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Antinociceptive effect of clomipramine in monoarthritic rats as revealed by the paw pressure test and the C-fiber-evoked reflex

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Abstract

The antinociceptive effect of clomipramine was studied in monoarthritic rats by using the paw pressure test and the C-fiber-evoked reflex. Monoarthritis was produced by intra-articular injection of complete Freund's adjuvant into the tibio-tarsal joint. Joint circumference as well as vocalization threshold to graded paw pressure were evaluated weekly during a 14-week period after the intra-articular injection. At week 8, monoarthritic and vehicle-injected control rats were given either clomipramine or saline and both the paw pressure threshold and inhibition of the C-fiber-evoked reflex response were evaluated. Results showed that (i) 1.5, 3.0, and 6.0 mg/kg, i.v. of clomipramine induced significantly greater dose-dependent antinociception to paw pressure testing in the monoarthritic group, as compared to the control one; and (ii) 0.75, 1.5, 3.0, and 6.0 mg/kg, i.v. of clomipramine exerted significantly higher dose-dependent inhibition of the C-reflex activity in monoarthritic rats than in controls. Results suggest that the higher sensitivity to clomipramine in monoarthritic rats could be related to adaptive changes occurring in monoamine metabolism or in other neurotransmitter systems during chronic pain. © 2001 Published by Elsevier Science B.V.

Keywords: Clomipramine; Chronic pain; Monoarthritis; Analgesia; Paw pressure; C-fiber reflex; (Rat)

1. Introduction

Antidepressant drugs are being increasingly utilized as pharmacological tools for achieving clinical analgesia in a variety of chronic painful syndromes (for reviews, see Eschalier, 1990; Magni, 1991; Onghena and Van Houdenhove, 1992; Max, 1994; Godfrey, 1996; McQuay et al., 1996; Smith, 1998; Sindrup and Jensen, 1999; Ansari, 2000). In this respect, animal studies have shown that tricyclic antidepressants induce only modest antinociceptive effects (or even no effect) in behavioral testing of acute pain, including pain of thermal, mechanical and chemical nature (for reviews, see Ardid, 1991; Eschalier et al., 1992). In contrast, antidepressants have been shown to be effective as antinociceptive drugs in animal models of chronic pain. Thus, studies have reported significant antinociceptive efficacy of amitriptyline and diazepam in autotomy behavior in rats following peripheral deafferentation (Abad et al., 1989; Seltzer et al., 1989), of desipramine in carrageenan-induced thermal hyperalgesia (Kawamata et al., 1999), of clomipramine, amitriptyline and desipramine in a rat model of diabetic neuropathy (Courteix et al., 1994), as well as of clomipramine (Ardid and Guilbaud, 1992), amitriptyline (Ardid and Guilbaud, 1992; Abdi et al., 1998; Esser and Sawynok, 1999; Yasuda et al., 1999), desipramine (Ardid and Guilbaud, 1992; Sawynok et al., 1999), fluoxetine (Sawynok et al., 1999), fenfluramine (Wang et al., 1999) and venlafaxine (Lang et al., 1996) in chronic mononeuropathic pain. These observations suggest that in contrast to acute pain, chronic pain induces adaptive changes in central neural substrates for nociception, and these neural changes can contribute to the antinociceptive efficacy of the antidepressant drugs. This notion is supported by the fact that systemically or in-

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trathecally administered antidepressant drugs appear to exert clear antinociceptive activity during painful stimulation of the injured limb (dorsal root or sciatic nerve ligation, intraplantar carrageenan injection), but not during painful stimulation of the uninjured contralateral limb (Lang et al., 1996; Esser and Sawynok, 1999; Kawamata et al., 1999). However, alternative explanations based on peripheral antinociceptive actions of antidepressants (Esser and Sawynok, 1999; Sawynok et al., 1999) cannot be discarded as a factor for a differential effectiveness of these drugs on acute and chronic experimental pain.

