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Antinociceptive effects of tricyclic antidepressants and their noradrenergic metabolites

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Abstract

This study evaluates the antinociceptive effect of several tricyclic antidepressants in four nociceptive tests which employ either thermal (hot plate and tail flick tests) or chemical (formalin and acetic acid tests) stimuli. Forced swimming test was also performed as a model of depression and an activity test was also performed. Mixed antidepressants in current clinical use: amitriptyline, imipramine and clorimipramine and their respective main secondary metabolites which preferentially inhibit noradrenaline reuptake: nortriptyline, desipramine and desmethylclorimipramine, were tested (2.5–20 mg/kg, i.p.) in mice. The results show a stronger antinociceptive effect in chemical tests induced by all the drugs, compared with thermal tests. The doses needed to produce antinociception were lower than those inducing an antidepressive effect, both effects being mutually independent. The overall results show that preferentially noradrenergic tricyclics induced an antinociceptive effect comparable with that of mixed tricyclics, indicating that noradrenaline reuptake plays an important role in tricyclic-induced antinociception.

Keywords: Tricyclics; Antidepressants; Pain; Antinociception; Noradrenaline; Serotonin

1. Introduction

It is generally accepted that there is a relationship between chronic pain and depression (Magni et al., 1987). In this sense, it is known that serotonin, noradrenaline (Basbaum and Fields, 1984) and opioids (Schmauss and Emrich, 1988; De Gandarias et al., 1999) are involved in both nociceptive and depressive disorders, as well as in the mechanism of action underlying the antinociceptive (Valverde et al., 1994) and antidepressive effects of antidepressants (Carlsson et al., 1969). In clinical practice, antidepressants—usually tricyclics—are widely used in several painful conditions, as well as opioids and other analgesics, and have been proven to be effective in the management of pain of diverse aetiology (Walsh, 1983; Onghena and Van Houdenhove, 1992; McQuay et al., 1996). On the other hand, it has been previously reported that opiate analgesics with monoaminergic reuptake inhibitory properties may induce an antidepressant-like effect in mice (Rojas-Corrales et al., 1998) accounting for the interrelationship between these two entities.

In spite of the wide use of antidepressants in painful disorders, the nature of antidepressant-induced analgesia remains to be elucidated. It has been suggested that antidepressant drugs have specific analgesic properties, and a body of clinical (Magni et al., 1987; Magni, 1991) and experimental (Spiegel et al., 1983; Tura and Tura, 1990; Casas et al., 1993, 1995) types of evidence together seems to demonstrate that the analgesic may be independent of the antidepressant effects. Furthermore, a wealth of clinical literature advocates the use of antidepressants in the management of certain pain states with or without co-existing depression. However, the relationship between antidepressive and analgesic effects of these drugs have not yet been fully elucidated (O'Malley et al., 2000). Regarding the mechanisms involved, it is well known that the major mechanism of action of tricyclic antidepressants is mainly related to interference with the reuptake of

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monoamines in the synapse (Carlsson et al., 1969), with the secondary amines such as nortriptyline (NOR) or desipramine (DMI) being more effective in the inhibition of noradrenaline than serotonin reuptake (Sulser and Mobley, 1980).

In the 1980s the selective serotonin reuptake inhibitors (SSRIs) were successfully introduced in the treatment of depression, and therefore they were also used to alleviate pain syndromes (Jung et al., 1997). The more beneficial profile of side effects of SSRIs, compared with tricyclics, contributed to the rapid introduction of these drugs in the treatment of affective disorders. Thereafter, these newer antidepressants offered the promise of an important therapeutic advance in the treatment of pain (De Angelis, 1992), as serotonin control is believed to be one of the primary mechanisms in pain modulation (Bowker and Abbott, 1990; Bardin et al., 2000). However, there was actually no evidence that SSRIs were better than the so-called 'older antidepressants' in pain treatment (Collins et al., 2000). In fact, an exacerbation of acute pain by SSRIs has been reported (Dirksen et al., 1998).

To date, tricyclics are still generally considered the more effective antidepressants in pain treatment, and in placebo-controlled trials they have proved to be currently still the drugs of first choice in treating several painful conditions such as polyneuropathy (Sindrup and Jensen, 2000). In particular, amitriptyline (AMT), imipramine (IMI) and clomipramine (CLO) are the antidepressants most frequently used in clinical studies of chronic pain (De Angelis, 1992) and AMT is further being considered a therapeutic standard in several painful conditions (Bryson and Wilde, 1996; Morello et al., 1999).

