# 3-ALFA-TROPANYL 2-(4-Cl-PHENOXY)BUTIRATE (SM21): A REVIEW OF THE PHARMACOLOGICAL PROFILE OF A NOVEL ENHANCER OF

*CNS Drug Reviews*
Vol. 3, No. 4, pp. 346–362


# 3-α-tropanyl 2-(4-Cl-phenoxy)butyrate (SM 21): A Review of the Pharmacological Profile of a Novel Enhancer of Cholinergic Transmission

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**Key Words:** Acetylcholine release—Analgesia—Antinociception—Cholinergic system—Learning and memory—Pain—SM 21.

## INTRODUCTION

Atropine-like preparations were used by ancient Romans to relieve pain; Pliny the elder, in his *Historia Naturalis*, reported that the juice of *Mandragora officinarum* or *Hyosciamus niger* was administered to patients before surgery to produce analgesia. Much more recently Ghelardini et al. (31) confirmed the paradoxical effect of atropine by reporting that, at very low doses, this compound induces central antinociception in rodents through an enhancement of cholinergic transmission. It is interesting to note that this antinociceptive activity, unlike that produced by direct muscarinic agonists and cholinesterase inhibitors, was not accompanied by typical cholinergic symptomatology symptoms (tremors, sialorrhea, diarrhea, rhinorrhea, lacrimation, etc.). Soon after, it was discovered that the R-(+)-enantiomer of atropine, R-(+)-hyoscyamine, was responsible for the antinociceptive activity of the racemate, while the S-(–)-enantiomer, S-(–)-hyoscyamine, was devoid of any antinociceptive action (33). R-(+)-hyoscyamine, in the same range of analgesic doses, was also able to prevent amnesia induced by antimuscarinic drugs (41). An investigation of the antinociceptive and antiamnesic effect of atropine, using microdialysis techniques has demonstrate that R-(+)- hyoscyamine, at cholinomimetic doses, produced an increase in the acetylcholine (ACh) release from the rat cerebral cortex *in vivo*, indicating that this compound has a presynaptic mechanism of action (41).

Based on these observations, a program to modify the chemical structure of atropine was started, aimed at developing cholinergic amplifiers endowed with more in-

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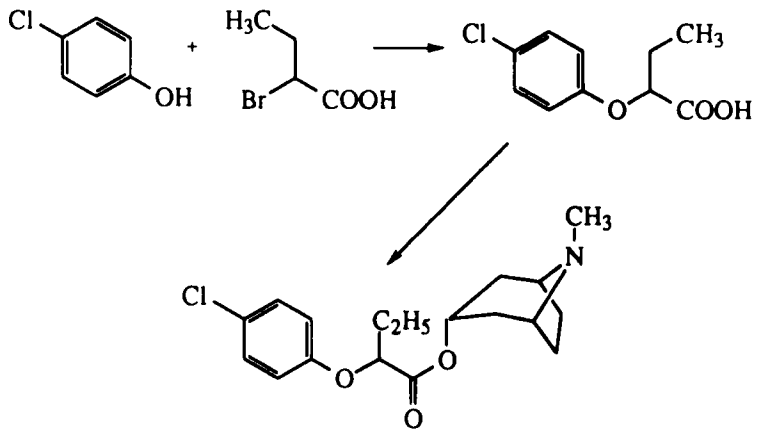


SM-21

**Fig. 1.** Chemical structure and synthesis of SM 21.

tensive antinociceptive and antiamnesic activity than atropine, but with the same lack of cholinergic side effects as atropine. These compounds would be potentially useful as analgesics and/or in the treatment of pathological conditions, such as Alzheimer's disease, characterized by cholinergic deficit. Of the many compounds synthesized and studied, SM 21 (3-α-tropanyl 2-[4-Cl-phenoxy]butyrate) (48) (Fig. 1) showed the best pharmacological profile.

## CHEMISTRY

Modification of the atropine structure by substituting the phenyl ring or the aminoalcohol moiety provided some potent compounds whose efficacy, compared with morphine, remained as low as that of atropine (48). Better results were obtained in the series of 2-phenylpropionic acid esters, which were synthesized to deal with the chemical instability of tropic acid. In this class the potency was much lower than that of atropine, but efficacy was definitely improved (48).

To restore high affinity, it was thought that the possibility of a hydrogen bond, present in atropine, should be reintroduced into the molecule. We synthesized several esters of substituted 2-(phenoxy)propionic acids. Further modifications of the molecule showed that 2-(phenoxy)butyric acid gave better results than the corresponding 2-(phenoxy)propionic acid and that the 3-α-tropanol was the best choice for the aminoalcohol moiety. In this class, SM 21 was selected as the most interesting compound (47). Its chemical structure and synthesis are illustrated in Fig. 1. Isosteric substitution of the oxygen atom with S, NH, $NCH_3$, or $CH_2$ was also performed (47).

Chemical modifications have led to compounds similar to SM 21, such as PG 9 (6), ET 142 (38), and SM 32 (40), with a pharmacological profile quite similar to that of

SM 21. Other chemical modifications, though informative regarding structure-activity relationships in the series, produced less interesting compounds (64).

SM 21 possesses a stereogenic center and, as a consequence, is normally obtained as a racemic mixture of two enantiomers. To develop the compound further, it was necessary to study the properties of the single enantiomers; therefore, we addressed the problem of obtaining the two enantiomers with acceptable optical purity (76).

SM 21 enantiomers show a certain enantioselectivity in pharmacological activity even if both stereoisomers are active (39) unlike what happens for atropine enantiomers, where only R-(+)-hyoscyamine shows analgesic and antiamnesic activity (41). In any case, the most potent and efficacious enantiomer is R-(+)-SM 21, which shares the same absolute configuration of R-(+)-hyoscyamine.