The present study was designed (i) to investigate the antinociceptive efficacy of intravenously administered clomipramine (a nonspecific monoamine uptake blocker with a preferential serotonergic profile) on a rat model of monoarthritic pain induced by intra-articular injection of complete Freund's adjuvant in one tibio-tarsal joint (Butler et al., 1991, 1992), and (ii) to compare the antinociceptive effect of clomipramine in monoarthritic rats to that obtained in sham control animals intra-articularly injected with the vehicle used to suspend the mycobacteria. In the present study, the contralateral uninjured limb was not used as control since there is evidence that after intra-articular adjuvant injection, changes in gene expression responsible for protein products, such as enzymes, receptors or neurotransmitters, do not remain solely in the dorsal horn ipsilateral to the arthritic limb as immunocytochemical staining for such agents reveals extensive bilateral changes of gene products after 4 weeks (Tölle et al., 1994). As a whole, these observations suggest that some functional changes may also develop in the uninjured side. Clomipramine-induced antinociception was evaluated by using the graded paw pressure test. To assess changes in pain transmission after clomipramine in the spinal cord dorsal horn, the C-fiber-evoked nociceptive reflex was employed.

2. Materials and methods

Experiments were performed in 114 Sprague–Dawley rats weighing 280–320 g. During this study, the guidelines on ethical standards for investigations of experimental pain in animals were followed (Committee for Research en Ethical Issues of the IASP, 1983). Complete Freund's adjuvant was prepared by adding 60 mg of killed *Mycobacterium butyricum* (Difco Laboratories, USA) to a mixture of 6 ml paraffin oil, 4 ml of NaCl 0.9% and 1 ml of Tween 80. The suspension was thoroughly mixed and autoclaved for 20 min at 120°C to rupture the cell walls of the mycobacteria. Monoarthritis was induced in rats ($N = 60$) by injecting 0.05 ml of complete Freund's adjuvant into the left tibio-tarsal joint under brief halothane anesthesia. Vehicle-injected control rats ($N = 54$) were given intra-articular injection of 0.05 ml of the vehicle used to suspend mycobacteria. The circumference of the injected tibio-tarsal joint as well as vocalization threshold to graded paw pressure (Randall-Selitto apparatus) were evaluated weekly after the intra-articular injection. Eight weeks after injecting the tibio-tarsal joint, once a stable vocalization threshold value to graded paw pressure was determined, 54 monoarthritic and 48 vehicle-injected animals were submitted to the pharmacological study.

2.1. Graded paw pressure

Eight weeks after injecting the tibio-tarsal joint with Freund's adjuvant or vehicle, 18 monoarthritic and 18 vehicle-injected control rats received 1.5, 3.0, or 6.0 mg/kg, i.v. of clomipramine, while six other monoarthritic or vehicle-injected rats were given i.v. saline (NaCl 0.9%). Clomipramine-induced changes in vocalization threshold to graded paw pressure were assessed 15, 30, 60, 90, and 120 min after drug administration by using a Randall-Selitto