Although tricyclics are especially useful in some situations associated with chronic pain, the greater part of our knowledge concerning the mechanism of their analgesic action is largely derived from animal experimentation, which includes procedures for assessing acute pain. In this context, previous studies have shown that the antinociceptive potency of reuptake inhibitors, as well as the mechanisms involved in their effect, varies according to the nature of the stimuli (Ardid et al., 1992; Dirksen et al., 1994; Casas et al., 1995; Micó et al., 1997). Furthermore, some of these experimental results suggest that monoaminergic specificity also influences the antinociceptive potency of reuptake inhibitors (Ardid et al., 1992), a finding that remains to be demonstrated definitively.

Taking into account that secondary amines are usually more selective than tertiary ones in the inhibition of noradrenaline reuptake, we considered it of interest to evaluate the effectiveness of three antidepressants frequently used in pain treatment: IMI, AMT and CLO, and their respective secondary, demethylated, main metabolites: DMI, NOR and desmethylclomipramine (DMCLO), in four antinociceptive tests. The tests used are intended to evaluate the response to pain induced by either thermal or chemical stimulus, which involve different levels of sensory–motor integration in the central nervous system (CNS). Finally, given the difficulty of exploring the emotional components of pain in animals, and considering that this aspect is very important when an antidepressant is evaluated as analgesic, we have tested the tricyclic antidepressants in a behavioral paradigm, the forced swimming test (FST). This test has been considered as a model of the depressive state and is widely used as a predictor of antidepressant activity in rodents (Porsolt et al., 1978). Using this set of tests, our aim is to study the antinociceptive efficacy of these frequently used tricyclics and the putative relationship between both antinociceptive and antidepressive effects of these drugs.

2. Methods

2.1. Animals

Male mice of the OF1 strain (20–25 g), obtained from a University Central Animal Service, were maintained on a 12-h light–dark schedule (light on at 08:00 h) with free access to food and water and at a constant environmental temperature (21±1 °C). Animals were housed 24 h before starting the experiments. We respected the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983) and our experimental protocol was approved by the Local Committee for Animal Experimentation (license number 079604). Experiments were performed blind on groups of 10 naive mice per group. Animals were used once and not reused thereafter.

2.2. Drugs

Imipramine HCl, desipramine HCl, amitriptyline HCl, nortriptyline HCl, clomipramine HCl and desmethylclomipramine HCl were purchased from Sigma–Aldrich (Barcelona, Spain). All the antidepressants or their vehicle (0.9% saline) were i.p. injected 30 min before starting the different tests. The doses tested of the antidepressants were 2.5, 5, 10 and 20 mg/kg. Injection volume was 0.1 ml per 10 g of body weight.

2.3. Nociceptive tests

2.3.1. Nociceptive tests employing thermal stimulus

2.3.1.1. Hot plate test (Woolfe and Macdonald, 1944)

Mice were placed on a hot plate (Digital DS-37 Socrel model), that was thermostatically maintained at 55±0.2 °C. A Plexiglas cylinder was used to confine the animal to the hot plate. The reaction time of each animal (either paw licking or jumping) was considered a pain response. The latency to reaction was measured. When none of these responses occurred within 30 s exposure

(cut-off), the test was terminated in order to avoid damage to the animal.

2.3.1.2. Tail flick test (D'Amour and Smith, 1941)

Mice were exposed to an overhead lamp (100 W) of an LI 7106 tail-flick model, which had a photoelectric sensor for automatic arrest and a digital time counter. Animals were kindly restrained by the investigator. This test was carried out on three different points of the mouse tail by focusing a light beam on each point, with 1-min intervals between each exposure. Determinations were done at those three different points and the average of these was considered as the pain latency. When the animals did not respond after 10 s of exposure (cut-off), the test was terminated in order to avoid damaging the animal's tissue.

2.3.2. Nociceptive tests employing chemical stimulus

2.3.2.1. Acetic acid test (Koster et al., 1959)

A 0.8% acetic acid solution was i.p. injected, 0.1 ml per 10 g of body weight, as an irritant stimulus. After a 6-min period, the animal was placed in a Plexiglas chamber for observation, and the number of abdominal contortions was recorded for 2 min.

2.3.2.2. Formalin test (Dubuisson and Dennis, 1977)

A 20-μl volume of a 1% formalin–saline solution was injected into the dorsal surface of the mouse's hind paw. Immediately after injection, the mouse was placed in a Plexiglas chamber for observation. The amount of time the animal spent licking the injected paw was recorded over a 2-min period immediately after formalin injection.