## CENTRAL PHARMACOLOGICAL PROFILE

### *In Vivo* Studies

#### *Antinociceptive Properties*

SM 21 induced antinociception in mice, rats, and guinea pigs. Antinociception was elicited regardless of which noxious stimulus was used: thermal (hot-plate and tail flick tests), chemical (abdominal constriction test), or mechanical (paw pressure test), performed according to O'Callaghan and Holtzman (69), D'Amour and Smith (17), Koster et al. (56), and Leighton et al. (59), respectively.

SM 21 produced a dose-dependent increase in the pain threshold in mice after systemic (subcutaneous [s.c.], intraperitoneal [i.p.], oral [p.o.], intravenous [i.v.]) injection, as illustrated by the hot-plate (Fig. 2) and abdominal constriction tests (Fig. 3a). SM 21 reached its maximum antinociceptive effect 15 min after administration and then slowly diminished (Fig. 2b,d). SM 21 produced an increase in the pain threshold not only in mice but also in rats, in the paw pressure (Fig. 3b), and tail flick (39) tests and in guinea pigs, in the paw pressure test (39), with a pharmacological profile similar to that exerted in mice.

SM 21 is endowed with central antinociceptive activity. It was, in fact, possible to reach the same intensity of analgesia by injecting directly into the cerebral ventricles (49) doses (5 to 20 μg/mouse) of SM 21 that were fifty times lower than those needed parenterally (Fig. 2c). That the antinociception depends on a retrodiffusion of the drug from the cerebral ventricles to the periphery can thus be ruled out.

Both enantiomers of SM 21, R-(+)-SM 21, and S-(–)-SM 21 induced antinociception in the mouse hot-plate and abdominal constriction tests in a dose-dependent manner; R-(+)-SM 21 was slightly more effective than S-(–)-SM 21 (39).

SM 21 showed good antinociceptive efficacy in comparison with that produced by R-(+)-hyoscyamine and some well known analgesic drugs such as morphine, diphenhydramine, and clomipramine. As a matter of fact, by comparing the areas under the curve of the above-mentioned compounds, tested at the highest doses that do not im-

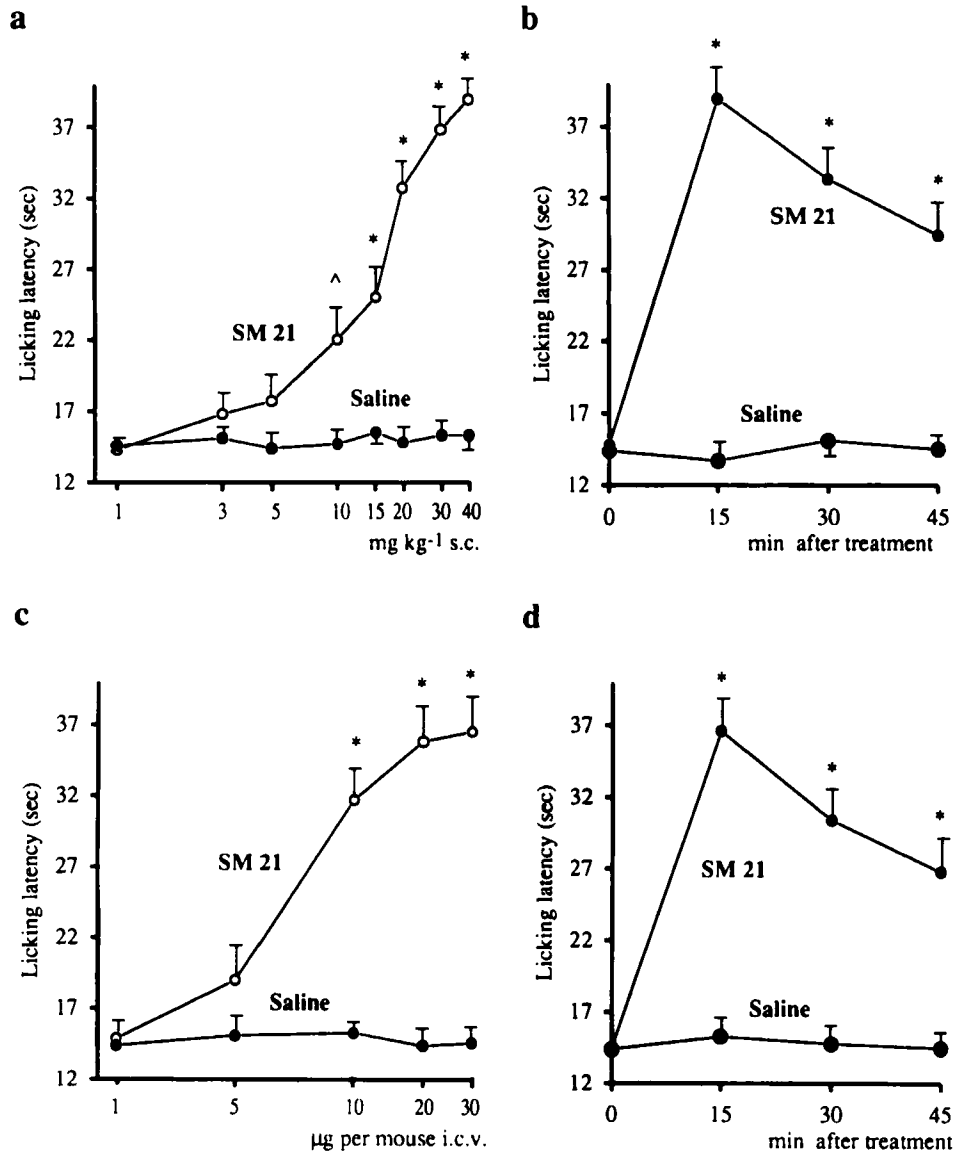


**Fig. 2.** Dose-response curves of SM 21 i.p. (a) and i.c.v. (c) injected in the mouse hot-plate test. The SM 21 time course of 40 mg/kg i.p. is reported in (b) and 30 μg per mouse i.c.v. in (d) from the same test. Vertical lines give S.E.M. Each point is the mean of at least 10 mice. $^{\wedge}P < 005$; $^{*}P < 0.01$ in comparison with saline controls. In (a) and (c) SM 21 was administered 15 min before the test.