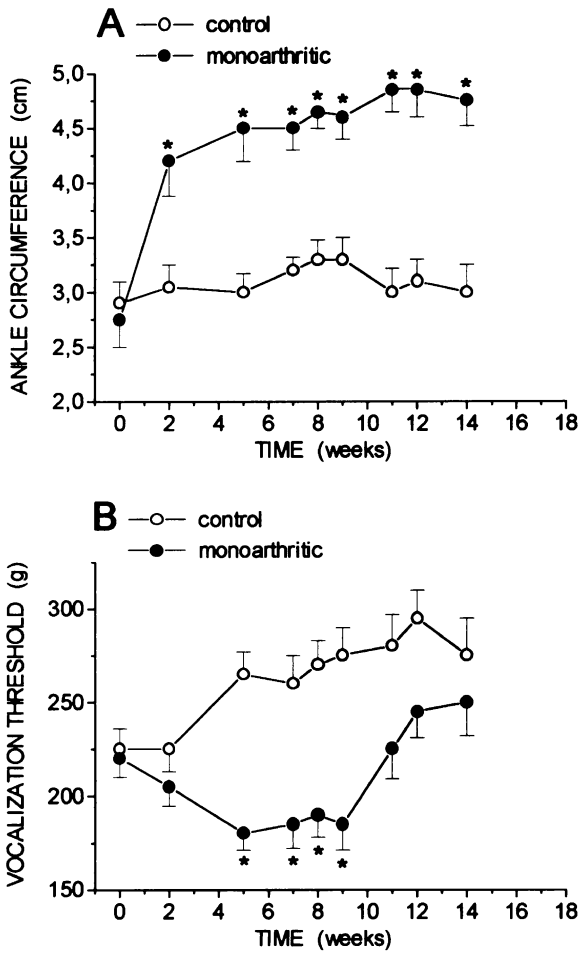

Fig. 1. Development of ankle circumference (A) and vocalization threshold to paw pressure (B) in monoarthritic and control rats during a 14-week period of assessment. Adjuvant or saline was intra-articularly injected at time 0. Values are means ± S.E.M. $N = 6$ in each group. Asterisks represent a significant change in the parameter assessed ($^{*}P < 0.001$), when values are compared to their respective control values (one-way ANOVA followed by nonpaired Student's *t*-test).

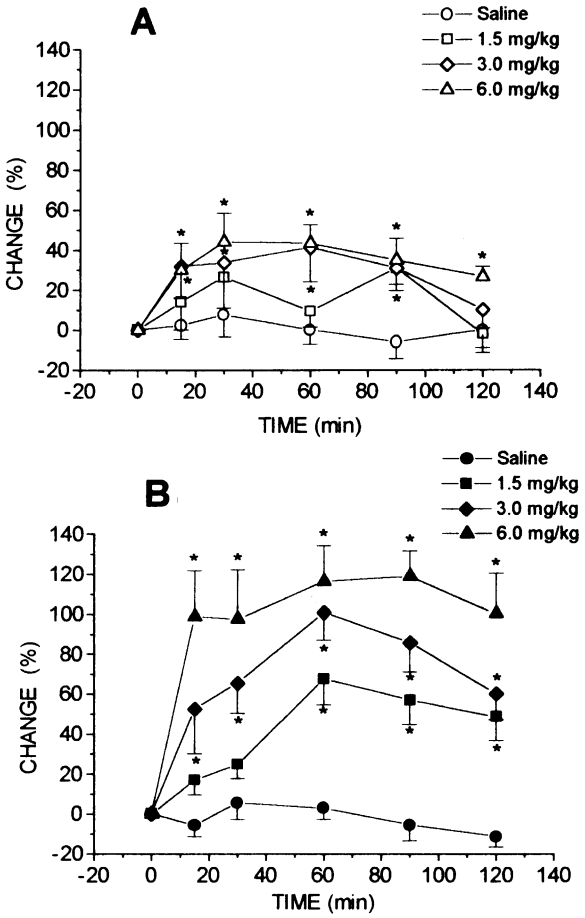

Fig. 2. Time-course of the increase of the vocalization threshold (as a percentage) to graded paw pressure after clomipramine or saline. Clomipramine or saline were injected at 0 min. Values are means ± S.E.M. $N = 6$ in each group. Asterisks represent a significant change in vocalization threshold ($^{*}P < 0.001$) when values under clomipramine are compared to their respective control values of saline series (one-way ANOVA followed by nonpaired Student's *t*-test). (A) Effect of 1.5, 3.0, and 6.0 mg/kg, i.v. of clomipramine in control rats. (B) Effect of 1.5, 3.0, and 6.0 mg/kg, i.v. of clomipramine in monoarthritic rats.

apparatus. The algesimetric measurements were performed blind, using the method of equal blocks ($N = 6$ in each group) to allow evaluation of the effect of different randomized treatments at the same time.

