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Long-lasting delayed hyperalgesia after subchronic swim stress

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Abstract

Rats subjected to an inescapable subchronic stress, consisting of 10–20 min of forced swimming for 3 days, showed a thermal hyperalgesia and an enhanced nociceptive behavior to the subcutaneous administration of formalin 24 and 48 h, respectively, after the last swim session. Hyperalgesia to thermal and chemical stimulants was still present 8 and 9 days after the last swim session, respectively. Chemical, but not thermal, nociception was negatively correlated with the swim effort or struggle times during the last swim session. The serotonin-selective reuptake inhibitors clomipramine (2.5 mg/kg/day, i.p., started 3 or 7 days before stress) and fluoxetine (0.25 mg/kg/day, i.p., started 7 days before stress), or serotonin precursor tryptophan (3 mg/kg/day, i.p., 24 h before each swim stress) blocked the development of both the thermal and the chemical hyperalgesia and increased swim effort times compared to vehicle-treated rats. These treatments did not affect nociceptive responses in control rats subjected to sham swimming. These findings suggest that repeated stress can produce a long-lasting increase in pain sensitivity to both phasic or tonic noxious stimuli by diminishing central serotonin activity. This model may help elucidate the underlying neural mechanisms that mediate the effects of repeated stress on pain sensitivity and affective states.

Keywords: Hyperalgesia; Stress; Antidepressants; Tryptophan; Clomipramine; Fluoxetine; Serotonin; Formalin test; Hot plate test

1. Introduction

Acute exposure to a variety of stressors produces an immediate analgesia in several pain tests [15]. Pain measurements done just after a chronic or prolonged stress can also reveal the presence of analgesia [18]. Yet, some studies have reported that under some experimental conditions both acute and chronic stress can elicit hyperalgesia instead of analgesia. For example, brief exposure to short emotionally arousing non-noxious stress, such as holding or novel environments produces an immediate and transient hyperalgesia that is followed by a longer analgesia in rats [24]. Similarly, prolonged stress produced by repeated exposure to a cold environment produces a 3-day-long hyperalgesia [21]. Unlike stress-induced analgesia, the mechanisms involved in the stress-induced hyperalgesia are less known. The activation of hypothalamic–pituitary–adrenal axis, as well as GABA and alpha2-adrenergic receptors, has been proposed to be involved in the short-lived hyperalgesia produced by acute stress [13,24]. On the other hand, substance-P- and calcitonin-gene-related peptide seem to be required, at least at the spinal level, for the induction of a long-lasting hyperalgesia evoked by a prolonged cold stress [21].

Changes in the activity of central serotoninergic systems might explain, at least in part, the bidirectional changes in nociception (analgesia and hyperalgesia) seen after different stress conditions. Bidirectional changes in the serotoninergic activity of nuclei known to be engaged in the stress response have been observed. For example, after acute exposure to different types of adverse psychological or physical stimuli, there is an increase in the extracellular concentrations of serotonin in several brain regions, especially in the raphe magnus [2]. Conversely, prolonged stress diminishes the efflux of serotonin in some brain structures known to be activated by stress, such as the amygdala and lateral septum [3,14]. Interestingly, the

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activity of serotoninergic descending fibers from the raphe magnus tonically inhibits pain transmission within the spinal cord [16] and the inhibition of serotonin synthesis at the spinal level produces hyperalgesia in rats [4]. The magnitude of tonic inhibition of pain transmission within the spinal dorsal horn appears to be dependent on the behavioral state of the organism (depressed mood, anxiety, fear) [16]. Thus, we hypothesized that long-lasting hyperalgesia seen after repeated moderate to severe stress [21] may be linked, at least in part, to a stress-induced decrease in tonic serotoninergic inhibition of nociception.

In the present study, we evaluated the effect of a subchronic inescapable, non-painful, moderate swim stress on nociception and the involvement of the serotoninergic system. We found that repeated swim stress procedures a long-lasting thermal and chemical hyperalgesic state, which is antagonized by pretreatment with serotoninergic antidepressants and tryptophan. These findings suggest the involvement of central serotoninergic systems in the prolonged hyperalgesic state produced by chronic stress.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Centro Biotecnológico IVIC, Caracas, Venezuela), weighing 150–300 g, were individually caged 3 days prior to testing. Procedures were performed between 7:00 AM–12:00 PM and were conducted in the same room where the animals were housed. Animals were housed at a room temperature of 24–26°C, and food and water were available ad libitum. The experimental protocol was approved by the Ethical Committee for Scientific Research on Animals at the University of Zulia.

2.2. Experimental protocol

In each experiment, 10 rats were subjected to a 12-day protocol. On day 1, basal or baseline thermal pain measures were obtained by measuring response latencies (in seconds) using the hot plate procedure as described by Carter [5]. In brief, animals were placed on a 52.5°C hot plate (IITC model 39D, USA), and the time to hind-limb licking or jumping behavior was measured. A cut-off time of 45 s was use to diminish the possibility of producing a burn. After a 30- to 60-min rest period, each rat was subjected to a forced swim procedure for 10 min by placing them in a plastic cylinder (diameter 30 cm, height 50 cm) that contained 20 cm of water at 24–26°C. On days 2 and 3 of the experiment, rats were subjected to a similar swim stress for 20 min. During the swimming sessions, the "immobility" and "struggling" times were recorded as previously described in Ref. [20]. Immobility occurred when the rat made bodily movements just sufficient to maintain its head above the water. Struggling occurred when the rat was actively diving, jumping, or vigorously moving all four limbs to break the surface of the water or when it attempted to climb the wall of the container with its forelimbs. Control rats were subjected to a sham-swimming session by allowing them to wade in the cylinder that contained only 2–4 cm of water at 24–26°C. Rats were allowed to dry in a warm environment (30–33°C) after each swimming session.

