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Salmon calcitonin potentiates the analgesia induced by antidepressants

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Abstract

Antidepressants are used in the treatment of a variety of pain syndromes. Most of them act by blocking noradrenaline (NA) and serotonin (5-HT) reuptake. It is also well known that the serotonergic system is also involved in calcitonin (CT) analgesia. Taking these two evidences into account, the modification of the analgesic effect of nortriptyline, amitriptyline, and paroxetine in the presence of salmon CT (s-CT) was examined in mice. The forced-swimming test was carried out in order to choose doses of each drug that did not induce an antidepressant effect under our experimental conditions (nortriptyline: 0.2–5 mg/kg ip, amitriptyline: 2.5–20 mg/kg ip, and paroxetine: 5–30 mg/kg ip). The analgesic effect of each antidepressant was then evaluated using the acetic acid test. At the doses tested, the antidepressants induced a dose-dependent analgesic effect. When mice were pre-treated with a subanalgesic dose of s-CT (2.5 IU/kg), the analgesic effect of amitriptyline and paroxetine was significantly increased though no modification was found for nortriptyline. In summary, s-CT was able to increase the analgesic effect of the antidepressant drugs that reduce the uptake of 5-HT, suggesting that the joint administration of antidepressants and CT may be an interesting alternative in pain management.

Keywords: Nortriptyline; Amitriptyline; Paroxetine; Calcitonin; Analgesia; Mouse

Several antidepressants have been used in the treatment of a variety of pain syndromes such as migraine, post-herpetic neuralgia, and painful peripheral neuropathies (Kalso et al., 1995; McQuay et al., 1996). They are extensively used alone or in association with opiates or anti-inflammatory agents when treatment with traditional analgesics fails. However, their usefulness is limited due to the frequent occurrence of adverse side effects.

The analgesic effect does not seem to be related to the antidepressant effect because analgesia is evident after acute administration at a dose lower than that required to induce antidepressant effects, and furthermore, their analgesic effectiveness has also been demonstrated in animals using different tests such as the formalin (Acton et al., 1992) and the tail-flick tests (Ventafridda et al., 1990) or autotomy behavior (Seltzer et al., 1989).

Most of the antidepressant drugs affect the noradrenaline (NA) and/or the serotonin (5-HT) levels. Those acting on the serotonergic system may modulate, either directly or indirectly, 5-HT metabolism or function (Zhu and Mcnaughton, 1994), inducing a decrease in the re-uptake of this neurotransmitter and increasing its synaptic concentration. This augmentation is autolimiting, since when the 5-HT concentration is increased, the neurotransmitter interacts with pre-synaptic $5HT_{1a}$ receptors that modulate the synthesis and release of the neurotransmitter. Animals treated with 5-HT uptake inhibitors look normal in gross appearance, but effects such as reduced aggressive behavior, decreased food intake and altered food selection, analgesia, anticonvulsant activity, and endocrine and neurochemical changes have been demonstrated and characterized (Fuller, 1995).

Calcitonin (CT) is a polypeptide hormone involved primarily in the regulation of blood calcium levels and bone calcium metabolism. The use of this hormone in osteoarticular disorders, principally in Paget's disease, was accompanied by an unexpected analgesic effect (Bijvoet and Hjansen, 1967). Since then, the analgesic effect of CT has been widely demonstrated in laboratory animals and in humans as well as being demonstrated in a variety of painful pathologies not always related to bone diseases (Ankrom

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and Shapiro, 1998; Baron et al., 1998; Gennari and Agnusdei, 1988; Hamamci et al., 1996; Kessel and Wörz, 1987).

Discrepancies still exist concerning the main mechanism involved in this analgesia. Different hypothesis have been postulated including anti-inflammatory (Cesarini et al., 1979; Guidobono et al., 1991), serotonergic (Bourgoin et al., 1988; Colado et al., 1994), and opioid (Martín et al., 1992, 1993; Welch et al., 1986) mechanisms.

With regard to the CT–serotonergic system interaction, previous data show that CT does not change the concentration of 5-HT or its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the central nervous system (CNS) of naive animals or animals treated with drugs altering the metabolism of 5-HT (Colado et al., 1994). Nevertheless, treatments that modify serotonergic function, such as administration of some 5-HT receptor antagonists (Clementi et al., 1984), degenerative lesions of the raphe dorsalis nucleus where the cell bodies of the serotonergic neurons are mainly located (Clementi et al., 1985), or lesions of the ascending and/or descending pathways (Colado et al., 1994; Hunskaar et al., 1986, 1987), have been shown to be capable of reducing the analgesic effect of CT. From these data, it may be suggested that the integrity of the serotonergic system is necessary in order to observe the analgesia induced by CT. Furthermore, data from our laboratory demonstrated that when 5-HT synthesis was increased by administration of 5-hydroxytryptophan, the 5-HT precursor, the analgesic effect of CT was significantly increased (Ormazábal et al., 1997).