2.2. C-fiber-evoked nociceptive reflex

Eight weeks after injecting Freund's adjuvant or vehicle, 30 monoarthritic and 24 vehicle-injected control animals were submitted to the electrophysiological study. The rats were anesthetized with urethane (1.0 g/kg, i.p.) and the right femoral vein was cannulated for subsequent intravenous administration of clomipramine or saline. The flexor C-reflex was elicited in the left hind limb as described previously (Strimbu-Gozariu et al., 1993; Falinower et al., 1994), by applying to toes four and five rectangular electric pulses of sufficient strength for threshold activation of C-fibers (5–7 mA strength, 2 ms duration), every 10 s, which were thereafter increased in strength by a threefold factor. The C-fiber-evoked reflex response was recorded from the ipsilateral biceps femoris muscle by means of two stainless steel needles inserted percutaneously. After amplification, the electromyographic recordings were lead to a computerized system for on-line digitization and stored in hard disk for later analysis. The digitized electromyographic recordings were full-wave rectified and the C-fiber-evoked responses were integrated within a time-window from 150 to 450 ms after the stimulus. Clomipramine was administered i.v. at doses of 0.75, 1.5, 3.0, and 6.0 mg/kg. Animals receiving saline i.v. served as controls. Clomipramine-induced changes in C-reflex activity were assessed 5, 10, 20, 35, 50, and 65 min after drug administration.

2.3. Analysis of results

Development of monoarthritis was assessed by measuring the ankle circumference (in cm) and the vocalization threshold to paw pressure (in g). Results from algesimetric measurements were expressed as time-course of percent increase of the vocalization threshold to paw pressure after clomipramine or saline. Results from electrophysiological experiments were expressed as time-course of percent inhibition of the C-reflex response, after clomipramine or saline administration. In both algesimetric and electrophysiological experiments, to appreciate the global effect of clomipramine over the total testing period and to correct the bias that may introduce the hyperalgesic status having monoarthritic rats, an estimated area (EA) between the curve obtained under drug and the curve obtained under saline was calculated. It is calculated as $EA = \Sigma SUD - \Sigma SUS$, where ΣSUD is the algebraic sum of the scores

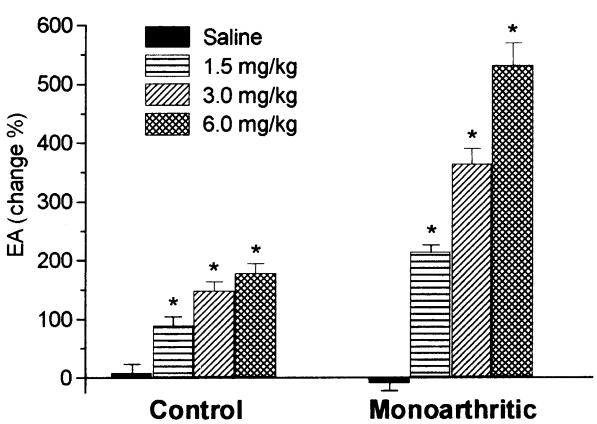

Fig. 3. Effect of clomipramine on the vocalization threshold (as a percentage) to graded paw pressure of control and monoarthritic rats, as revealed by estimated areas under the curves (EA). Values are means ± S.E.M. $N = 6$ in each group. Asterisks represent a significant change ($^{*}P < 0.001$) compared to saline series (one-way ANOVA followed by nonpaired Student's *t*-test).