On day 4, thermal nociception was again evaluated with the hot plate (52.5°C, cut-off time 45 s). On day 5, chemical nociception was assessed with the formalin test [6]. Briefly, 0.1 ml of 1% formalin (37.5% formaldehyde in 0.9% saline) was subcutaneously injected in the plantar pad of the right hind paw and the nociceptive behavior was observed for 30 min; pain ratings were recorded at 180-s intervals. A pain rating scale, as described by Dubuisson and Dennis [6], was used in this study. Briefly, pain intensity was rated according to the following scale, 0 = weight is evenly distributed among all paws when the rat is still or in locomotion;

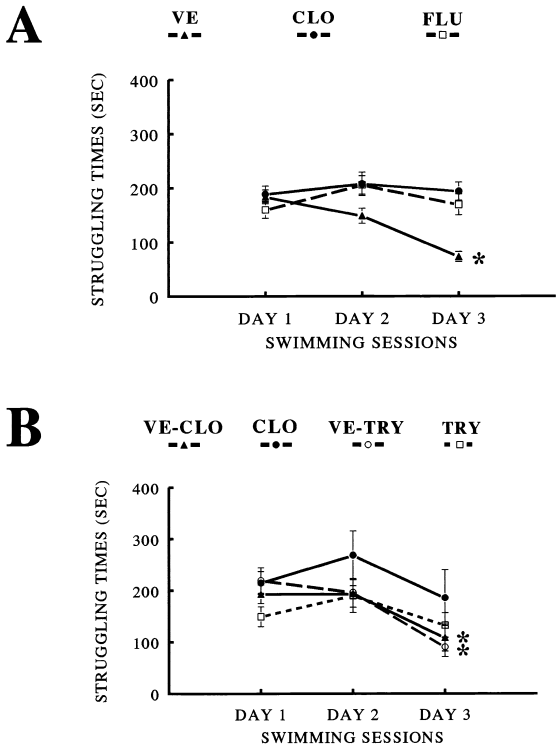

Fig. 1. Progressive decline in struggling times after repeated forced swimming sessions and its blockade with low doses of serotoninergic agents. Rats were subjected to a 10-min swimming on day 1 and to a 20-min swimming on days 2 and 3. Panel A: VE = vehicle (0.9% NaCl, i.p., once a day); CLO = clomipramine (2.5 mg/kg, i.p., once a day); and FLU = fluoxetine (0.25 mg/kg, i.p., once a day) both started 7 days before the first swimming session. Panel B: VE-CLO = vehicle (0.9% NaCl, i.p., once a day) and CLO = clomipramine (2.5 mg/kg, i.p., once a day) both started 3 days before the first swimming session; VE-TRY = vehicle (0.9% NaCl, i.p.) and TRY = tryptophan (3 mg/kg, i.p.) both injected 24 h before each swimming session. * Significant difference compared to the first swimming session ($p < .05$, one-way ANOVA followed by Duncan's test).

1 = little or no weight is placed upon the injected paw; 2 = injected paw is elevated; 3 = injected paw is licked, bitten, or shaken. Numerical ratings were calculated from the following formula: pain rating = (T1 + 2T2 + 3T3)/180, where T1, T2, and T3 were the duration (in seconds) spent in categories 1, 2, or 3, respectively, during each 180-s observation interval. A computer-assisted scoring of the nociceptive behavior was done by an observer unaware of the treatment.

Thermal nociception was again evaluated with the hot plate on day 11. For this reassessment, the time to licking of the left hind paw or jumping were used to determine the response latency on the hot plate since the pain sensitivity of the right hind paw may have been altered by the injection of low-dose formalin 6 days prior. Chemical nociception was reevaluated with the formalin test conducted on the left hind paw on day 12.

2.3. Drug treatments

A group of rats were treated with daily i.p. injections of the tricyclic antidepressant clomipramine, 2.5 mg/kg/day started either 3 or 7 days before the first forced swimming session and maintained daily throughout the experimental protocol (day 11). Another group was treated with daily i.p. injections of the selective serotonin uptake inhibitor fluoxetine, 0.25 mg/kg i.p. started 7 days before the swimming session and maintained daily throughout the experimental protocol (day 11). A third group of rats was injected with tryptophan, 3 mg/kg i.p. 24 h before each swimming session (days 1–3). Control rats for each group were injected i.p. with a similar volume of the drug vehicle (0.9% NaCl). Since high doses of tricyclic antidepressants, including clomipramine, have been found to have analgesic efficacy