The use of antidepressant as analgesic drugs is limited by the behavioral side effects, and that most of the antidepressants block monoamine re-uptake, some of them being selective 5-HT re-uptake inhibitors. We examined the modification of the analgesic effect of three antidepressants induced by pre-treatment with CT, bearing in mind that the serotonergic system is also involved in the analgesic effect of CT. The drugs tested were nortriptyline, which preferentially inhibits NA re-uptake; amitriptyline, which is not selective and inhibits both NA and 5-HT re-uptake; and paroxetine, a selective 5-HT re-uptake inhibitor (Fuller, 1995).

1. Methods

CD1 male mice weighing 25–30 g were used. All the animals were supplied with food and water "ad libitum" and were housed in a temperature-controlled room at 23°C. Lighting was on a 12/12-h light/dark cycle. The mice were housed for at least 1 day in the test room before experimentation. All the experimental procedures are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

In the first place, in order to choose doses of antidepressants that did not modify the behavior of mice, the forced-swimming test was carried out. The Porsolt forced-swimming test is a paradigm of behavioral depression widely used and accepted to evaluate antidepressant activity (Korzeniewska and Plaznik, 1998; Pare and Kluczynski, 1997; Porsolt, 1997; Weiss et al., 1998).

Once the doses were chosen, the experimental procedure was:

1. Evaluation of the levels of 5-HT and its metabolite to confirm the effect of the antidepressants on the serotonergic system,
2. Analysis of the analgesic effect of antidepressants administered alone and in combination with salmon CT (s-CT).

When CT increased the analgesic effect of the antidepressants, the forced-swimming test was carried out in order to discard any antidepressant influence on the analgesia.

1.1. Antidepressant tests

In order to study the analgesia using doses of the antidepressants without antidepressant effects under our experimental conditions, the Porsolt test (Porsolt et al., 1978; Willner, 1984) was carried out in mice intraperitoneally (ip) treated with saline solution (control group), nortriptyline (0.2–5 mg/kg), amitriptyline (2.5–20 mg/kg), and paroxetine (5–30 mg/kg). This test was also performed after the treatment with CT (2.5 IU/kg) and amitriptyline (2.5–20 mg/kg) or paroxetine (5–30 mg/kg).

In the forced-swimming test, mice were placed in a cylinder (25 cm high, 10 cm diameter) containing 6 cm of water maintained at 23°C. The total duration of the immobility periods was evaluated during 4 min starting 2 min after the mouse was placed on the water. An animal was judged to be immobile whenever it made only the movements needed to keep its head just above the surface.

The number of animals used to evaluate the antidepressant effect of each dose was $n = 8–12$. Data are presented as mean ± S.E.M. time of immobility in treated or in control animals.

1.2. Analysis of 5-HT and metabolite

Levels of 5-HT and 5-HIAA were measured in the brain of mice treated with saline, nortriptyline (2.5–10 mg/kg), amitriptyline (2.5–10 mg/kg), paroxetine (10–30 mg/kg), and CT (2.5 IU/kg). The ratio 5-HIAA/5-HT was used as an indication of 5-HT turnover. The effect of the treatments was evaluated in the midbrain, medulla oblongata, hippocampus, striatum, cortex, and spinal cord.

Mice were sacrificed by decapitation, the brains were removed quickly, and the midbrain, medulla oblongata, hippocampus, striatum, cerebral cortex, and spinal cord rapidly dissected out at 4°C and stored at −80°C. The tissues were homogenized in perchloric acid (0.2 M) containing sodium metabisulfite (0.1%), cysteine (0.1%), and EDTA (0.01%) (Green et al., 1992). Homogenates were

centrifuged at 15 000 × *g* for 20 min at 4°C. Aliquots of the supernatant were taken for analysis of 5-HT and 5-HIAA content by high-performance liquid chromatography (HPLC) with electrochemical detection.

The mobile phase for 5-HT and 5-HIAA analysis consisted of KH_2PO_4 (0.05 M), octanesulfonic acid (1 Mm), EDTA (0.1 mM), and methanol (16%), and the pH was adjusted to 3 with phosphoric acid. The mobile phase was filtered and degassed. The flow rate was 1 ml/min, and the working electrode potential was set at 0.7 V.

The HPLC system consisted of a pump (Waters 510) linked to an automatic sample injector (Waters 712 WISP), a stainless-steel reversed phase column (Resolve C18, 5 μm, 3.9 mm × 15 cm) with a precolumn (Resolve C18) and an amperometric detector (Waters M460). The current produced was monitored by using an integrator (Waters M745).

The animals used for the biochemical determinations were separated groups from those used to evaluate antinociceptive effects. Data are the mean of values obtained from six to eight tissues.