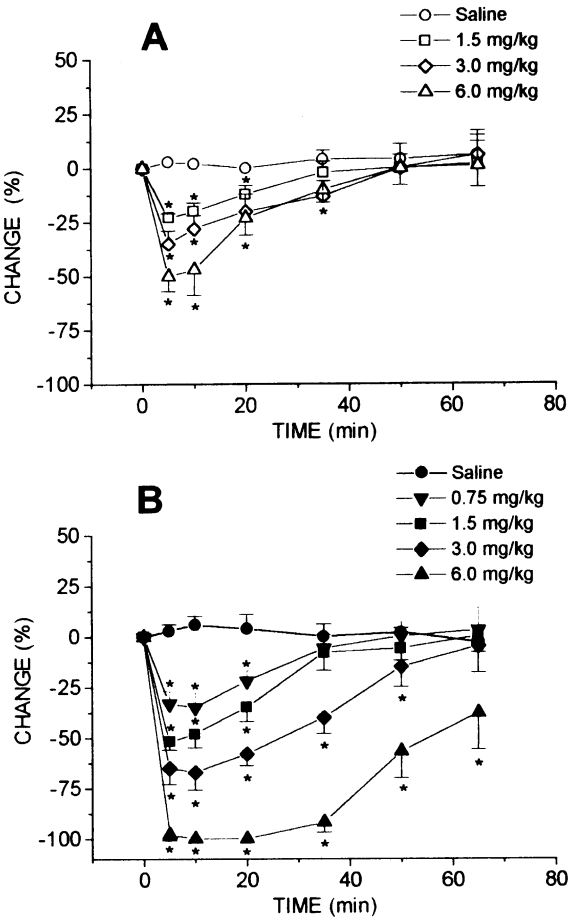

Fig. 4. Time-course of the inhibition of the C-reflex response (as a percentage) after i.v. administration of clomipramine or saline. Clomipramine or saline were injected at 0 min. Values are means ± S.E.M. $N = 6$ in each group. Asterisks represent a significant change in C-reflex activity ($^{*}P < 0.001$) when the values under clomipramine are compared to their respective control values of saline series (one-way ANOVA followed by nonpaired Student's *t*-test). (A) Effect of 1.5, 3.0, and 6.0 mg/kg, i.v. of clomipramine in control rats. (B) Effect of 0.75, 1.5, 3.0, and 6.0 mg/kg, i.v. of clomipramine in monoarthritic rats.

under drug (SUD) and ΣSUS is the algebraic sum of the scores under saline (SUS) over the total period of testing that followed drug administration.

Results were expressed as means ± S.E.M. An analysis of variance followed by a Student's *t*-test for independent samples were made to compare different treatments. Values of *t* with a probability less than 0.05 were considered to indicate statistically significant difference between means.

3. Results

3.1. Development of monoarthritis

The injection of complete Freund's adjuvant into the tibio-tarsal joint produced clear signs of inflammation in the ankle of the rat. Monoarthritis was indicated by an increase in ankle circumference (Fig. 1A) and hyperalgesia to graded paw pressure (Fig. 1B). Increase in joint circumference was well developed by week 2 after intra-articular adjuvant injection and was maintained throughout the observation period until week 14. Significantly enhanced pain sensitivity to paw pressure in the injected hind limb was clearly observed on weeks 5 to 9 after induction of monoarthritis (Fig. 1B).

3.2. Paw pressure testing

Fig. 2A shows that 8 weeks after injection of Freund's adjuvant or vehicle, clomipramine i.v. induced a dose-dependent increase of the vocalization threshold in both control and monoarthritic rats, the effect being markedly higher in the monoarthritic group. Thus, the maximum enhancement of the vocalization threshold was about 45 ± 14% in control animals after 6.0 mg/kg of clomipramine (Fig. 2A), while a 118 ± 20% increase was observed in the monoarthritic group following administration of the same dose of drug (Fig. 2B). Results expressed as summed scores over the total period of drug testing (estimated area or EA) indicate that clomipramine exerted a significantly higher dose-dependent increase of the vocalization threshold in monoarthritic rats than in controls (Fig. 3).