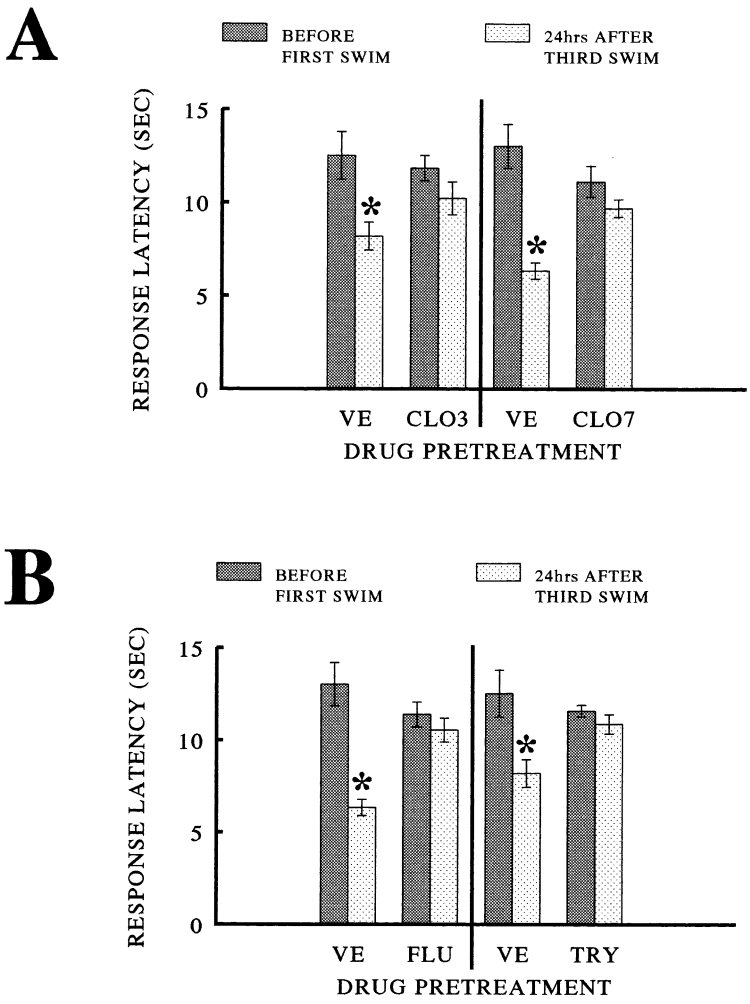

Fig. 2. Subchronic stress-induced thermal hyperalgesia and its blockade by serotoninergic agents. Thermal nociception was measured, about 2 h before the first swimming and about 24 h after the third swimming session, using the hot plate procedure. The ordinate shows response latencies (in seconds). Panel A: VE = respective vehicle (0.9% NaCl, i.p., once a day); CLO3 and CLO7 = clomipramine (2.5 mg/kg, i.p., once a day) started 3 and 7 days, respectively, before the first swimming session. Panel B: VE = respective vehicle (0.9% NaCl, i.p.); FL = fluoxetine (0.25 mg/kg, i.p., once a day) started 7 days before the first swimming session; TRY = tryptophan (3 mg/kg, i.p.) 24 h before each swimming session. * Significant difference compared to controls before stress ($p < .01$, one-way ANOVA followed by Duncan's test).

[7,23], we used doses that were low enough not to produce hypoalgesia.

2.4. Data reduction and statistical analysis

For the formalin test, the 3-min intervals of observation were averaged for each group. Three phases of behavior were observed in response to formalin administration. An early active phase (early phase) was observed during the fist two intervals (6 min) of observation (intervals 1 and 2). A quiescent phase (interphase) was observed during intervals 3, 4, and 5. A second period of activity (late phase) was observed during intervals 6, 7, 8, 9, and 10. Pain's scores for each phase were calculated by summing the pain's score across the intervals associated with each phase. Statistical evaluation was performed using either a one-way or two-way analysis of variance (ANOVA), followed by post hoc analysis with Duncan's multiple range test. N is the number of animals used. Significance was assumed at $P < .05$. Linear correlation analyses were conducted between struggling times and pain measurements (thermal and formalin evoked measures).

2.5. Materials

Clomipramine hydrochloride, fluoxetine hydrochloride, and L-tryptophan were purchased from RBI (Natick, MA, USA).

3. Results

For vehicle-treated animals, struggling times progressively decreased during the three forced swimming sessions