pair mouse normal behavior, SM 21 was as effective as morphine, and more effective than R-(+)-hyoscyamine, diphenhydramine and clomipramine (39).

SM 21, at doses lower than 1 mg/kg, was able to reduce the number of abdominal constrictions induced by intraperitoneal injection of a 0.3% acetic acid solution and to

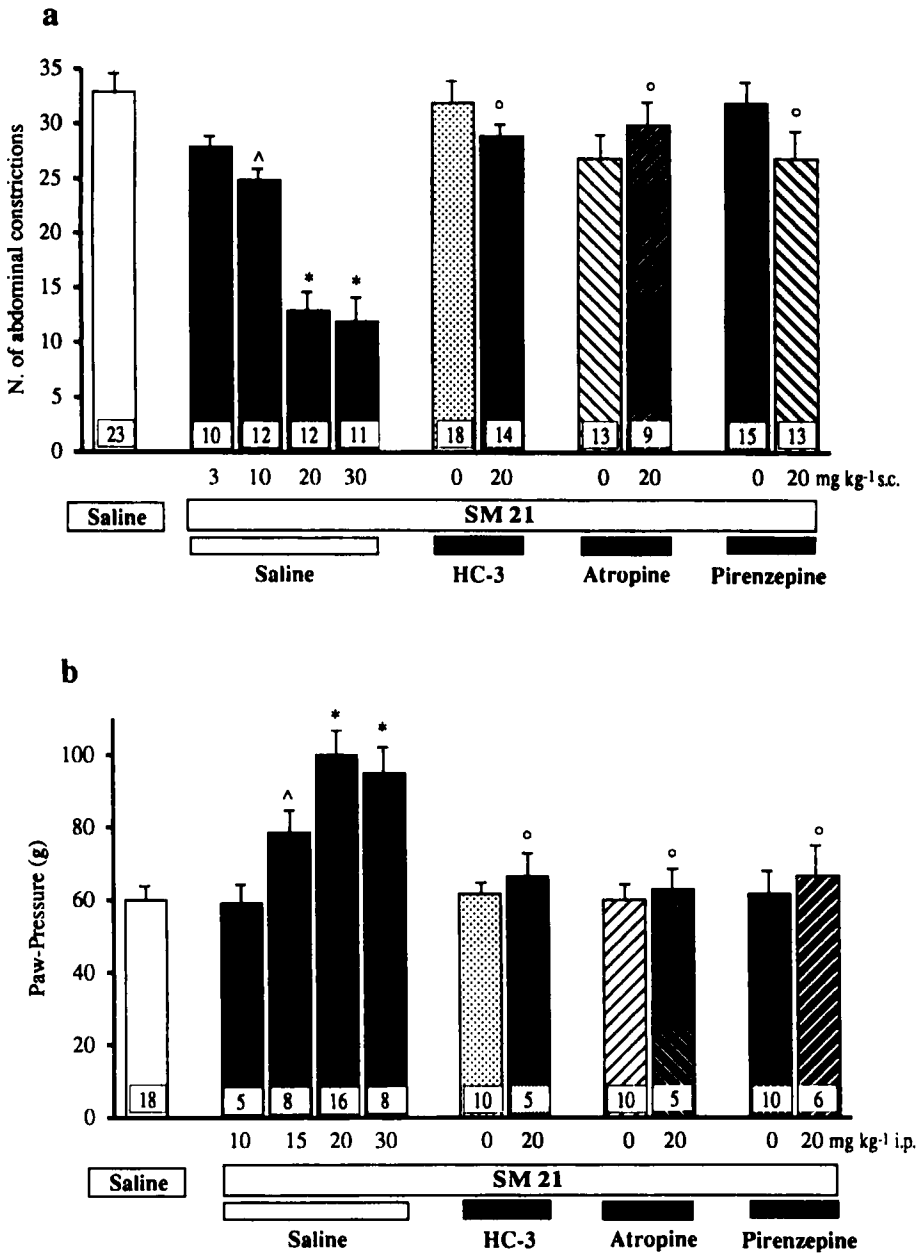


**Fig. 3.** (a) Antinociceptive effect of SM 21 and antagonism of hemicholinium-3 (HC-3) (1 μg per mouse i.c.v.), atropine (5 mg/kg i.p.) and pirenzepine (0.1 μg per mouse i.c.v.) on the enhancement of pain threshold induced by SM 21 (20 mg/kg s.c.) in the mouse abdominal constriction test induced by 0.6% acetic acid (a) and in the rat paw-pressure tests (b) HC-3, atropine and pirenzepine were injected respectively 5 h, 15 min, and 10 min before testing. In the abdominal constriction test the nociceptive responses were recorded 15 min after SM 21 administration. Vertical lines show S.E.M. $^{\wedge}P < 0.05$; $^{*}P < 0.01$ in comparison with saline controls. $^{\circ}P < 0.01$ in comparison with SM 21 (20 mg/kg s.c.). Numbers inside the columns indicate the number of mice or rats.

reverse the hyperalgesia induced by morphine withdrawal (data not shown). SM 21 antinociception is not due to an anti-inflammatory action. SM 21, at concentrations up to $10^{-4}$ M, did not inhibit inducible COX activity in comparison with indomethacin ($IC_{50}$: $23 \times 10^{-6}$ M) and, at analgesic doses, failed to suppress paw edema in response to carrageenan administration (Table 1).

