104.2 ± 5.5
Oxotremorine	0.1	11	106.2 ± 8.2	76.5 ± 7.3*	63.6 ± 9.6**	64.4 ± 8.7**
Physostigmine	0.2	9	93.4 ± 5.7	61.4 ± 6.8**	54.5 ± 8.1**	52.3 ± 8.8**

*$P < 0.05$.
**$P < 0.01$ in comparison with saline controls. The number of mice is shown in parentheses.

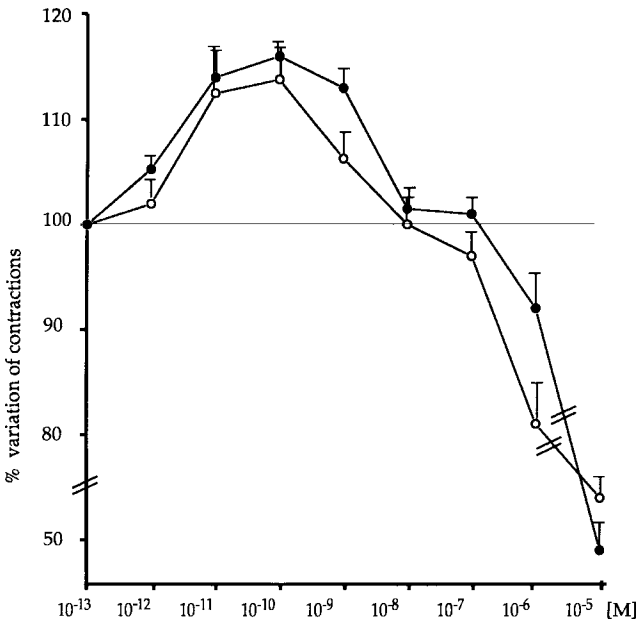

Figure 6.

Fig. 6. Dose-response curves of both ET-142 (closed symbols) and SS-20 (open symbols) on electrically (0.1 Hz; 0.5 ms; double-threshold voltage)-evoked contractions of guinea pig ileum myenteric plexus longitudinal muscle strip expressed as percentage variation of contractions. Each point represents the mean of at least four experiments and vertical lines give s.e.m.

dium was raised to 10^{-8} M. ET-142 and SS-20 began to inhibit both types of evoked contractions at 10^{-6} M.

Finally, ET-142 and SS-20 had a weak antiacetylcholinesterase activity, with an IC_{50} value on electrical eel acetylcholinesterase of 3×10^{-4} M (data not shown).

DISCUSSION

ET-142 and SS-20 were able to induce antinociception in mice regardless of which noxious stimulus was used: thermal (hot-plate test), chemical (abdominal constriction test), and mechanical (paw pressure). ET-142 and SS-20 antinociception was obtained without producing any visible modification in animal gross behavior.

Moreover, motor coordination on the rotarod test was completely intact in mice treated with ET-142 and SS-20. The analgesic effect of the two compounds was also compared with that induced by well-known analgesic drugs such as morphine, clomipramine, ketorolac, and diphenhydramine at the highest doses that did not impair rotarod performance. By comparing the maximum antinociceptive activity, the enhancement of the pain threshold produced by ET-142 and SS-20 was greater than all the reference drugs.

ET-142 and SS-20 exerted their antinociceptive effect by acting centrally. It was possible to enhance the pain threshold by injecting directly into the cerebral ventricles doses (30 μg per mouse) of ET-142 and SS-20 which were 1,000 times lower than those needed parenterally. Furthermore, a central cholinergic mechanism of action for ET-142 and SS-20 was hypothesized since their antinociception was prevented by the muscarinic antagonist, atropine, the selective M_1-antagonists, dicyclomine and pirenzepine, and the ACh depletor, HC-3. Taking into account that HC-3 and pirenzepine were able to antagonize ET-142 and SS-20 antinociception after icv injection and that atropine methylbromide administered ip did not prevent ET-142 and SS-20 enhancement of the pain threshold, this supports the hypothesis that the analgesic site of action of the investigated compounds is localized in the central nervous system (CNS). A presynaptic mechanism facilitating cholinergic transmission is potentially involved in ET-142 and SS-20 antinociception as revealed by the antagonism of HC-3. A postsynaptic mechanism of action can potentially be ruled out since, as reported by Bartolini et al. [1987, 1992], HC-3 was not able to antagonize antinociception induced by agonists of postsynaptic muscarinic receptors such as oxotremorine, McN-A-343, and AF-102B.

The in vitro experiments supported the hypothesis that ET-142 and SS-20 amplify cholinergic neurotransmission since, ranging from 10^{-12}–10^{-9} M, they increased presynaptically induced (electrical-evoked) contractions of longitudinal muscle of guinea pig ileum. ET-142 and SS-20 are also endowed with very low anticholinesterase activity, as demonstrated by the in vitro evaluation of their IC_{50} value ($IC_{50} = 3 \times 10^{-4}$ M). It is possible that ET-142 and SS-20 are able to amplify cholinergic neurotransmission through a presynaptic mechanism and that this effect is in turn potentiated by their low cholinesterase inhibitory activity. However, we cannot exclude that other mechanisms able to potentiate the endogenous cholinergic system may be involved in the antinociception induced by ET-142 and SS-20.

Opioid and GABAergic neurotransmitter systems are not involved in ET-142 and SS-20 antinociception since the opioid antagonist naloxone and the $GABA_B$ antagonist CGP-35348 were unable to prevent the effect of ET-142 and SS-20. The doses and administration schedules of the above-mentioned drugs were ideal for preventing antinociception induced by morphine [Ghelardini et al., 1992] and the $GABA_B$ agonist baclofen [Malcangio et al., 1991].

In conclusion, our results indicate that ET-142 and SS-20 are able to produce dose-dependent antinociception in mice and rats by potentiating endogenous cholinergic activity and without impairing motor coordination or spontaneous motility. Furthermore, both compounds show a very similar pharmacological profile, indicating that the substitution of a heteroatom (oxygen with sulphur) does not modify the antinociceptive activity of ET-142 and SS-20.

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