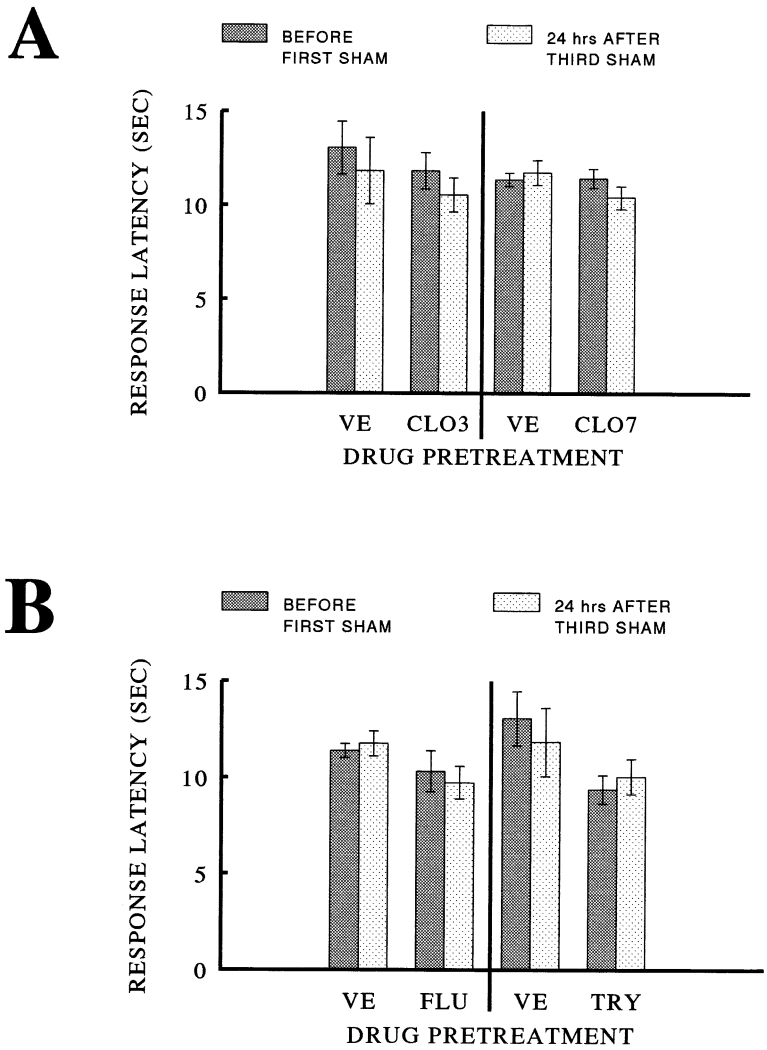

Fig. 3. Lack of analgesic efficacy of the serotonin uptake inhibitors (clomipramine and fluoxetine) and the serotonin precursor tryptophan on thermal pain. Control rats were subjected to a sham-swimming procedure by placing them in a container with a water depth of 2–4 cm. The ordinate shows response latencies (in seconds) to the hot plate procedure. Panel A: VE = respective vehicle (0.9% NaCl, i.p.); CLO3 and CLO7 = clomipramine (2.5 mg/kg, i.p., once a day) started 3 and 7 days, respectively, before the first swimming session. Panel B: VE = respective vehicle (0.9% NaCl, i.p.); FLU = fluoxetine (0.25 mg/kg, i.p., once a day) started 7 days before the first swimming session; TRY = tryptophan (3 mg/kg, i.p.) 24 h before each swimming session.

(Fig. 1A: $F_{8,81}=6.59$, $P<.0001$; Fig. 1B: $F_{11,108}=4.37$, $P<.0001$). In contrast, a decline in the struggling times during the forced swimming was not seen in rats pretreated with low doses of either clomipramine, fluoxetine, or tryptophan (Fig. 1). In addition, there was no significant difference between the struggling times of the rats pretreated with any of the serotoninergic agents and vehicle across the three swimming sessions.

3.1. Thermal nociception

Fig. 2 shows hot plate response latencies measured before the first swimming and 24 h after the third (last) swimming sessions, day 1 and day 4 of the experimental protocol, respectively. A 50% reduction in the thermal pain latency was observed in vehicle-treated animals when comparing the measurements before the first and after the last swimming session (p's$<.01$). Correlation analysis between struggling times during the third swimming session (day 3) and the response latencies in the hot plate test, measured 24 h after the last swimming session (day 4), were not significantly correlated in vehicle-treated rats ($r=-0.162$; $P=.329$; $N=39$; Fig. 4A). In contrast to the vehicle-treated animals, pretreatment with clomipramine, fluoxetine, or tryptophan impaired the swim stress-induced thermal hyperalgesia observed on

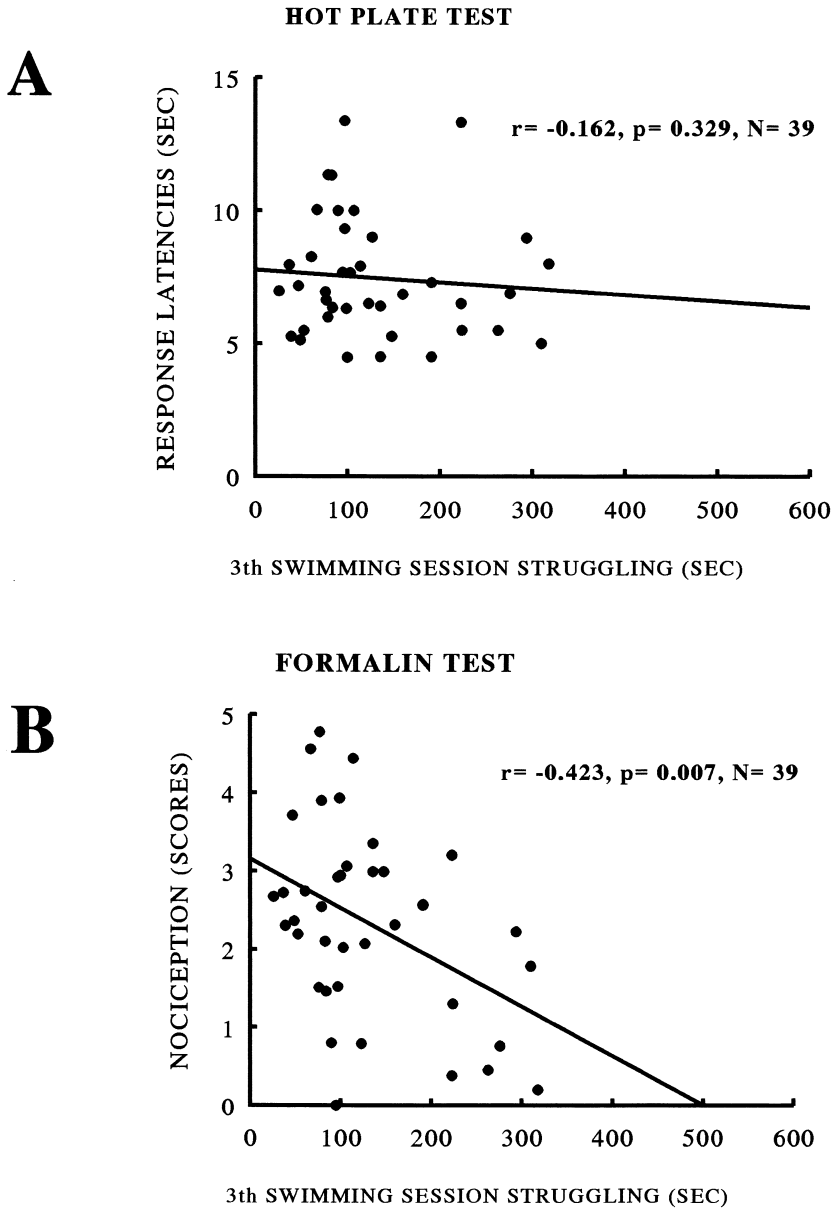

Fig. 4. Correlation between thermal and chemical nociception and swim time. A linear correlation analysis was done between the struggle (swim) times during the third swimming session and either the hot plate response latencies (Panel A) or nociception scores during the interphase of the formalin test (Panel B). r=correlation coefficient, P=probability, N=number of rats.