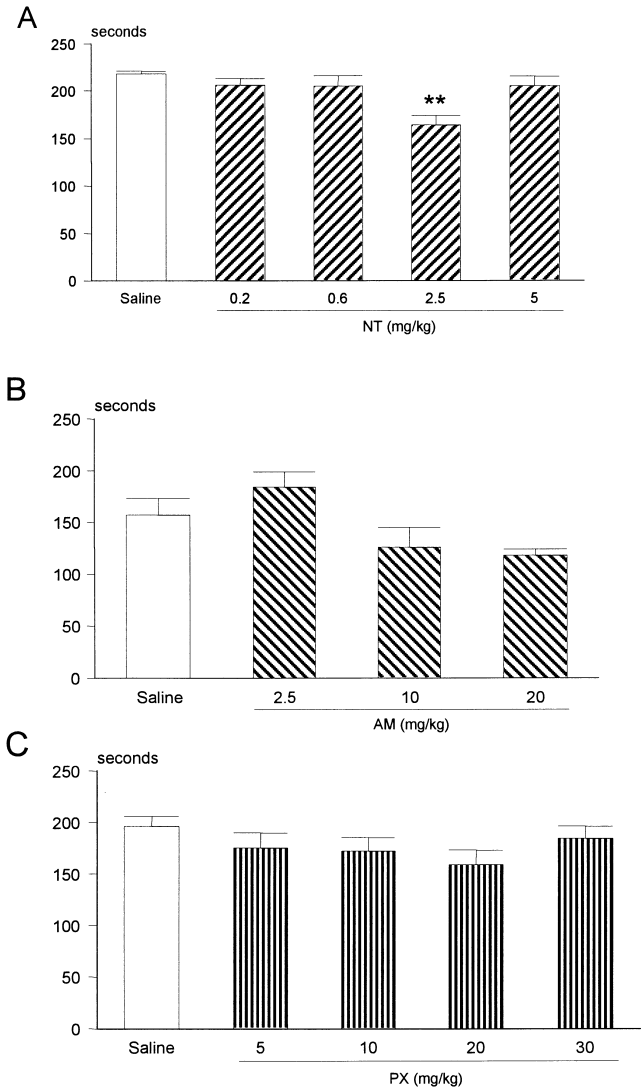

Fig. 1. Effect of antidepressants in the forced-swimming test. Bars represent the mean ± S.E.M. time of immobility recorded in control mice (open bars) and in mice treated with different doses of (A) nortriptyline (NT), (B) amitriptyline (AM), and (C) paroxetine (PX; $8 < n < 12$). $^{**}P < .01$ vs. control.

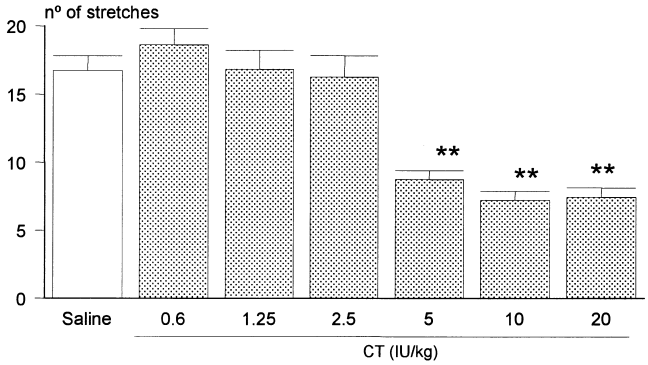

Fig. 2. Effect of CT in the acetic acid test. Bars represent the mean ± S.E.M. number of stretches induced by 2% acetic acid intraperitoneally administered in animals treated with different doses of s-CT. $^{**}P < .01$ vs. control. ($10 < n < 12$).

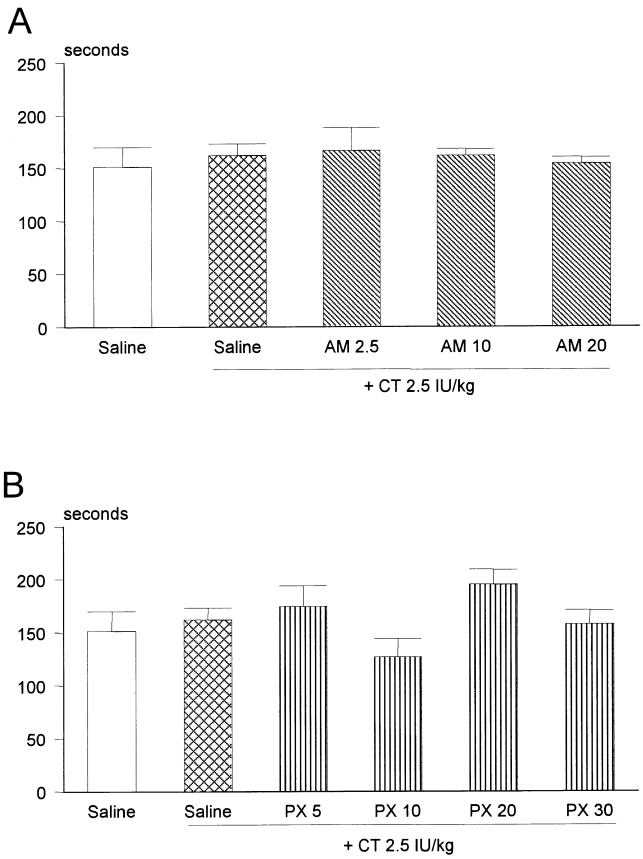

Fig. 3. Effect of antidepressants + CT in the forced-swimming test. Bars represent the mean ± S.E.M. time of immobility recorded in control mice (open bars), in mice treated with CT (double-hatched bars), and in mice treated with CT and different doses of (A) amitriptyline (AM) or (B) paroxetine (PX; $8 < n < 12$).