2.4. Behavioral tests

2.4.1. The forced swimming test (Porsolt et al., 1977)

Naive mice were dropped individually into glass cylinders (height=25 cm, diameter=10 cm) containing water 6 cm deep at 22±1 °C, and left there for 6 min. The total duration of immobility during the last 4 min was recorded. Reduction of immobility in this test was considered to indicate antidepressant activity. A mouse was judged to be immobile when it remained floating in the water making only the movements necessary to keep its head above the water.

2.4.2. Activity test

A S.M.A.R.T. (Spontaneous Motor Activity Recording and Tracking) apparatus provided by LETICA Scientific Instruments was used for motor activity measurement. Each animal was placed in a Plexiglas chamber (20 cm× 20 cm×15 cm). After 30 min, the apparatus began to record the total activity of each animal over a 10-min period. Motor activity was assessed following the arbitrary units established by the S.M.A.R.T.

2.5. Statistical analysis

Statistical analysis was performed on raw data. The results are expressed as the mean±S.E.M. in the text. The figures show values of % of maximum possible effect (%MPE): %MPE=((response−control)/(cut-off−control)×100). Response is the mean of the parameter measured in the different tests for each group of treatment. Control is the mean of the parameter measured in the different tests for control groups. Cut-off was established for each test as the maximum effect (30 s for hot plate test, 10 s for tail flick test, 0 contortions for acetic acid test, 0 s for formalin test, 0 s for FST and 0 arbitrary units for activity test). Differences between groups were analysed by Student–Newman–Keuls post hoc test after significant one-way ANOVA. A P value of <0.05 was considered to be significant.

3. Results

3.1. Imipramine and desipramine

The %MPE values for imipramine are shown in Fig. 1A. In the hot plate test ($F_{(4,45)}=3.7289$, $P=0.0105$) only the highest dose (20 mg/kg) of IMI was effective in enhancing hot plate latency (10.9±1.08 vs. 7.2±0.76 s of a saline control treated group, $P<0.05$), while none of the tested doses induced a significant effect in tail flick test ($F_{(4,45)}=1.1585$, $P=0.3418$). On the other hand, IMI displayed a strong and dose-related antinociceptive effect in both tests employing chemical stimulus: acetic acid ($F_{(4,45)}=17.7124$, $P=0.0000$; saline: 8.40±1.00 contortions) and formalin ($F_{(4,45)}=10.6577$, $P=0.0000$; saline: 35.40±3.50 s) tests. A significant immobility time-reduction was observed with 10 (92.10±12.07 s, $P<0.05$) and 20 (85.80±13.61 s, $P<0.05$) mg/kg of IMI versus saline (149.30±8.57 s) in the FST ($F_{(4,45)}=21.5600$, $P=0.0000$), while in the activity test ($F_{(4,45)}=5.3616$, $P=0.0013$) the reduction in spontaneous motor activity was significant after 5 mg/kg (12.19±4.98 vs. 27.84±5.06 arbitrary units of control group, $P<0.05$) of IMI.

The results obtained with the secondary amine DMI are summarized as %MPE in Fig. 1B. In the tail flick test ($F_{(4,45)}=14.1145$, $P=0.0000$) only 20 mg/kg induced a significant effect (7.20±0.76 vs. 3.50±0.33 s of a saline control treated group, $P<0.01$), while no significant effect was induced in the hot plate test ($F_{(4,45)}=1.5903$, $P=0.1933$; saline: 7.00±1.00 s). However, DMI induced a dose-related antinociceptive effect ($P<0.01$) in both acetic acid ($F_{(4,45)}=12.1218$, $P=0.0000$; saline: 9.90±0.79 contortions) and formalin ($F_{(4,45)}=18.3207$, $P=0.0000$; saline: 29.1±3.47 s). In the FST ($F_{(4,45)}=8.0554$, $P=0.0001$) only the highest dose was able to reduce significantly the immobility time (31.67±9.03 vs. 123.10±15.05 s of a