day 4. The hyperalgesic responses in vehicle-pretreated rats were not due to repeated thermal testing and the drugs used in this study did not produce analgesic effects because response latencies measured 24 h after the last sham swim were not significantly different from the latencies obtained prior to the first sham swim in rats pretreated with either vehicle, clomipramine, fluoxetine, or tryptophan (Fig. 3). Moreover, in vehicle-treated rats, response latencies measured 1 day after the last swimming session (stress swim group) were significantly lower than those measured 1 day after the sham-swimming session (sham swim group) (compare between vehicles of Figs. 2 and 3, P's $< .05$). In addition, the response latencies of vehicle-treated rats, measured 8 days after the last swimming session (on day 11 of the experimental protocol), were similar to those measured 1 day after the last swimming session and about 50% lower than basal measurements done before the first swimming session ($f_{5,54} = 17.26$; $P < .0001$; Fig. 8A). These findings show that three days of non-noxious swimming stress produces a delayed and long-lasting thermal hyperalgesia that cannot be simply related to learning or adaptation responses to repeated hot plate testing and which can be impaired by centrally acting serotoninergic agents.

3.2. Chemical nociception

Fig. 5A shows the effects of repeated forced swim on the phasic nociceptive behaviors elicited by the subcutaneous injection of formalin in the hind paw of naive rats. The response pattern was similar to that previously described by other workers [6]. Maximal nociceptive responses were observed during the first interval of the early phase and during the ninth interval at the late phase.

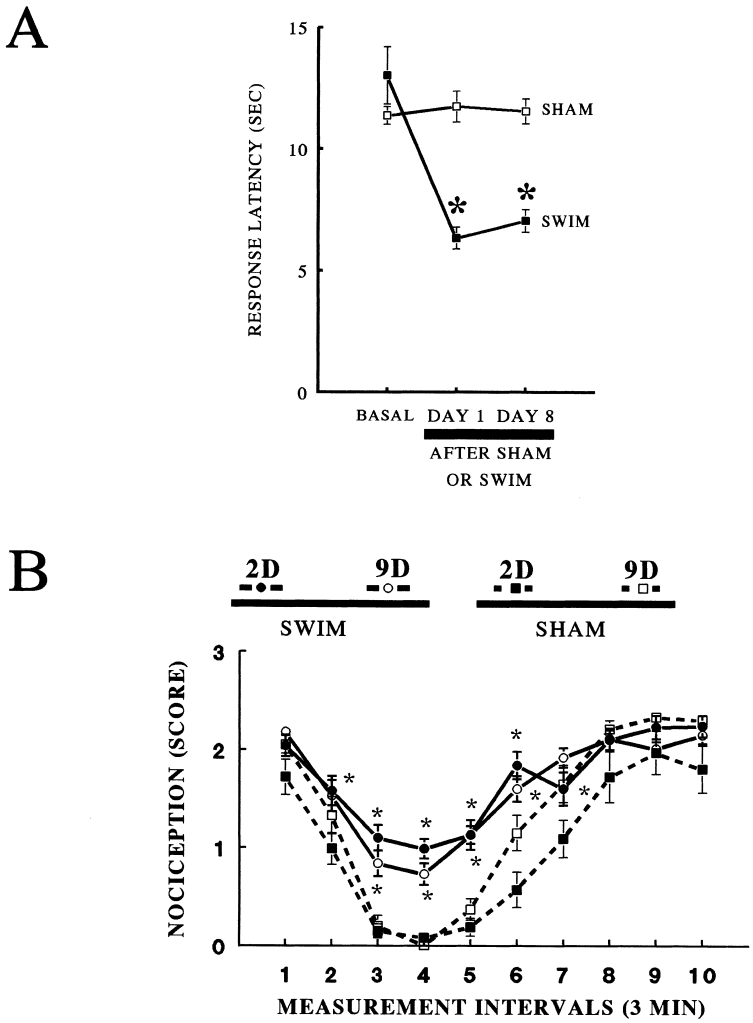

Fig. 8. Long-lasting thermal and chemical hyperalgesia induced by repeated swim stress. Panel A: Thermal nociception was assessed, with the hot plate test, 1 and 8 days after the third swimming session and compared to baseline responses (basal) measured before the first swimming session. Control rats were subjected to a sham swimming by placing them in a container with a water depth of 2–4 cm. Test day is shown on the abscissa. Panel B: Chemical nociception in rats subjected to either swim stress or sham swim (controls) was assessed with the formalin test 2 and 9 days after the third sham or swimming session. * Significant difference compared to either basal responses (Panel A) or sham swim controls (Panel B) ($p < .05$, one-way ANOVA followed by Duncan's test).