1.3. Analgesic test

The analgesic test used was the writhing test: intraperitoneal injection of diluted solutions of acetic acid is a well-established animal model for tonic visceral pain in rodents (Martínez et al., 1999). This tonic visceral test has been extensively used in mice to determine the analgesic activity of drugs. While the precise afferent pathway underlying pain responses in the writhing test are not known, the involvement of both visceral and somatic afferents has been suggested (Gebhart and Sengupta, 1996).

The mice were injected intraperitoneally with 0.3 ml of a 2% acetic acid solution to produce the typical writhing reaction, which is characterized by a wave of contraction of the abdominal musculature followed by extension of the hind limbs. After acetic acid administration, mice were placed in individual transparent containers, and 5 min later, the number of writhes was counted during a 10-min period. Each mouse was used only once. An observer, who was unaware of the treatment, performed the test and data recording.

The test was carried out after following treatments:

- Saline solution, control group;
- s-CT 2.5–20 IU/kg, 90 min before testing;
- Antidepressants:
  - nortriptyline 0.2–2.5 mg/kg, 30 min before testing,

Table 1
Levels of 5-HT, 5-HIAA, and turnover (5-HIAA/5-HT) expressed in ng/g in different structures of CNS in mice treated with different doses of nortriptyline ($6 < n < 8$)

	5-HT (ng/g)	5-HIAA (ng/g)	5-HIAA/5-HT (ng/g)
Midbrain			
Saline	516 ± 16	201 ± 11	0.39 ± 0.02
Nortriptyline 0.2 mg/kg	542 ± 13	190 ± 10	0.35 ± 0.02
Nortriptyline 0.6 mg/kg	561 ± 15	159 ± 9*	$0.28 \pm 1.3 \times 10^{-2}$**
Nortriptyline 2.5 mg/kg	529 ± 18	184 ± 13	$0.35 \pm 2.4 \times 10^{-2}$
Nortriptyline 5 mg/kg	583 ± 17*	156 ± 6*	$0.27 \pm 9.9 \times 10^{-3}$**
Medulla oblongata			
Saline	608 ± 26	352 ± 27	0.57 ± 0.03
Nortriptyline 0.2 mg/kg	583 ± 55	337 ± 44	$0.56 \pm 3.2 \times 10^{-2}$
Nortriptyline 0.6 mg/kg	623 ± 21	312 ± 13	$0.50 \pm 1.1 \times 10^{-2}$
Nortriptyline 2.5 mg/kg	642 ± 33	395 ± 27	$0.62 \pm 4.4 \times 10^{-2}$
Nortriptyline 5 mg/kg	654 ± 25	316 ± 14	0.49 ± 0.01
Spinal cord			
Saline	351 ± 30	61 ± 8	$0.18 \pm 1.8 \times 10^{-2}$
Nortriptyline 0.2 mg/kg	332 ± 17	63 ± 8	$0.19 \pm 1.6 \times 10^{-2}$
Nortriptyline 0.6 mg/kg	329 ± 18	52 ± 6	$0.16 \pm 9.1 \times 10^{-3}$
Nortriptyline 2.5 mg/kg	303 ± 15	56 ± 8	$0.18 \pm 2 \times 10^{-2}$
Nortriptyline 5 mg/kg	339 ± 8	47 ± 4	0.15 ± 0.01
Cortex			
Saline	172 ± 10	78 ± 4	$0.46 \pm 2.08 \times 10^{-2}$
Nortriptyline 0.2 mg/kg	181 ± 6	106 ± 9*	$0.59 \pm 4.74 \times 10^{-2}$*
Nortriptyline 0.6 mg/kg	164 ± 12	70 ± 7	0.42 ± 0.03
Nortriptyline 2.5 mg/kg	204 ± 10	95 ± 12	$0.47 \pm 5 \times 10^{-2}$
Nortriptyline 5 mg/kg	197 ± 12	63 ± 5	$0.32 \pm 2.24 \times 10^{-2}$*
Hippocampus			
Saline	314 ± 11	424 ± 13	$1.37 \pm 7.4 \times 10^{-2}$
Nortriptyline 0.2 mg/kg	343 ± 29	447 ± 24	1.34 ± 0.1
Nortriptyline 0.6 mg/kg	316 ± 13	333 ± 30*	$1.04 \pm 7.5 \times 10^{-2}$
Nortriptyline 2.5 mg/kg	310 ± 17	391 ± 32	$1.23 \pm 9.3 \times 10^{-2}$
Nortriptyline 5 mg/kg	306 ± 25	345 ± 22	$1.16 \pm 7.8 \times 10^{-2}$
Striatum			
Saline	443 ± 40	140 ± 19	$0.31 \pm 2.4 \times 10^{-2}$
Nortriptyline 0.2 mg/kg	419 ± 17	135 ± 12	0.32 ± 0.03
Nortriptyline 0.6 mg/kg	405 ± 28	107 ± 15	$0.26 \pm 2.2 \times 10^{-2}$
Nortriptyline 2.5 mg/kg	431 ± 20	132 ± 15	$0.31 \pm 3.9 \times 10^{-2}$
Nortriptyline 5 mg/kg	451 ± 21	116 ± 15	$0.26 \pm 2.2 \times 10^{-2}$

* $P < .05$ vs. saline.
** $P < .01$ vs. saline.