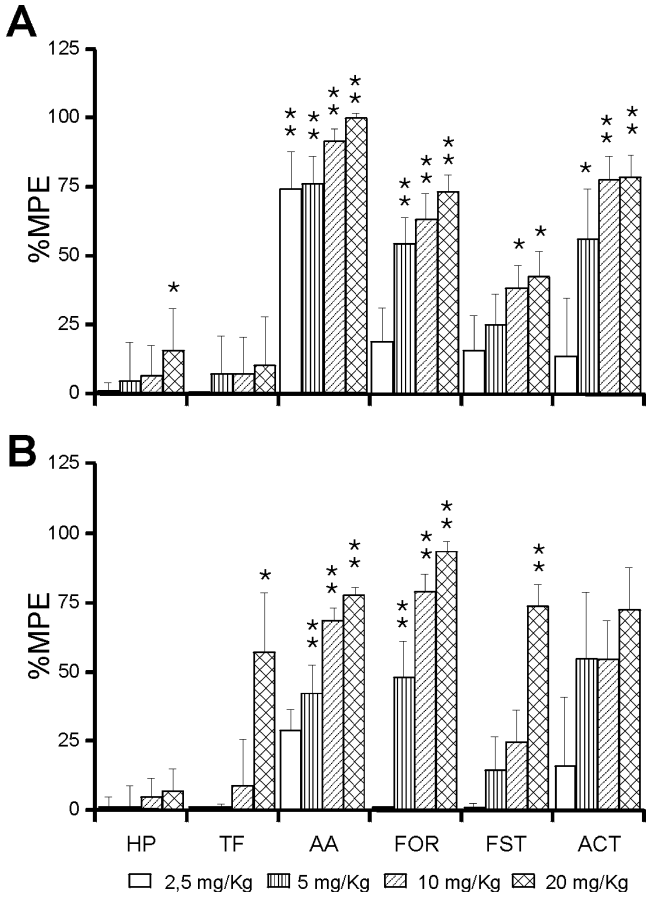

Fig. 1. Effect induced by (A) imipramine, IMI, (i.p. administered 30 min before test) and (B) desipramine, DMI, (i.p. administered 30 min before test) in four nociceptive and two behavioral tests. HP, hot plate test; TF, tail flick test; FOR, formalin test; AA, acetic acid test; FST, forced swimming test; ACT, activity test. Bars and error bars express percentage of maximum possible effect (%MPE), Student Newman–Keuls after significant ANOVA was performed on raw data, $*P<0.05$ vs. saline, $**P<0.01$ vs. saline.

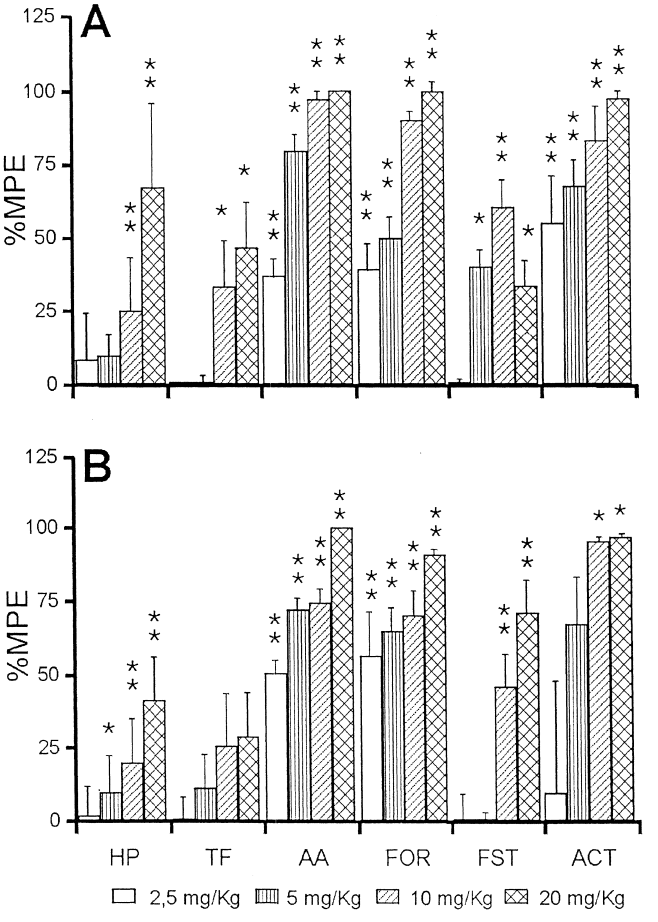

Fig. 2. Effect induced by (A) amitriptyline, AMT, (i.p. administered 30 min before test) and (B) nortriptyline, NOR, (i.p. administered 30 min before test) in four nociceptive and two behavioral tests. HP, hot plate test; TF, tail flick test; FOR, formalin test; AA, acetic acid test; FST, forced swimming test; ACT, activity test. Bars and error bars express percentage of maximum possible effect (%MPE), Student–Newman–Keuls after significant ANOVA was performed on raw data, $*P<0.05$ vs. saline, $**P<0.01$ vs. saline.

saline control treated group, $P<0.01$), while no significant effect was induced in the activity test ($F_{(4,45)}=1.4160$, $P=0.2441$; saline: 15.27±6.27 arbitrary units).