3.3. Effects on the C-reflex response

Fig. 4 shows that 8 weeks after injection of Freund's adjuvant or vehicle, clomipramine i.v. exerted a dose-dependent inhibitory effect on the C-fiber nociceptive reflex in both control and monoarthritic rats. The maximum inhibition of the C-reflex was only 50 ± 9% in control animals following 6.0 mg/kg of clomipramine (Fig. 4A), whereas monoarthritic rats exhibited a 100% inhibition of

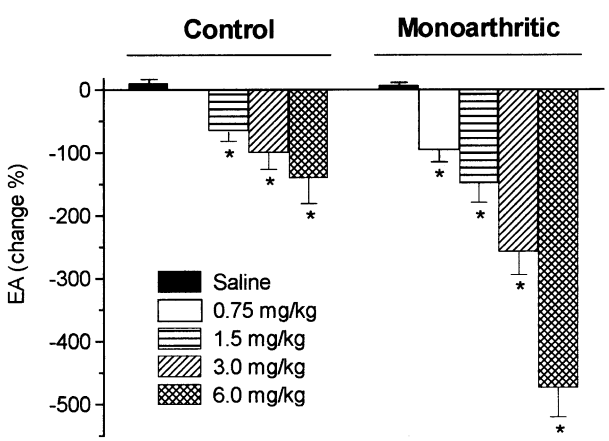

Fig. 5. Effect of clomipramine on the C-reflex response of control and monoarthritic rats, as revealed by estimated areas under the curves (EA). Values are means ± S.E.M. $N = 6$ in each group. Asterisks represent a significant change ($^{*}P < 0.01$), compared to saline series (one-way ANOVA followed by nonpaired Student's *t*-test).

the C-reflex response at the same dose of drug (Fig. 4B). Results expressed as summed scores over the total period of testing (EA) indicate that clomipramine induced a significantly greater dose-dependent inhibitory effect in C-reflex activity from monoarthritic rats than that from controls (Fig. 5).

4. Discussion

Intra-articular injection of Freund's adjuvant into the tibio-tarsal joint, as previously described (Butler et al., 1991, 1992), induced a limited form of arthritis that was stable for several weeks. This was characterized by increased ankle circumference and hyperalgesia to graded paw pressure that developed 2 to 4 weeks after the intra-articular injection of Freund's adjuvant. The regulatory neuropeptide substance P has been implicated in the development of joint inflammation (McDougall et al., 1995). This is in agreement with the fact that abolition of the neural discharge of the sciatic nerve by local anesthesia could prevent increases of preprotachykinin messenger RNA expression in dorsal root ganglia involved in neurogenic inflammation (Donaldson et al., 1994).

Results showed that clomipramine exhibited a greater dose-dependent antinociceptive efficacy in monoarthritic rats than in control animals, as revealed by paw pressure testing and C-fiber-evoked reflex evaluation. The EA measurement corrects the bias introduced by the hyperalgesic state showed by monoarthritic rats, being then a representative index of the change in both threshold for vocalization and C-reflex activity produced by the drug treatment over the total testing period.

Serotonin reuptake inhibition has been considered for many years as the main mechanism underlying the antinociceptive effect of antidepressant drugs (Messing and Lytle, 1977; Loldrup et al., 1989; Imahita and Shimizu, 1992), although noradrenaline reuptake inhibitors also may induce important analgesic effects (Ardid and Guilbaud, 1992; Onghena and Van Houdenhove, 1992; Mestre et al., 1997). Clomipramine probably exerts antinociception by acting on supraspinal monoaminergic neurons, since this drug leads to significant antinociceptive activity in animals after systemic (Ardid, 1991; Mestre et al., 1997) and intracerebroventricular (Sierralta et al., 1995; Mestre et al., 1997) administration, but not after intrathecal injection (Ardid, 1991; Mestre et al., 1997). This is consistent with other reports indicating that intracerebroventricular administration of fenfluramine, a preferential serotonergic reuptake blocker (Wang et al., 1999), as well as microinjection of zimelidine into the nucleus raphe magnus, another serotonin reuptake inhibitor (Llewelyn et al., 1984), produce antinociception in algesimetric tests.