- amitriptyline 0.15–10 mg/kg, 60 min before testing,
- paroxetine 1.25–30 mg/kg, 30 min before testing;

- s-CT (2.5 IU/kg, 90 min before test) plus each antidepressant.

The number of animals used to evaluate the analgesic effect of each dose was $n = 10–12$. Data are presented as analgesic effect (percentage of inhibition of the stretches vs. control values) ± S.E.M.

1.4. Data analysis

Statistical evaluation of the data was carried out by one- or two-way analysis of variance (ANOVA) followed by LSD and Newman–Keul's tests. The probability level of $P < .05$ was considered to be statistically significant.

1.5. Drugs

s-CT was kindly provided by Rhône-Poulenc-Rorer. Nortriptyline and amitriptyline were obtained from Sigma, Madrid, Spain, and paroxetine was a gift from SmithKline-Beecham, Spain.

All drugs were dissolved in saline and intraperitoneally injected in a volume of 10 ml/kg. Doses in the text refer to the free base of a given drug.

2. Results

In the first stage, the antidepressant effect of the tested drugs was analyzed in order to select doses without antidepressant activity to discard the influence of this effect in

Table 2
Levels of 5-HT, 5-HIAA, and turnover (5-HIAA/5-HT) expressed in ng/g in different structures of CNS in mice treated with different doses of amitriptyline ($6 < n < 8$)

	5-HT (ng/g)	5-HIAA (ng/g)	5-HIAA/5-HT (ng/g)
Midbrain			
Saline	692 ± 29	295 ± 21	$0.42 \pm 2.1 \times 10^{-2}$
Amitriptyline 2.5 mg/kg	723 ± 23	274 ± 13	$0.38 \pm 1.5 \times 10^{-2}$
Amitriptyline 5 mg/kg	673 ± 36	266 ± 20	$0.39 \pm 1.5 \times 10^{-2}$
Amitriptyline 10 mg/kg	745 ± 33	245 ± 11	0.33 ± 0.014**
Medulla oblongata			
Saline	516 ± 22	166 ± 11	$0.32 \pm 9.7 \times 10^{-3}$
Amitriptyline 2.5 mg/kg	510 ± 16	167 ± 6	$0.33 \pm 5.2 \times 10^{-3}$
Amitriptyline 5 mg/kg	507 ± 19	165 ± 9	$0.32 \pm 8.4 \times 10^{-3}$
Amitriptyline 10 mg/kg	510 ± 20	134 ± 7	$0.27 \pm 5.4 \times 10^{-3}$**
Hippocampus			
Saline	294 ± 7	148 ± 10	$0.50 \pm 3 \times 10^{-2}$
Amitriptyline 2.5 mg/kg	286 ± 14	136 ± 8	$0.48 \pm 2.5 \times 10^{-2}$
Amitriptyline 5 mg/kg	288 ± 12	135 ± 12	$0.47 \pm 4.5 \times 10^{-2}$
Amitriptyline 10 mg/kg	289 ± 11	121 ± 8	$0.42 \pm 1.6 \times 10^{-2}$
Striatum			
Saline	333 ± 14	176 ± 8	$0.53 \pm 2 \times 10^{-2}$
Amitriptyline 2.5 mg/kg	353 ± 18	176 ± 6	$0.51 \pm 4 \times 10^{-2}$
Amitriptyline 5 mg/kg	369 ± 4	188 ± 8	$0.51 \pm 2.2 \times 10^{-2}$
Amitriptyline 10 mg/kg	365 ± 6	159 ± 6	$0.43 \pm 1.4 \times 10^{-2}$*
Cortex			
Saline	138 ± 9	49 ± 3.5	$0.36 \pm 1.7 \times 10^{-2}$
Amitriptyline 2.5 mg/kg	143 ± 7	45 ± 3	$0.32 \pm 3 \times 10^{-2}$
Amitriptyline 5 mg/kg	149 ± 12	45 ± 2.5	$0.32 \pm 2.8 \times 10^{-2}$
Amitriptyline 10 mg/kg	154 ± 8	41 ± 3	$0.27 \pm 2 \times 10^{-2}$
Spinal cord			
Saline	426 ± 31	80 ± 6	$0.19 \pm 7.2 \times 10^{-3}$
Amitriptyline 2.5 mg/kg	424 ± 26	73 ± 5	$0.17 \pm 4.2 \times 10^{-3}$
Amitriptyline 5 mg/kg	409 ± 26	69 ± 5	$0.17 \pm 4.5 \times 10^{-3}$
Amitriptyline 10 mg/kg	452 ± 41	62 ± 3	$0.14 \pm 4.5 \times 10^{-3}$**

* $P < .05$ vs. saline.
** $P < .01$ vs. saline.

the analgesia. After the single administration of amitriptyline, nortriptyline, or paroxetine at the tested doses, no significant modification in the forced-swimming test was found: only one dose of nortriptyline slightly reduced the duration of the time of immobility (Fig. 1).