3.2. Amitriptyline and nortriptyline

AMT (Fig. 2A) induced a dose-related antinociceptive effect at 10 (11.20±0.90 s, $P<0.01$) and 20 (21.60±1.46 s, $P<0.01$) mg/kg versus saline (5.00±0.25 s) in the hot plate test ($F_{(4,45)}=58.2495$, $P=0.0000$). Similarly, in the tail flick test ($F_{(4,45)}=6.5362$, $P=0.0003$) both 10 (6.50±0.75 s, $P<0.05$) and 20 (7.20±0.74 s, $P<0.05$) mg/kg were effective in enhancing pain threshold versus saline-treated mice (7.20±0.74 s). In both chemical tests, all the administered doses exhibited a significant effect ($P<0.01$), reaching MPE values of 100% (0 contortions) and 99.68% (0.10±0.89 s) at 20 mg/kg in acetic acid ($F_{(4,45)}=99.6326$, $P=0.0000$; saline: 9.80±0.57 contortions) and formalin ($F_{(4,45)}=25.8869$, $P=0.0000$; saline: 31.20±4.08 s) tests, respectively. In the FST ($F_{(4,45)}=7.7410$, $P=0.0001$) AMT induced the highest reduction in immobility time at 10 mg/kg (43.50±9.92 vs. 109.33±15.03 s of a saline control treated group, $P<0.01$), while all the doses diminished the spontaneous motor activity in a dose-dependent fashion ($F_{(4,45)}=7.9589$, $P=0.0001$; saline: 11.00±2.24 arbitrary units).

NOR (Fig. 2B), the main metabolite of AMT, induced a significant and dose-dependent antinociceptive effect in the hot plate test ($F_{(4,45)}=31.2879$, $P=0.0000$) from 5 mg/kg (8.30±0.72 vs. 5.80±0.47 s of a saline control treated group, $P<0.05$). In the tail flick test ($F_{(4,45)}=2.9728$, $P=0.0292$), a slight enhancement of the latency was observed, but it was not significant at 20 mg/kg of NOR (5.60±0.58 vs. 3.80±0.35 s of a saline control treated group, $P>0.05$). On the other hand, all the doses produced a significant antinociceptive effect ($P<0.01$) in both acetic

acid ($F_{(4,45)}$=44.0515, P=0.0000; saline: 9.30±0.83 contortions) and formalin ($F_{(4,45)}$=10.9747, P=0.0000; saline: 36.40±3.94 s) tests. In the FST ($F_{(4,45)}$=9.2130, P=0.0000), NOR induced an immobility time-reduction effect at 10 (68.00±14.83 s, P<0.05) and 20 mg/kg (36.20±14.88 s, P<0.01) vs. saline (125.70±12.10 s). Significant reductions in spontaneous motor activity ($F_{(4,45)}$=4.8864, P=0.0023) were observed from 10 mg/kg (1.19±0.64 vs. 24.40±4.95 arbitrary units of a saline control treated group, P<0.01).

3.3. Clomipramine and desmethylclomipramine

As it is shown in Fig. 3A, CLO significantly enhanced hot plate latencies ($F_{(4,45)}$=5.5706, P=0.0010) from 5 mg/kg (7.90±0.81 s, P<0.05) to 20 mg/kg (8.40±0.71 s, P<0.05) versus saline (5.70±0.27 s), while none of these doses produced an antinociceptive effect in the tail flick test ($F_{(4,45)}$=1.8246, P=0.1407). The number of contortions in the acetic acid test ($F_{(4,45)}$=27.6440, P=0.0000; saline: 8.40±1.09 contortions) was diminished dose-dependently by the three highest doses tested (P<0.01). The time spent licking in the formalin test ($F_{(4,45)}$=14.4114, P=0.0000; saline: 35.40±3.5 s) was significantly reduced by all the doses tested. CLO was ineffective in reducing immobility time at the administered doses in the FST ($F_{(4,45)}$=0.7050, P=0.5927); while in the activity test ($F_{(4,45)}$=3.4982, P=0.0143) a significant reduction in activity was observed at 10 mg/kg (6.67±2.18 vs. 23.92±4.79 arbitrary units).