## *Antiamnesic Activity*

SM 21 ameliorated cognitive processes in mice and rats. This compound was able to prevent amnesia induced by treatment with drugs such as scopolamine (Fig. 4), dicyclomine (Fig. 4), diazepam (27), and AF64A (26), or exposure to hypoxic environment (67) in the passive avoidance test. The antiamnesic effect of SM 21 was dose-dependent and the first active dose was lower than that able to enhance the pain threshold. A complete prevention of amnesia was, in fact, obtained at a dose (10 mg/kg) that was weakly analgesic only in the hot-plate test. The time-course of the antiamnesic activity of SM 21 was equal to that observed for the antinociceptive action, reaching its maximum effect between 15 and 30 min after injection. Therefore, in the passive avoidance experiments SM 21 was administered 20 min before the training session.

In the passive avoidance test an improvement in cognition of animals that have no memory impairment is difficult to demonstrate. SM 21, as well as well-known nootropic drugs, such as piracetam and aniracetam, or cholinomimetics, such as physostigmine and oxotremorine, do not show any memory facilitation in unamnesic animals (45,16).

A procognitive activity of SM 21 was unmasked by using a social learning test, performed according to Mondadori et al. (68), but in which adults rats with unimpaired memory were used. SM 21, as well as piracetam, exerted beneficial effects on the cognitive performance by prolonging the time spent by rats to delete mnemonic information (25).

## *Subacute Treatment*

SM 21 induced tolerance after repeated administration. SM 21, injected twice daily for two weeks at doses at which it demonstrates a full antiamnesic and antinociceptive

*TABLE 1. Effect of SM 21 on carrageenan-induced paw edema in rats*

| Pretreatment | Treatment | Dose, mg/kg i.p. | Paw volume ml ± S.E.M. |
|---|---|---|---|
| Saline | Saline | | 1.37 ± 0.08 |
| Carrageenan | Saline | | 2.21 ± 0.09 |
| Carrageenan | SM 21 | 20 | 2.27 ± 0.06 |
| Carrageenan | SM 21 | 30 | 2.19 ± 0.05 |
| Carrageenan | Indomethacin | 1 | 1.45 ± 0.07* |

Indomethacin was used as positive control; $n = 5$ rats per group. *$P < 0.05$ in comparison with carrageenan-saline controls. SM 21 was injected 15 min before the test.

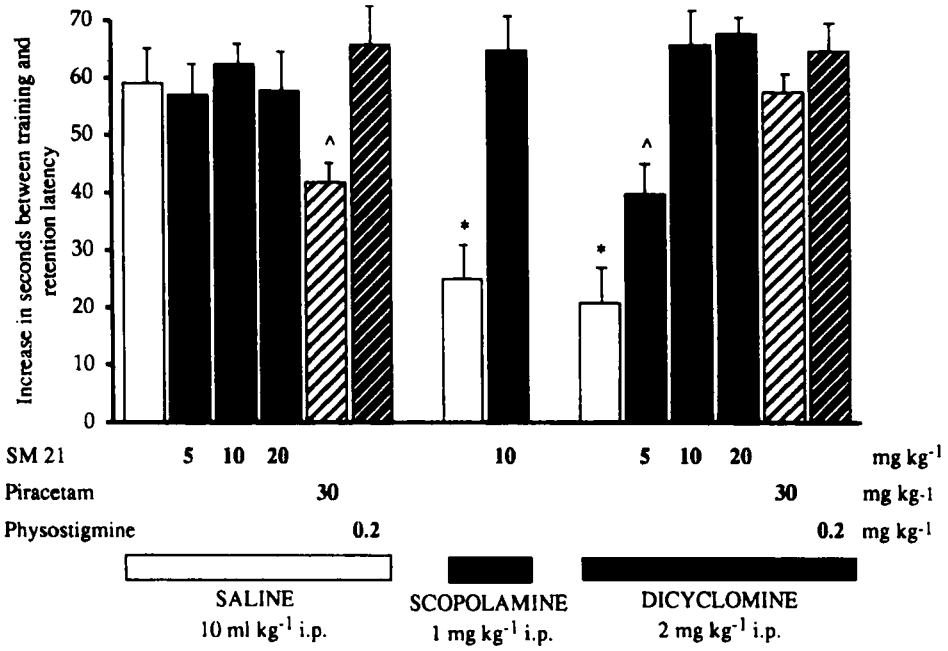


**Fig. 4.** Effect of i.p. SM 21, piracetam, and physostigmine on dicyclomine-induced amnesia in mouse passive avoidance test and, under the same experimental conditions, effect of SM 21 on scopolamine amnesia. Punishment consists of a fall into cold water (10°C). SM 21, piracetam, and physostigmine were injected 20 min before the training session. Scopolamine and dicyclomine were injected immediately after the training session. $^{\wedge}P < 0.05$; $^{*}P < 0.01$ in comparison with saline controls. Each column represents the mean of at least 25 mice.

activity (10 and 30 mg/kg i.p., respectively), produced a complete loss of both behavioral effects. Following the same administration schedule, however, other analgesic drugs, such as morphine, oxotremorine, and baclofen, develop tolerance toward their analgesic effect (61,62). Subacute treatment with SM 21 (30 mg/kg i.p.) did not produce loss of body weight or the symptomatology typical of the withdrawal syndrome.