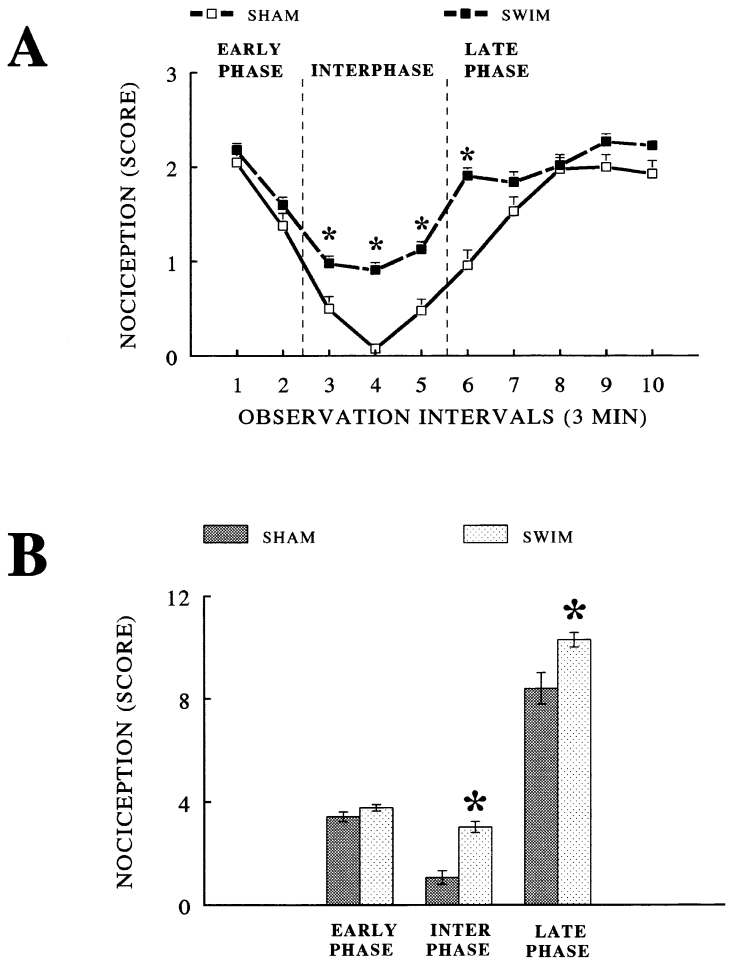

Fig. 5. The effects of swim stress on formalin-evoked pain behaviors. Assessment was done approximately 48 h after the third swimming session, using the formalin test. Control rats were subjected to sham swimming by placing them in a container with a water depth of 2–4 cm. The ordinate on Panel A shows the pain scores based on the rating scale as described by Dubuisson and Dennis [6]; consecutive 3-min observation intervals are shown on the abscissa. Panel B shows the average pain scores associates with the early phase (intervals 1 and 2), interphase (intervals 3, 4, and 5), and late phase (intervals 6, 7, 8, 9, and 10). * Significant difference compared to the non-stressed controls ($p < .05$, one-way ANOVA followed by Duncan's test).

The early and late phases were separated by a quiescent interphase (observation intervals 3, 4, and 5) where the animals showed very little nociceptive behavior. Compared to the sham swim group, the nociceptive behavior in rats tested 48 h after the last forced swim (day 5) showed a marked increase in formalin-evoked responses from interval 3 to 6, that is, from minute 9 to 21 ($f_{79,720} = 22.32$; $p < .0001$; Fig. 5A). The total pain score for the interphase (intervals 3, 4, and 5) increased about three times compared to responses obtained from the sham swim group ($f_{5,54} = 53.48$; $P < .0001$; Fig. 5B). Similarly, the total pain score for the late phase was increased by approximately 40% for the forced swim group compared to the sham swim group. This was mainly due to higher pain score during the 6th interval, which occurred at the beginning of the late phase ($f_{5,54} = 53.48$; $P < .0001$; Fig. 5B). Correlation analysis showed that struggling times obtained at the third swimming session were negatively correlated with the interphase pain scores ($r = -0.423$; $P = .007$; $N = 39$; Fig. 4B) but were not correlated with either the early phase ($r = -0.256$; $P = .116$; $N = 39$) or the late phase ($r = -0.018$; $P = .915$; $N = 39$). The hyperalgesia observed during the interphase and the beginning of the late phase of the formalin test was not seen in rats following pretreatment with either clomipramine, fluoxetine, or tryptophan (Fig. 6A: $F_{29,270} = 25.86$, $P = .0001$; Fig. 6B: $F_{39,360} = 20.46$, $P = .0001$). In contrast, in control rats subjected to sham swimming, pretreatment with either clomipramine or fluoxetine did not significantly change the phase-dependent pain scores when compared to their respective vehicles (Fig. 7). Yet, pretreatment of control rats subjected to sham swimming with tryptophan significantly reduced the nociceptive behavior during 3th, 6th, and 7th intervals compared to the respective vehicle ($f_{39,365} = 15.82$, $P < .0001$; Fig. 7B). Similar to the swim stress-induced thermal hyperalgesia, the enhanced chemical nociception by stress swim was long-lasting since it