The results obtained with DMCLO are summarized in Fig. 3B. In the hot plate test ($F_{(4,45)}$=6.9540, P=0.0002) a significant antinociceptive effect was observed from doses ranging from 5 mg/kg (9.20±0.80 s, P<0.05) to 20 mg/kg (10.50±0.79 s, P<0.01) compared with saline-treated mice (6.60±0.68 s). In the tail flick test ($F_{(4,45)}$=20.9507, P=0.0000) a significantly enhanced pain threshold was observed at 20 mg/kg (6.10±0.33 vs. 3.30±0.33 s of a saline control treated group, P<0.01). However, all the doses except the lowest were able to diminish significantly (P<0.01) the number of contortions in the acetic acid test ($F_{(4,45)}$=33.4269, P=0.0000; saline: 10.60±1.18 contortions). The time spent in paw licking in the formalin test was reduced significantly by all the doses ($F_{(4,45)}$=18.5978, P=0.0000; saline: 33.10±3.46 s). In the FST ($F_{(4,45)}$=4.3398, P=0.0047) the dose of 20 mg/kg was able to induce a reduction in immobility time (46.56±14.10 vs. 128.75±10.68 s of a saline control treated group, P<0.01). No significant effect was induced in the spontaneous motor activity ($F_{(4,45)}$=1.4241, P=0.2415; saline: 15.30±6.26 arbitrary units).

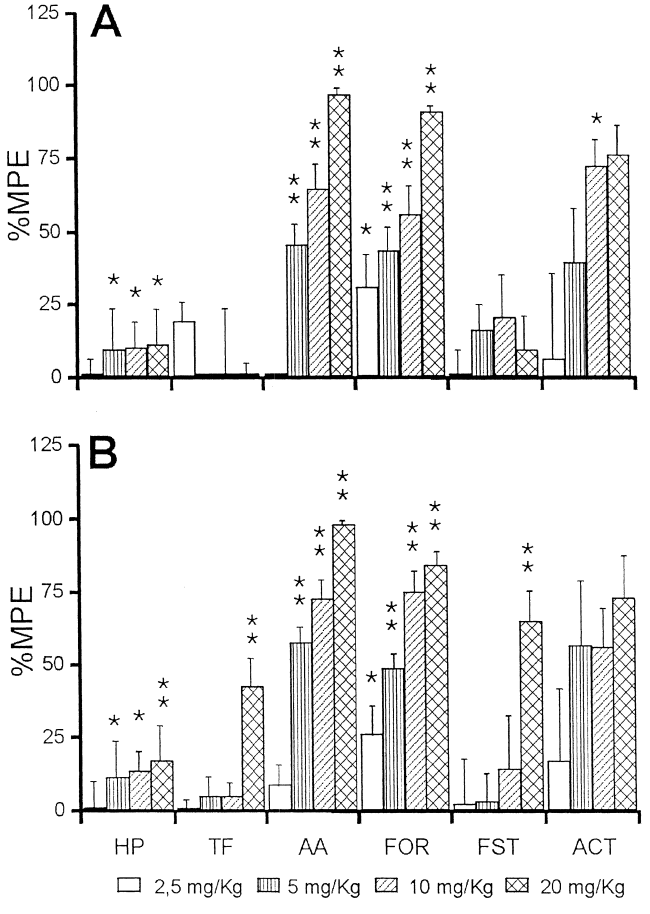

Fig. 3. Effect induced by (A) clomipramine, CLO, (i.p. administered 30 min before test) and (B) desmethylclomipramine, DMCLO, (i.p. administered 30 min before test) in four nociceptive and two behavioral tests. HP, hot plate test; TF, tail flick test; FOR, formalin test; AA, acetic acid test; FST, forced swimming test; ACT, activity test. Bars and error bars express percentage of maximum possible effect (%MPE), Student–Newman–Keuls after significant ANOVA was performed on raw data, *P< 0.05 vs. saline, **P<0.01 vs. saline.

4. Discussion

This study evaluates the antinociceptive effect of tricyclic antidepressants in four painful conditions integrated at different levels in the CNS (Mogil et al., 1999). The FST was selected to correlate the effectiveness of the drugs in both pain and depression models. An activity test was performed to measure the putative impairment of motor function, since sedation is a frequent side effect of tricyclics. By these means we were able to study the relationship of this impairment with immobility in the FST and with responses to pain in the nociceptive tests.