## *Effect of SM 21 on Animal Behavior*

The maximum antinociceptive effect of SM 21 was obtained at 40 mg/kg s.c. without producing any visible modification in mouse or rat gross behavior. At the same dose, SM 21-treated mice showed a complete integrity of motor coordination on the rotarod test, tested according to Kuribara et al. (57) (Table 2). Under these experimental conditions, SM 21 was compared with equiactive doses of oxotremorine and physostigmine (Table 2). The muscarinic agonist and the inhibitor of cholinesterase both produced a statistically significant reduction in endurance time on the rotating rod. Normal spontaneous motility, evaluated by the Animex apparatus (data not shown), as well as exploratory behavior, revealed by the hole-board test (39), were

*TABLE 2. Effect of SM 21, oxotremorine, and physostigmine in the rotarod test*

| Treatment s.c. | Endurance time on rotarod (s) | | | |
|---|---|---|---|---|
| | Before treatment | After treatment | | |
| | | 15 min | 30 min | 45 min |
| Saline | 98.5 ± 5.1 (16) | 92.7 ± 6.2 (16) | 103.7 ± 5.0 (16) | 97.4 ± 7.2 (16) |
| SM 21, 40mg/kg | 103.2 ± 5.6 (10) | 97.5 ± 6.2 (10) | 99.6 ± 5.4 (10) | 100.2 ± 4.3 (10) |
| Oxotremorine, 40 μg/kg | 106.2 ± 8.2 (11) | 76.5 ± 7.3* (11) | 63.6 ± 9.6* (11) | 64.4 ± 8.7* (11) |
| Physostigmine, 200 μg/kg | 93.4±5.7 (9) | 61.4 ± 6.8* (9) | 54.5 ± 8.1* (9) | 52.3 ± 8.8* (9) |

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

also observed after subcutaneous administration of SM 21 at 40 mg/kg s.c. and intracerebroventricular (i.c.v.) administration of 30 μg/mouse. Impaired motor coordination and spontaneous motility were revealed in mice starting at 100 mg/kg s.c. The $LD_{50}$ was at 400 mg/kg s.c., corresponding to 883 μmol/kg.

## PERIPHERAL PHARMACOLOGICAL PROFILE

### Effect on Smooth Muscle

#### *Effect on Intestinal Motility*

SM 21, administered at analgesic and antiamnesic doses, did not modify transit in the intestinal tract of the mouse, performed according to Reynell and Spray (74) (data not shown). In contrast, other analgesic drugs, such as morphine, significantly retarded gastrointestinal propulsion, the cholinesterase inhibitor neostigmine accelerated net propulsion (80). The lack of effect of SM 21 on intestinal motility indicates that this compound, with the same analgesic activity, has advantage over opioid analgesics, which produce constipation, or classical cholinomimetics, which produce diarrhea.

#### *Effect on Isolated Guinea Pig Ileum*

SM 21 added to the organ bath at concentrations ranging from 1 pM to 1 nM potentiated the contractions evoked by both nicotine (4 μM) and electrical stimulation at 0.1 Hz, 0.5 ms, voltage double threshold, performed according to Paton and Vizi (71) (Fig. 5). The effect was larger (area under the curve ratio) on the contractions induced by nicotine than those induced by electrical stimulation. The potentiation was no longer observed when the concentration of SM 21 in the medium was raised to 10 nM.