The writhing test was carried out in order to select a subanalgesic dose of CT. Its antinociceptive effect was tested (Fig. 2), and a subanalgesic dose of 2.5 IU/kg was selected for use together with the antidepressants.

The joint treatment with CT (2.5 IU/kg) did not modify the effect of amitriptyline or paroxetine on the forced-swimming test (Fig. 3).

Next, in order to assess the modifications of the serotonergic function induced by the different treatments, the levels of 5-HT and its metabolite 5-HIAA were determined in the midbrain, hippocampus, striatum, cortex, medulla oblongata, and spinal cord.

As expected, nortriptyline induced minor modifications that did not reach significant value in the level of 5-HT or its metabolite. When the ratio 5-HIAA/5-HT was calculated as turnover index, it was significantly reduced in the midbrain and in the cortex, but this effect was only found after treatment with the highest tested dose (Table 1).

The administration of amitriptyline did not induce significant changes either in 5-HT or 5-HIAA levels, however, the modification in the turnover index was greater than for nortriptyline and was significantly reduced in the midbrain, striatum, medulla oblongata, and spinal cord (Table 2).

The selective inhibitor of 5-HT re-uptake, paroxetine, was able to significantly reduce 5-HIAA levels. Furthermore, a statistically significant reduction of the turnover index was observed with all the tested doses in all the tested tissues (Table 3).

Table 3
Levels of 5-HT, 5-HIAA, and turnover (5-HIAA/5-HT) expressed in ng/g in different structures of CNS in mice treated with different doses of paroxetine ($6 < n < 8$)

	5-HT (ng/g)	5-HIAA (ng/g)	5-HIAA/5-HT (ng/g)
Midbrain			
Saline	772 ± 37	314 ± 10	$0.41 \pm 1.6 \times 10^{-2}$
Paroxetine 10 mg/kg	856 ± 26	265 ± 20	$0.31 \pm 1.9 \times 10^{-2}$**
Paroxetine 20 mg/kg	951 ± 48*	273 ± 13*	0.29 ± 0.015**
Paroxetine 30 mg/kg	913 ± 37*	216 ± 10**	$0.24 \pm 1.1 \times 10^{-2}$**
Medulla oblongata			
Saline	435 ± 23	166 ± 10	$0.38 \pm 1.1 \times 10^{-2}$
Paroxetine 10 mg/kg	513 ± 21*	152 ± 8	$0.30 \pm 1.2 \times 10^{-2}$**
Paroxetine 20 mg/kg	566 ± 15**	151 ± 6	$0.27 \pm 7.5 \times 10^{-3}$**
Paroxetine 30 mg/kg	547 ± 22**	143 ± 9	$0.26 \pm 1.6 \times 10^{-2}$**
Hippocampus			
Saline	370 ± 9	168 ± 4	$0.46 \pm 1.6 \times 10^{-2}$
Paroxetine 10 mg/kg	388 ± 11	141 ± 8*	0.36 ± 0.014**
Paroxetine 20 mg/kg	388 ± 16	143 ± 6**	$0.37 \pm 1.5 \times 10^{-2}$**
Paroxetine 30 mg/kg	384 ± 20	132 ± 7**	$0.34 \pm 1.5 \times 10^{-2}$**
Striatum			
Saline	580 ± 13	179 ± 8	0.31 ± 0.013
Paroxetine 10 mg/kg	621 ± 37	138 ± 9**	$0.22 \pm 9.2 \times 10^{-3}$**
Paroxetine 20 mg/kg	607 ± 44	126 ± 10**	$0.21 \pm 5.5 \times 10^{-3}$**
Paroxetine 30 mg/kg	599 ± 36	120 ± 6**	$0.20 \pm 6.5 \times 10^{-3}$**
Cortex			
Saline	147 ± 7	39 ± 1	$0.27 \pm 1.2 \times 10^{-2}$
Paroxetine 10 mg/kg	170 ± 11	29 ± 2**	$0.18 \pm 1.1 \times 10^{-2}$*
Paroxetine 20 mg/kg	168 ± 10	30 ± 2**	$0.21 \pm 3.3 \times 10^{-2}$*
Paroxetine 30 mg/kg	178 ± 16	28 ± 3**	$0.16 \pm 9.6 \times 10^{-3}$**
Spinal cord			
Saline	542 ± 39	79 ± 4	$0.15 \pm 4.7 \times 10^{-3}$
Paroxetine 10 mg/kg	571 ± 29	61 ± 4**	$0.11 \pm 4.1 \times 10^{-3}$**
Paroxetine 20 mg/kg	638 ± 25	66 ± 3*	$0.10 \pm 4.1 \times 10^{-3}$**
Paroxetine 30 mg/kg	555 ± 33	53 ± 3**	$0.09 \pm 1.9 \times 10^{-3}$**

* $P < .05$ vs. saline.
** $P < .01$ vs. saline.