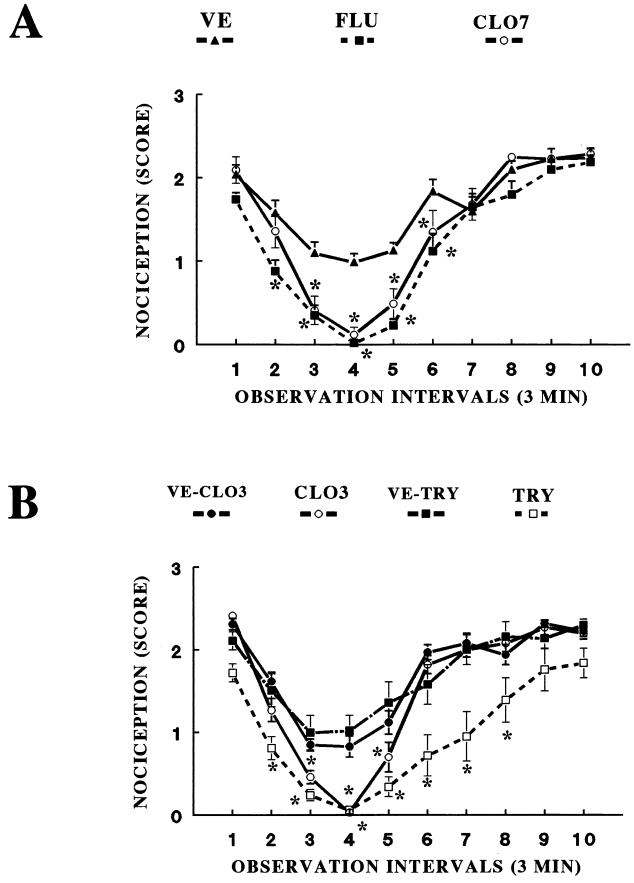

Fig. 6. Blockade of the stress-induced chemical hyperalgesia by the serotonin uptake inhibitors (clomipramine and fluoxetine) and the serotonin precursor tryptophan. Chemical nociception was measured, approximatel 48 h after the third swimming session, using the formalin test. Panel A: VE = vehicle (0.9% NaCl, i.p., once a day); CLO7 = clomipramine (2.5 mg/kg, i.p., once a day); and FLU = fluoxetine (0.25 mg/kg, i.p., once a day) started 7 days before the first swimming session. Panel B: VE-CLO3 = vehicle (0.9% NaCl, i.p., once a day) and CLO3 = clomipramine (2.5 mg/kg, i.p., once a day) both started 3 days before the first swimming session; VE-TRY = vehicle (0.9% NaCl, i.p.) and TRY = tryptophan (3 mg/kg, i.p.) both injected 24 h before each swimming session. * Significant difference compared to swim-stressed rat pretreated with vehicle ($p < .05$, one-way ANOVA followed by Duncan's test).

was still observed 9 days after the third swimming session ($f_{39,360} = 25.36$; $P < .0001$; Fig. 8B).

4. Discussion

In the present study, we were able to induce a long-lasting hyperalgesia following a repeated non-noxious stress procedure. In agreement with our results, previous studies have also found that stress can elicit hyperalgesia rather than hypoalgesia. For example, brief exposure to an emotionally arousing stressor, such as inescapable holding or exposure to a novel environment, produces an immediate and transient hyperalgesia, which is followed by a longer analgesia to thermal and electrical stimuli [24]. Acute thermal hyperalgesia has also been induced in the rat following exposure to vibration [13]. It has also been shown that chronic stress produced by repeated exposure to a cold environment (4°C for 30 min every 1 h for 1 day) is capable of producing a 3-day-long mechanical hyperalgesia [21].

4.1. Neurochemical mechanisms involved in the stress-induced hyperalgesia

The mechanisms involved in transient hyperalgesias after a stress procedure are poorly understood. Vidal and Jacob [24] reported that hypophysectomy enhances the hyperalgesia induced by inescapable holding and reduces the hyperalgesia triggered by exposure to novelty. This suggested the

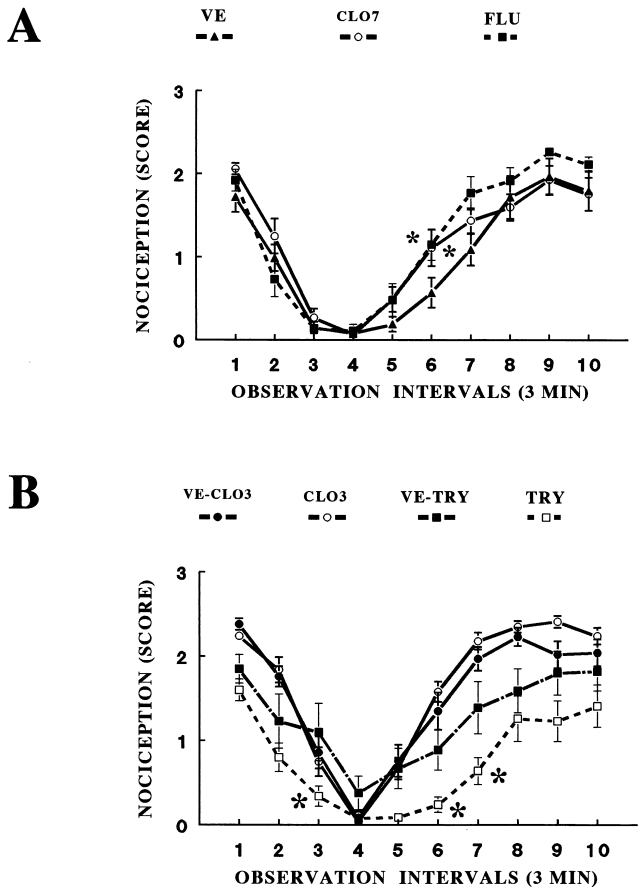

Fig. 7. Assessment of the analgesic efficacy of the serotonin uptake inhibitors (clomipramine and fluoxetine) and the serotonin precursor tryptophan using control rats subjected to a sham swimming, i.e., placing them in a container with a water depth of 2–4 cm. Panel A: VE = vehicle (0.9% NaCl, i.p., once a day); CLO7 = clomipramine (2.5 mg/kg, i.p., once a day); and FLU = fluoxetine (0.25 mg/kg, i.p., once a day) started 7 days before the first swimming session. Panel B: VE-CLO3 = vehicle (0.9% NaCl, i.p., once a day) and CLO3 = clomipramine (2.5 mg/kg, i.p., once a day) started 3 days before the first swimming session; VE-TRY = vehicle (0.9% NaCl, i.p.) and TRY = tryptophan (3 mg/kg, i.p.) injected 24 h before each swimming session. * Significant difference compared to non-swim-stressed rat pretreated with vehicle ($p < .05$, one-way ANOVA followed by Duncan's test).