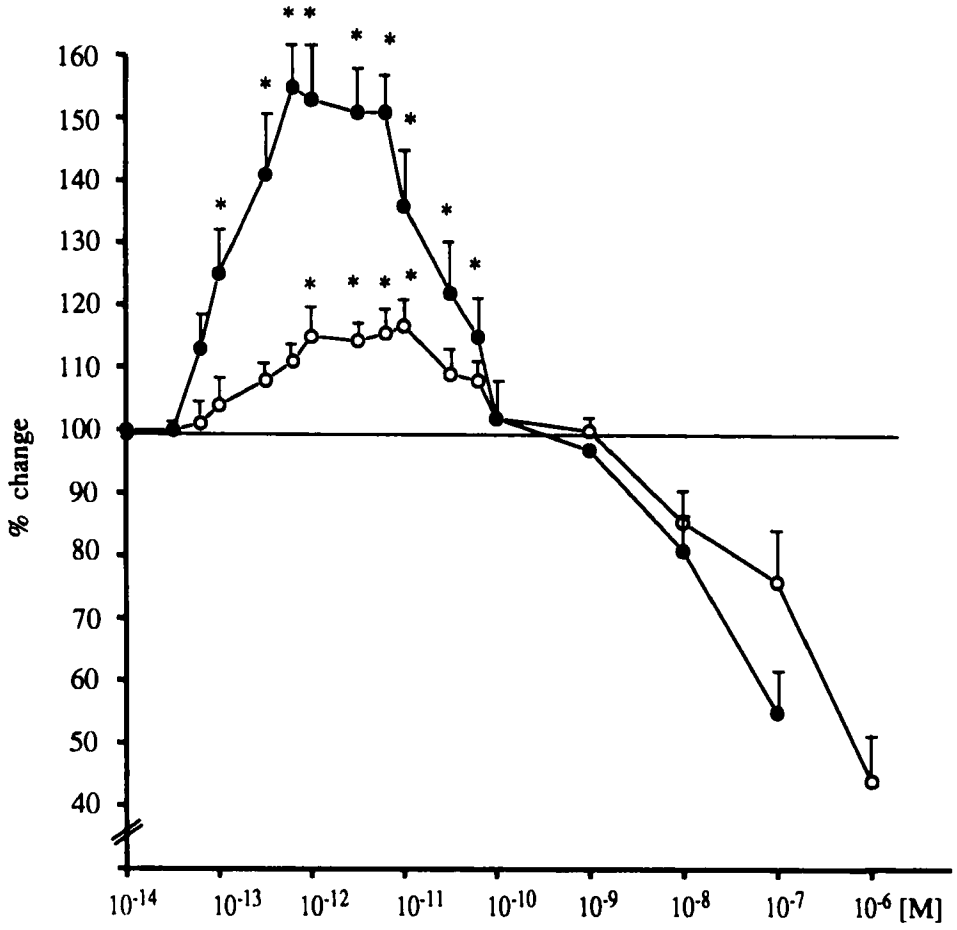


**Fig. 5.** Dose-response curves of SM 21 on nicotine-(4 μM; closed symbols) and electrically (0.1 Hz; 0.5 ms; double threshold voltage; open symbol evoked contractions) of guinea pig ileum myenteric plexus longitudinal muscle strip expressed as percentage variation of contractions. Each point represents the mean of at least 6 experiments and vertical lines give S.E.M. $^{*}P < 0.05$ calculated in the range between 0.1 pM and 0.1 nM.

SM 21 began to inhibit both types of evoked contractions at 1 μM. Nicotine-evoked ileum contractions were about four times greater than those electrically evoked (Fig. 5). This is probably due to the simultaneous activation, during electrically-evoked contractions, of both intramural cholinergic and sympathetic fibers, whereas during nicotine-evoked contractions only cholinergic neurons are likely to be activated. Noradrenaline released during electrical stimulation could be responsible for limiting the effect of the ACh released by low doses of SM 21. The higher amplification by SM 21 of the nicotine-evoked contractions of guinea pig ileum as compared

with those elicited by electrical stimulation depended on the inhibitory control exerted by norepinephrine, which is released only during electrical stimulation (31).

## Effect on Striated Muscle

### *Rat Phrenic Nerve-Hemidiaphragm Preparation*

SM 21 (1 μM to 1 mM) potentiated the hemidiaphragm contractions evoked by electrical stimulation of the left phrenic nerve, performed according to the method described by Bülbring (10) and modified by Wessler and Kilbinger (83), and did not modify the contractions evoked through direct stimulation of the diaphragm muscle (data not shown). At concentrations lower than 1 μM, SM 21 was always inactive. A potentiation of hemidiaphragm contractions is exerted by numerous muscarinic antagonists, such as atropine, pirenzepine, dicyclomine, and glycopyrrolate (73,84) by blocking the muscarinic autoreceptors. SM 21 may, therefore, exert its effect on the phrenic nerve by antagonizing muscarinic receptor subtypes. One must consider, however, that inhibitors of cholinesterase can also amplify hemidiaphragm contractions (1). Since SM 21 is endowed with very low anticholinesterase activity ($IC_{50}$ = 110 μM), it may be possible that its action underlies antimuscarinic and/or anticholinesterase activity. The lack of inhibition of electrical stimulation of hemidiaphragm contractions rules out the possibility that SM 21 acts as a local anesthetic. In fact, local anesthetics such as lidocaine and procaine inhibit the electrically stimulated contractions of the same preparation up to complete abolishment in a dose-dependent manner (1).

## MECHANISM OF ACTION

SM 21 antinociception was found to be dependent on cholinergic activation, since this analgesia is antagonized by the muscarinic antagonist atropine (Fig. 3a,b), the $M_1$-antagonist pirenzepine (Fig. 3a,b), the ACh depletor HC-3 (Fig. 3a,b), and by lesion of the nucleus basalis magnocellularis (NBM) (6), which is the primary source of ACh for the cerebral cortex (75). Moreover, the antagonism exerted by intracerebroventricular injection of HC-3 and pirenzepine in mice and NBM lesions in rats on SM 21-induced antinociception confirms that the site of action of SM 21 is centrally located.

A presynaptic mechanism facilitating cholinergic transmission is involved in SM 21 activity as revealed by microdialysis studies performed according to Giovannini et al. (43). SM 21 increased ACh release from rat cerebral cortex, which peaked from 45 to 60 min after administration and returned to basal values within 120 min (Fig. 6). This effect was sensitive to tetrodotoxin (Fig. 6). The SM 21-induced increase in ACh release occurred at the same range of doses (10 to 20 mg/kg i.p.) at which SM 21 exerted its antinociceptive and antiamnesic activities. The greater latency required to reach the maximum amplification of ACh release compared to that