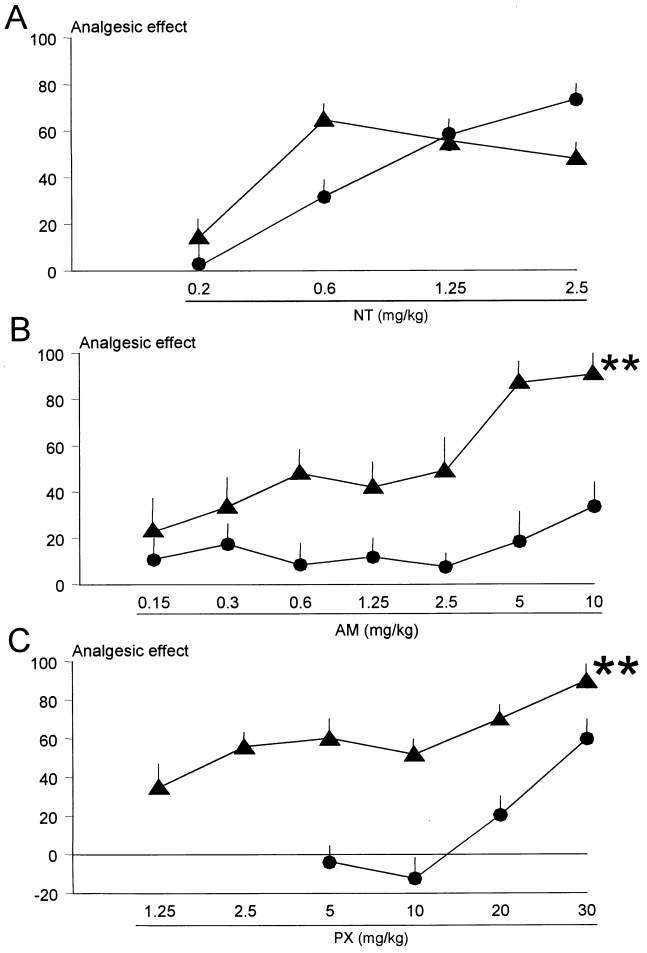

Fig. 4. Effect of antidepressants + CT in the acetic acid test. Lines represent the analgesic effect (% inhibition vs. control) ± S.E.M. of different doses of (A) nortriptiline (NT), (B) amitriptiline (AM), and (C) paroxetine (PX) in control animals (circles) and in animals treated with s-CT (triangles). ** $P < .01$ vs. control. ($10 < n < 12$).

Regarding the analgesic activity, the effect of the three antidepressants was evaluated when administered to naive animals or after the administration with the previously selected dose of CT (2.5 IU/kg). The use of a dose of CT lacking in effectiveness permits us to discard any additive effect. Nortriptyline and paroxetine at the doses tested induced dose-dependent analgesia; amitriptyline induced analgesia only at the highest dose tested. The effect of higher doses could not be evaluated because of the presence of motor disturbances. Pre-treatment with CT did not modify the analgesia induced by nortriptyline (Fig. 4A) but produced a statistically significant shift to the left of the dose–response curves of amitriptyline and paroxetine.

3. Discussion

One of the generally accepted uses of antidepressants is the treatment of some kinds of pain such as rheumatic pain (Usha et al., 1996), diabetic neuropathy and post-herpetic neuralgia (Onghena and Van Houdenhove, 1992), and neuropathic post-mastectomy pain (Kalso et al., 1995). They are used especially when the problem can not be solved satisfactorily with other conventional analgesics, and they are frequently administered together with anti-inflammatory or opioid drugs. Their antinociceptive activity has also been demonstrated in animal tests (Acton et al., 1992; Seltzer et al., 1989; Ventafridda et al., 1990). Our results are in agreement with these data and show that amitriptyline, nortriptyline, and paroxetine induce dose-dependent analgesia in the acetic acid test in mice, a test that is widely used in the first stages of the evaluation of antinociceptive drugs (Savelon et al., 1998) and is related with visceral pain (Savelon et al., 1998).

Although the use of antidepressant drugs as analgesics is quite common, the mechanisms involved in this effect are not fully understood. They seem to be based on their pharmacological effect of blocking the re-uptake of neurotransmitters such as NA, 5-HT, or both, although a catecholaminergic hypothesis is insufficient to explain the actions of antidepressants in relieving pain in the absence of depression (Merskey, 1997); in fact, there is not a linear relationship between the analgesic effectiveness and the inhibitory monoamine re-uptake potency (Rafieian-kopaei and Sewell, 1999). Moreover, antidepressants have also been reported to enhance opioid analgesia (Botney and Fields, 1983), and there are data demonstrating that some antidepressants such as imipramine may alter the expression of μ-opioid receptors in rat forebrain (de Gandarias et al., 1998) or improve the analgesia induced by μ-selective opioid agonists without modification of the effect of κ agonists, as does nefazodone (Pick et al., 1992). From this, it could be suggested that relationships between antidepressants and the opioid system could also play a role in the antinociceptive effects of these drugs.