The results show a significant antinociceptive effect induced by all the tricyclic antidepressants tested. The doses needed to obtain antinociception were lower than those effective in inducing an antidepressive effect, according to some clinical data (McFarlane et al., 1986). Our results did not provide evidence for a relationship between the overall antinociceptive effectiveness of the drugs and their monoaminergic specificity. Nevertheless, some differ-

ences were observed in the potency of these antidepressants depending on the test performed.

Previous reports have shown that antidepressant drugs elicit an analgesic effect in a wide variety of nociceptive tests (Spiegel et al., 1983; Tura and Tura, 1990; Casas et al., 1993, 1995) and it has been demonstrated that noradrenergic antidepressants, such as DMI and NOR, are more effective in doing so when chemical rather than thermal tests are used (Ardid et al., 1992). Results from controlled clinical studies carried out by Atkinson et al. (1999), among others, suggest that at standard dosages noradrenergic agents may provide more effective analgesia in back pain than do SSRIs. Furthermore, Max et al. (1992), have demonstrated that DMI was as efficient as AMT in diabetic neuropathy, suggesting that inhibition of reuptake of noradrenaline mediates the analgesic effect of antidepressant drugs in this painful disorder. Furthermore, experimental studies have provided data which suggests that the interaction with noradrenaline transporter mediates the potentiation of opiate-analgesia induced by antidepressants (Bohn et al., 2000). Nevertheless, the assumption that noradrenergic antidepressants are more potent as analgesics, compared with antidepressants with a pronounced serotonergic effect, remains controversial (Aigner and Bach, 2000).

The overall results in this study show us that the greater differences in the effects induced by the drugs were related to the type of test performed rather than to their selectivity on monoamine reuptake. Previous studies (Eschalier et al., 1992) have suggested that the positive results obtained with antidepressants in nociceptive tests range from chemical (best) to thermal (worst). In agreement with this finding, we here demonstrate that all antidepressants tested elicited a strong antinociceptive effect in chemical tests. However, it is hard to establish the relative potency of mixed versus noradrenergic drugs. IMI displayed higher effect (%MPE=100.00±1.19) than DMI (%MPE= 77.78±2.93) in the acetic acid test; however, DMI induced higher %MPE values in the formalin test versus acetic acid test (%MPE=93.81±2.99 vs. 73.16±6.07). On the other hand, similar effects were found between AMT and CLO compared with their main active secondary metabolites, NOR and DMCLO, in the formalin test. These results suggest that noradrenergic antidepressants induce similar effects to mixed antidepressants and, therefore, that the noradrenergic component plays an important role. However, in other types of studies, AMT was effective in enhancing the antinociceptive effect induced by adrenal medullary transplants into the spinal cord of the rat, while selective compounds like DMI and fluvoxamine, were not so effective (Ortega-Alvaro et al., 1997).

Some authors have indicated previously that both serotonin and endogenous opioids are involved in the analgesic effect of AMT and CLO (Eschalier et al., 1981; Hamon et al., 1987; Sacerdote et al., 1987). On this point, antinociception induced by opiates with monoaminergic properties has been reported to be enhanced by manipulation of the serotonin system (Rojas-Corrales et al., 2000). However, in our study DMCLO induced similar %MPE values to CLO in chemical tests, the latter being more selective in the inhibition of serotonin reuptake (Hyttel, 1982). Similarly, the effects induced by DMI and NOR were found to be similar to those of IMI and AMT. These results suggest that the serotonergic component is not the primary component involved in the antinociceptive effect of tricyclic antidepressants, although we cannot rule out a role for this component. In fact, mixed antidepressants such as IMI and AMT displayed the highest effects, and were able to eliminate all contortions completely, reaching MPE values near to 100% at 20 mg/kg. This is consistent with other clinical data in which mixed antidepressants are more efficient than selective compounds (Onghena and Van Houdenhove, 1992).

It is interesting that the doses which induced an elevation of the pain threshold in chemical tests were near to those which induced a sedative effect. However, IMI, AMT and NOR were effective at 2.5 mg/kg in the acetic acid test, whereas none of the antidepressants, apart from AMT, induced significant sedation at this dose. Previous reports have suggested that changes in motor activity could not account for the antinociceptive effect induced by antidepressants, at least as far as the hot-plate and writhing tests are concerned (Ardid et al., 1992). Nevertheless, the relationship of sedation and chemical antinociception remain to be further investigated.