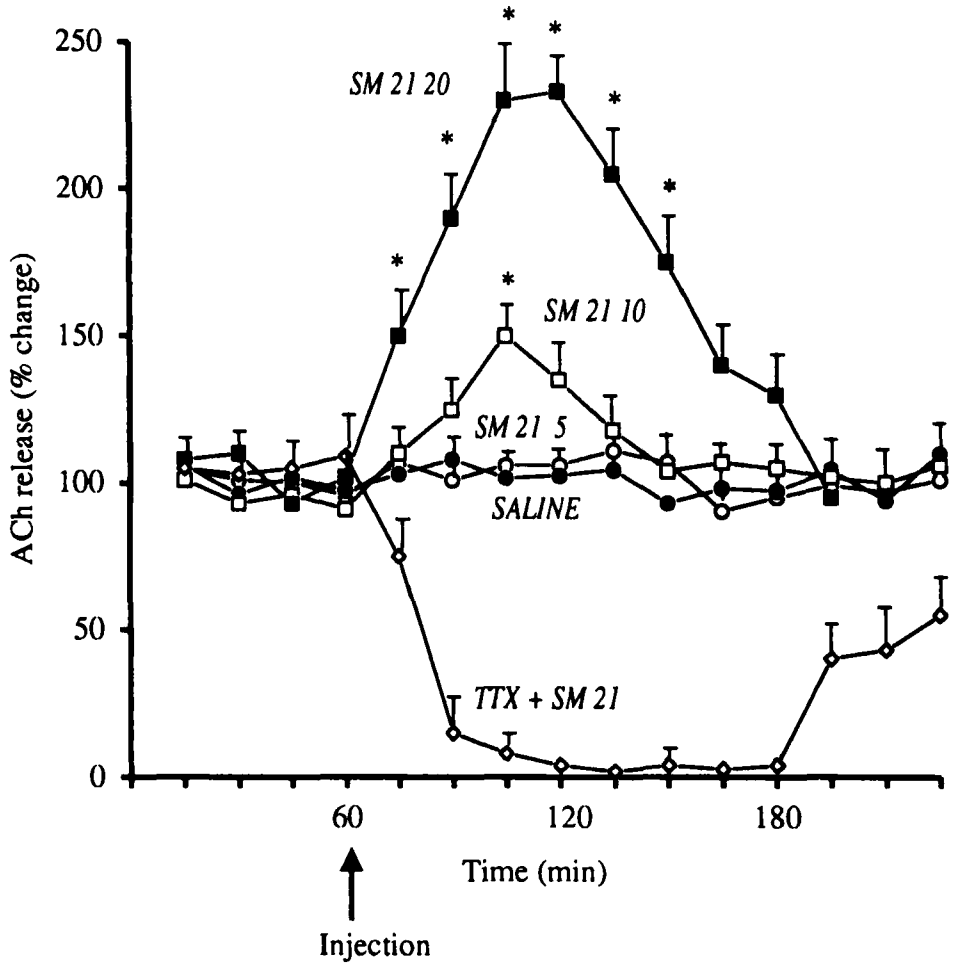


**Fig. 6.** Dose-response curves of SM 21 on ACh release from parietal cortex and antagonism of SM 21 (20 mg/kg i.p.) by TTX (0.5 μM). All values are expressed as changes over basal output. SM 21 was administered at 60 min as shown by the arrow. Vertical lines give S.E.M. Each point represents the mean of at least 5 independent experiments. Doses of SM 21 are expressed as mg/kg i.p. Significant differences were evaluated by comparing the percentage variation vs. the mean ± S.E.M. of all pre-drug determinations. $^{*}P < 0.05$ in comparison with controls.

required to be active could be ascribed to the time taken by ACh to diffuse from the synaptic cleft to the microdialysis tube.

The hypothesis of a presynaptic cholinergic mechanism for SM 21 is confirmed by: 1) the SM 21-induced amplification of electrically and chemically evoked contractions of guinea pig ileum myenteric plexus longitudinal muscle strips (Fig. 5) without modifying its basal tone; 2) the antagonism of SM 21-induced antinociception by the ACh depletor HC-3. A postsynaptic mechanism of action for SM 21 can be ruled out since, as reported by Bartolini et al. (3,5), HC-3 was not able to antagonize antinociception

*TABLE 3. Affinity profiles of SM 21, R-(+)-hyoscyamine, and AFDX-116 at $M_1 - M_4$ muscarinic receptors and binding affinities of SM 21 and AFDX-116 for $m_1 - m_4$ muscarinic receptor subtypes expressed in Chinese hamster ovary cells (CHO-K1)*

| | $pA_2$ values | | | |
|---|---|---|---|---|
| | $M_1$ rabbit vas deferens | $M_2$ rat left atrium | $M_3$ rat ileum | $M_4$-putative guinea pig uterus |
| SM 21 | 5.97 ± 0.11* | 6.63 ± 0.10* | 6.35 ± 0.04* | 6.26 ± 0.05* |
| R-(+)-hyoscyamine | 7.05 ± 0.05[a] | 7.25 ± 0.04[a] | 6.88 ± 0.05[a] | 9.56 ± 0.01[a] |
| AFDX 116 | 6.84±0.14[b] | 7.12 ± 0.11[b] | 6.34 ± 0.13[c] | 6.70 ± 0.06 |
| | $pK_i$ values | | | |
| | $m_1$ | $m_2$ | $m_3$ | $m_4$ |
| SM 21 | 6.90 ± 0.16 | 6.28 ± 0.12 | 6.62 ± 0.10 | 6.53 ± 0.05 |
| AFDX 116 | 6.84 ± 0.14[b] | 7.12±0.11[b] | 6.34±0.13[b] | 6.70 ± 0.06 |

Each value represents the mean ± S.E.M.; * $pK_B$ values were obtained with SM 21 1 μM. From ref. [a]34, [b]23, [c]24.

induced by agonists of postsynaptic muscarinic receptors such as oxotremorine, McN-A-343, and AF-102B; and 3) SM 21 did not elicit the typical cholinergic symptoms (tremors, sialorrhea, diarrhea, rhinorrhea, lacrimation, etc.) produced by injection of direct postsynaptic muscarinic agonists (9). It is also to be noted that there is a wide gap between the low concentrations at which SM 21 is thought to inhibit the presynaptic muscarinic receptors (Fig. 5) and the high concentrations that are needed to block the postsynaptic muscarinic receptors (Table 3).