It is well established that the analgesic effectiveness of these drugs is not closely related to their effect on mood (Feinmann, 1985; Gorard et al., 1995; Panerai et al., 1991; Poulsen et al., 1995), and that the dose required to produce analgesia is lower than that required to induce an antidepressant effect (Max, 1994a,b). In order to assess if the antidepressant effect may have an influence on the analgesia under our experimental conditions, the Porsolt test was performed. This test is widely used to predict potential antidepressant action in humans, since the immobility time is reduced by clinically relevant doses of tricyclic and atypical antidepressants and 5-HT re-uptake inhibitors in rats and mice (Guo et al., 1995; Kitada et al., 1981; Lucki et al., 1994; Porsolt, 1997). Our results conform to previous data and show that the antinociceptive doses of nortriptyline, amitriptyline, and paroxetine lack antidepressant effectiveness.

On the other hand, the adverse effects of the antidepressants restrict their indication as analgesics, and even having gained good pain relief, they could be the cause of the discontinuation of the treatment. To improve compliance,

the use of smallest effective dose has been proposed (Kalso et al., 1995). Thus, the possibility of improvement of the analgesia induced by low doses of antidepressant became an attractive target.

It is well known that CT induces analgesia in pathologies of diverse etiology: osteoarticular disorders, Paget's disease (Bijvoet and Hjansen, 1967), phantom limb pain (Jaeger and Maier, 1992), or reflex sympathetic dystrophy (Gobelet et al., 1992) among others, and as with the antidepressants, it is often used when other treatments fail. It has also been previously demonstrated (Clementi et al., 1984, 1985; Martín et al., 1992; Welch et al., 1986), and present data confirm that CT induces dose-dependent analgesia in animals. The mechanism underlying this effect is not completely known, but the involvement of the serotonergic system appears to be important because when the integrity of the serotonergic pathways or its function are disturbed, the analgesic effect is reduced or even disappears (Clementi et al., 1984, 1985; Colado et al., 1994). Furthermore, CT is able to increase the in vitro release of 5-HT in spinal medulla (Bourgoin et al., 1988), although in vivo treatment did not modify the levels of 5-HT, 5-HIAA, or the turnover evaluated as the ratio metabolite/neurotransmitter in CNS as demonstrated by previous (Ormazábal et al., 1997) and present data. Another common point between CT and antidepressants is the fact that both may interact with the opioid system (Martín et al., 1992; Ormazábal et al., 1997; Welch et al., 1986), enhancing opioid effects, although the underlying mechanisms are in both cases unknown.

The joint administration of a subanalgesic dose of CT was able to significantly increase the analgesic effect of amitriptyline and paroxetine, so a single additive interaction could be discarded especially when it is considered that analgesic effectiveness was reached even when subanalgesic doses of the antidepressants were used. These results suggest that CT potentiates the antinociceptive effect of antidepressants, and it is important that it does so without changing the antidepressant activity of the drugs. It is also interesting to remark that whereas the effect of amitriptyline and paroxetine was increased, the analgesia induced by nortriptyline was not significantly modified. To try to discern the mechanisms that could be involved in the CT–antidepressant interaction, the importance of the serotonergic system in the analgesic effect of CT described above and the difference in the effect of the antidepressants in the 5-HT re-uptake must be considered. Interestingly, both amitriptyline and paroxetine are drugs accepted as inhibitors of 5-HT re-uptake, however, nortriptyline also inhibits the re-uptake of NA. The inhibition of re-uptake leads to a decrease in the level of the main 5-HT metabolite 5-HIAA because the metabolism of 5-HT takes place in the neuron. On the other hand, the increase in the extracellular concentrations of 5-HT activates autoreceptors on 5-HT cell bodies, which in turn limits the excessive release of the neurotransmitter. Consequently and in accordance with this, our results show the turnover (metabolite/neurotransmitter) of 5-HT significantly reduced after treatment with amitriptyline and paroxetine.

The other antidepressant studied, nortriptyline, reduces NA re-uptake, having little or no effect on 5-HT levels. Our results are in agreement with this and show significant reduction only in one of the structures and only with the highest dose used.

From this data, it could be suggested that the serotonergic system plays an important role in the CT–antidepressant interaction although other possibilities cannot be discarded.

In summary, this experiment was designed to investigate if CT may be able to increase the analgesic effect of the antidepressant drugs that reduce the uptake of 5-HT, and our data confirm this hypothesis. CT clearly potentiates the analgesia induced by amitriptyline (a non-selective inhibitor of the re-uptake of NA and 5-HT) and paroxetine (inhibitor of the 5-HT re-uptake), whereas the analgesic effect of nortriptyline was not significantly modified. Inasmuch as the use of the antidepressants in analgesia is often limited because of their adverse effects that are more frequent when high doses are used, it could be suggested that the joint administration of small doses of antidepressants modifying 5-HT re-uptake and CT may be an alternative that merits further consideration and study.

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