In thermal tests, the responses to pain were variable, especially in the tail flick, where the drugs induced only a weak effect, with IMI and CLO inducing no effect. The secondary amines seemed to be more efficient in this test, but AMT was the more effective antidepressant, and lost statistical significance after demethylation. The variable results in the tail flick test may be related to the kind of response required, mainly integrated at spinal level, while the other tests required a more elaborate response (Abbott et al., 1982). Contrary to this hypothesis, Spenger et al. (2000) have demonstrated recently a cortical involvement in the response to tail stimulation. Moreover, Dirksen et al. (1994) have suggested that the site of action of AMT varies among the pain modalities, even when similar (thermal) stimuli are employed.

It has been postulated that two factors are involved when a nociceptive test is used to assess drug effects (Dennis et al., 1980): the physical and temporal properties of the noxious stimulus and the pattern of the required motor response. Thus, the different results obtained among the nociceptive tests might then reflect the differential processing of the noxious stimuli, with different physical and temporal properties, and which trigger different motor responses. Unfortunately, the specific modulation of these responses by the drugs affecting the different monoaminergic systems is still unknown.

Given the wide variety of antidepressants and their

relative potencies in respect of the inhibition of noradrenaline and/or serotonin reuptake (Hyttel, 1982), as well as the existence of different thermal and chemical nociceptive tests, the relationship of these factors needs to be investigated further. Actually, although tricyclic antidepressants play an important role in several painful disorders, no clear relationships have been observed between the mechanism of action of the drugs and the effect in different pain conditions (Sindrup and Jensen, 1999), thus relationships between drug and pain mechanisms need to be elucidated.

In relation to the behavioral effects, antinociception induced by IMI, AMT, citalopram, and maprotiline have been demonstrated to be independent of their antidepressive effect in rats (Korzeniewska-Rybicka and Plaznik, 1998). Our results show that both effects induced by AMT, IMI, NOR, DMI and DMCLO are also independent of each other in mice, and that antinociception is produced at lower doses than those needed to obtain an antidepressive-like effect. Analysing the reduction of immobility in the FST, i.e. the antidepressant-like effect, we found that CLO was not effective in this test at the doses and time tested, and that IMI produced a lower effect (%MPE= 36.19±11.30) than the other drugs. Similar results have been obtained by other authors for drugs with serotonergic properties in this test, where they were not always effective (Porsolt et al., 1979; Borsini et al., 1981; Semba and Takahashi, 1988). Secondary amines were more effective in the FST, inducing higher % values of MPE than the parental drugs. Drugs with a relatively important noradrenergic component appear to be more effective than those with a serotonergic one, at least in mice. On the other hand, the immobility reduction action induced by tricyclics in the FST were not related to an enhanced motor activity, since all the antidepressants tested displayed sedative properties, and thus diminished the spontaneous motor activity from low doses. It is possible that this sedation makes AMT less effective at the highest dose tested (see Fig. 2A) in the FST. On the other hand, this sedative effect of tricyclic antidepressants may be clinically beneficial for the well-being of some patients with chronic pain syndromes. However, sedation is not desirable in many patients.

In summary, we support the conclusion that antinociceptive and antidepressive effects of tricyclics are mutually independent. There is no clear relationship found between monoaminergic specificity and effectiveness in the different antinociceptive tests performed. Moreover, serotonin does not seem to display a crucial role in the antinociceptive effect of tricyclics, since the secondary amines, known to be more selective in noradrenaline reuptake, retain a strong antinociceptive effect compared with their parental drugs, mixed reuptake inhibitors. Nevertheless, mechanisms involved in the antinociceptive effect of SSRIs may be different from those of tricyclics. On the other hand, the secondary amines seem to be more effective than the tertiary one in the forced swimming test, at least as far as mice are concerned.

Finally, it is surprising that, in spite of their efficacy, noradrenergic tricyclics are not marketed in many countries. A possible explanation is that these drugs have been developed by pharmaceutical companies focusing on their antidepressive effect, and have not been designed for pain treatment. According to clinical experience and our results, we can state that the mixed antidepressant AMT seems to be the most effective as well as the most sedative of those tested. Nevertheless, the benefits of antidepressants in pain treatment are hard to predict and also would depend on the kind of pain in both experimental and clinical conditions. Moreover, studies in chronic pain models and also chronic administration are needed to closely resemble the clinical situation.

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