It is well known that activation of the nicotinic system induces antinociception. SM-21, even if it increases extracellular levels of ACh, produces an enhancement of the pain threshold that is not prevented by mecamylamine, excluding a mechanism of action involving the interaction with nicotinic receptors (data not shown). This hypothesis is also supported by the fact the antimuscarinic drugs, at doses able to antagonize muscarinic antinociception, do not prevent nicotinic antinociception (33).

It has long been known that activation of the cholinergic system induces antinociception (72,30,52,51,13,50,60), as well as a facilitation of cognitive processes (16). It is plausible, therefore, that enhancement of extracellular levels of ACh can be considered responsible for the antinociceptive effect of SM 21. Moreover, the SM 21-induced amplification of endogenous ACh release may counteract the amnesic effect produced by the antimuscarinic drugs scopolamine and dicyclomine.

ACh release can be increased by blocking $M_2/M_4$ muscarinic autoreceptors (58,81,65,79). The affinity profile of SM 21 vs. $M_1$ (rabbit vas deferens, according to Eltze [23] and modified by Dei et al. [18]), $M_2$ (guinea pig atrium, according to Eltze et al. [22] and modified by Dei et al. [18]), $M_3$ (guinea pig ileum, according Eltze and Figala [24]), and putative $M_4$ receptors (prepuberal guinea pig uterus, according to Dörje et al. [20]) shows low $M_4/M_1$ (1.9 times) and $M_2/M_1$ (4.6 times) selectivity ratios as reported in Table 3. In this study, SM 21 selectivity was compared with that of

the selective $M_4$ antagonist R-(+)-hyoscyamine (34) and the selective $M_2$ antagonist AFDX-116* (42). It is possible that a selectivity ratio of 4.6, even if small, may be high enough to enhance the pain threshold and to reverse amnesia as a consequence of ACh release. The $M_2$ muscarinic antagonists, AFDX-116 (42), methoctramine (66), and AQRA-741 (19), which are endowed, like SM 21, with cholinergic presynaptic antinociceptive (4,32,46) and antiamnesic (2) properties, and which are able to increase ACh release (58,81), have an $M_2/M_1$ selectivity ratio comparable to that of SM 21. However, binding studies performed on the $m_1$ to $m_4$ human muscarinic receptor subtypes expressed in CHO cells (21,12) did not confirm the results obtained by functional studies, as shown in Table 3. Other mechanisms able to potentiate the endogenous cholinergic system may be involved in the antinociceptive and antiamnesic effect induced by SM 21.

It has been demonstrated that $D_2$ dopaminergic (44,82,78,54), $A_1$ adenosinergic (55,11), $H_3$ histaminergic (14), 5-$HT_4$ serotoninergic heteroreceptors (15), and 5-$HT_{1A}$ receptors (8), increase ACh release. However, the above-mentioned receptors are not involved in an SM 21 mechanism of action. In fact, SM 21 is able to interact with $D_2$, $H_3$, 5-$HT_4$, and 5-$HT_{1A}$ only at concentrations higher than $10^{-6}$ M, as revealed by binding studies (data not shown). These results are supported by the fact that quinpirole ($D_2$ agonist), $N^6$-cyclopentyladenosine ($A_1$ agonist), R-($\alpha$)-methylhistamine ($H_3$ agonist), GR-48125 (5-$HT_4$ antagonist), and NAN 190 (5-$HT_{1A}$ antagonist), at doses able to prevent the antinociception induced respectively by haloperidol (33), caffeine (37), thioperamide (63), BIMU 1 and BIMU 8 (36), and 5-$HT_{1A}$ agonists (35,29), failed to prevent SM 21 antinociception (39).

Neurotransmitter systems other than the cholinergic are not involved in SM 21 antinociception. This compound interacts with the following receptor subtypes: $\alpha_1$-, $\alpha_2$-, $\beta_1$-, $\beta_2$-adrenoceptors, $D_1$, $GABA_A$, $GABA_B$, $H_1$, $NK_1$, $\delta$-, $\kappa$-, $\mu$-opioid, 5-$HT_{1D}$, 5-$HT_2$, 5-$HT_3$, and $K^+$ channels: ATP-sensitive $K^+$ channel, voltage-dependent $K^+$ channel, $Ca^{2+}$-activated $K^+$ channel only at concentrations higher than $10^{-6}$ M (data not shown). The lack of prevention of SM 21 antinociception by the opioid antagonist naloxone, the $GABA_B$ antagonist CGP-35348 and the biogenic amine depletor reserpine (39) is in agreement with the binding data. Pertussis toxin (PTX) pretreatment was able to prevent opioid (70), catecholaminergic, GABAergic (53), histaminergic (28), and purinergic (77) analgesia, but not muscarinic antinociception (28). Since SM 21 antinociception was not prevented by pretreatment with pertussis toxin (7), the hypothesis of a cholinergic mechanism underlying the SM 21 mechanism of action is further supported.

## SUMMARY

SM 21 is a 2-phenylpropionic acid ester, structurally related to atropine, that produces a central antinociceptive and antiamnesic effect in mice and rats. These activities are exerted without impairing motor coordination and without producing typical cholinergic symptomatology. SM 21 is also able to amplify the evoked contractions

* Chemival name of AFDX-116 is: 11,2-(diethylamino)methyl-1-piperidinil acetyl-5,11-dihydro-6H-pyrido 2,3b 1,4-benzodiazepine-6-one.

of smooth and striated muscle. A potentiation of endogenous cholinergic activity, by enhancing ACh extracellular levels, can be considered responsible for the action of SM 21 on both the central and peripheral nervous system. However, at this point the exact mechanism by which ACh levels are increased is not entirely elucidated.

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