Concerning the greater antinociceptive efficacy of clomipramine in monoarthritic rats than in control animals, it has been shown that monoamine synthesis is increased in bulbospinal structures of rats submitted to some forms of persistent chronic pain (Godefroy et al., 1987; Feria et al., 1992; Colado et al., 1994). More recently, it has been reported that chronic monoarthritis induced by adjuvant inoculation into the tibio-tarsal joint increases tyrosine hydroxylase mRNA levels in the pontine noradrenergic cell groups (Cho et al., 1995). Since some of these monoaminergic neurons are involved in bulbospinal control of pain (Proudfit and Clark, 1991; Kwiat and Basbaum, 1992), it is tempting to speculate that serotonergic and/or noradrenergic hyperactivity in these pathways could be implicated in the enhanced sensitivity of monoarthritic rats to clomipramine. As has recently been demonstrated, the antinociceptive effect of i.v. clomipramine is prevented by lesion of the dorsolateral funiculus of the spinal cord (Ardid et al., 1995), the neural pathway that contains the majority of descending monoaminergic axons involved in spinal cord modulation of pain. More recently, it has been reported that activation of α_2-adrenoceptors is involved in antidepressant-induced antinociception, irrespective of the serotonergic or noradrenergic specificity of the drug for inhibiting reuptake (Gray et al., 1999). However, other studies have shown that pretreatment with the 5-HT receptor antagonist mianserin produced rightward and downward shifts in the clomipramine-induced antinociception dose–effect curve, suggesting that the effects of clomipramine were mediated by 5-HT receptors (Gatch et al., 1998). Further investigation utilizing antidepressant drugs of different monoamine spectra combined with intrathecal administration of selective antagonists for 5-HT receptors and adrenoceptors is required to elucidate the role of adaptive changes in bulbospinal monoamine systems on the mechanisms of antidepressant-induced antinociception in monoarthritis and other forms of chronic pain.

Central nervous system neurons of monoarthritic animals, in addition to the modifications in monoamine turnover, could develop other neurochemical changes that may be related to the increased clomipramine-induced antinociception observed in these animals. In fact, it has been shown that the spinal cord of monoarthritic rats exhibits complex changes in δ- and κ-opioid binding sites (Besse et al., 1992), increased γ-aminobutyric acid levels and glutamic acid decarboxilase gene transcription (Castro-Lopes et al., 1994), decreased amount of substance P and calcitonin gene-related peptide (Mapp et al., 1993), and increased forskolin-stimulated cAMP formation (Przewlocka et al., 1992). Whether these changes are involved in the enhanced sensitivity to clomipramine in monoarthritic rats remains unclear. Another source of complexity in interpreting antinociceptive effects of antidepressant drugs in rat models of chronic pain is that some of these compounds may act as NMDA receptor antagonists (Eisenach and Gerbhart, 1995). Thus, spinal cord NMDA receptors play an important role in mediating the hyperalgesia developed during persistent pain, and this represents

part of the complex neural adaptive mechanisms occurring in the spinal cord of animals submitted to chronic pain (Coderre et al., 1993).

In conclusion, the present study shows that monoarthritic rats exhibited higher sensitivity to clomipramine than normal rats when tested with both nociceptive paw pressure and nociceptive electrical stimulation, a behavior that could be related to adaptive changes in monoamine metabolism or in other neurotransmitter systems occurring during chronic pain. Enhanced antinociceptive effects of antidepressants during chronic pain could support the empiric clinical point of view that tricyclic antidepressants may constitute first-line agents for pain relief in some chronic painful syndromes, in harmony with the modest antinociceptive action of these drugs in acute pain.

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