involvement of the hypothalamic–pituitary–adrenal axis in these processes. In addition, diazepam does not alter holding hyperalgesia but abolishes both novelty hyperalgesia [24] and vibration-induced hyperalgesia [13], suggesting a modulatory role for GABA in some forms of stress-evoked hyperalgesia. Clonidine, an alpha2-adrenoceptor agonist, reduces vibration-induced hyperalgesia in rats by a mechanism that is blocked by the alpha2-adrenoceptor antagonist yohimbine [13]. Thus, pituitary factors, GABA, and alpha2-adrenoceptors seem to be involved in the induction and/or in the modulation of transient hyperalgesias produced by acute stress.

The mechanisms underlying long-lasting hyperalgesias, induced by prolonged stressors, has been even less studied. Satoh et al. [21] found that the long-lasting mechanical hyperalgesia produced by prolonged cold stress is blocked by the intrathecal administration of antisera to substance-P- and calcitonin-gene-related peptide, but not antiserum to galanin, suggesting the involvement of peptide-containing primary afferents. The outcome of the present study suggests that the increased thermal and chemical nociception, observed after subchronic swim stress, might be mediated by changes in the activity of the central serotoninergic system. We found that the forced swimming hyperalgesias are completely blocked by acute pretreatment with low doses of tryptophan, a precursor of serotonin, and prolonged pretreatment (3 to 7 days) with low doses of the serotonin-selective uptake inhibitors, clomipramine, and fluoxetine. Microdialysis studies in freely moving rats have shown an increased serotonin release in several brain regions especially in the raphe magnus after 5 min [2,9] and 15 min of forced swimming [10]. Conversely, a longer forced swimming, for 30 min, diminishes the efflux of serotonin in some brain structures such as the amygdala and lateral septum [14]. Thus, we speculate that subchronic swim stress produces an initial increase in the activity of central serotonin systems which is followed by a decrease in release in response to a prolonged stress.

4.2. Site of action for the serotoninergic agents

The sites where the centrally acting serotoninergic agents used in this study exert their actions remain to be elucidated. Since serotonin-selective uptake inhibitors and tryptophan were systemically administered, their antihyperalgesic effect could be exerted on the different areas innervated by the central serotoninergic system. A major component of the antihyperalgesic effect of the serotoninergic agents could be due to their action on serotoninergic neurons in the raphe magnus that send descending fibers to the superficial layers of the spinal dorsal horn. Indeed, raphe magnus neurons have been found to inhibit formalin-evoked pain responses [1]. Moreover, the interphase of the formalin test is thought to depend on the endogenous activity of descending inhibitory fibers terminating in the dorsal spinal cord rather than on the lack of peripheral excitatory input [26]. Interestingly, we found that subchronic swim stress induced an increased nociceptive behavior in the formalin test mainly during the interphase.

Supraspinal sites may also represent another putative site of action where serotoninergic agents produce their antihyperalgesic effects. Formalin-evoked pain responses can be inhibited by electrical stimulation of the periaqueductal gray matter [6]. Thus, subchronic swim stress might produce hyperalgesia by impairing the inhibitory control of the periaqueductal gray and this can be prevented by pretreatment with the serotoninergic agents. More rostrally, blockade of several structures of the limbic circuit also reduces pain behaviors evoked by formalin [22]. Stressors are known to activate several regions of the limbic system, such as the paraventricular nucleus of the hypothalamus and the hippocampus [3]. Thus, subchronic stress may increase nociception by increasing the activity of limbic structures (e.g., hypothalamus and hippocampus) to produce hyperalgesia and serotoninergic agents may act in these regions to inhibit the limbic system mediated stress-evoked hyperalgesia.

4.3. Clinical implications

High doses of tricyclic antidepressants, including clomipramine, have analgesic efficacy in the formalin test [23] but not in the hot plate [7]. In the present study, clomipramine and fluoxetine, but not tryptophan, at the very low doses, lacked analgesic activity in the hot plate and the formalin tests. Thus, the blockade of the development of hyperalgesia after the swim stress by these serotoninergic agents was not due to their analgesic properties. We observed a progressive decline in struggling times after repeated exposure to inescapable swim stress and this decrease in survival behavior was directly correlated with the hyperalgesia observed during the interphase period of the formalin test. Treatment with clomipramine, fluoxetine and tryptophan, drugs known for their antidepressant efficacy [8], abolished both the deficit in the survival behavior (swimming) and the hyperalgesia to noxious thermal and chemical stimuli. The relationship between the escape behavioral deficit and hyperalgesia were observed for both clomipramine treatment schedules, further supporting the close linkage between swim stress-induced hyperalgesia and behavioral manifestations of helplessness or a lack of desire to survive. The escape behavioral deficit, induced by inescapable swim stress, has been termed "behavioral despair", and is thought to be a surrogate model of human depression [20]. Chronic stress produces functional and biochemical changes in the limbic–hypothalamic–pituitary axis that are remarkably similar to those observed during severe depression and that have been considered a necessary feature of this syndrome [3]. Pain sensitivity is increased in individuals with either minor or major depression [17,19] and depressed individuals report a higher prevalence of clinical pain problems [12,25]. In addition,

the Fawn–Hooded rat strain shows an exaggerated escape deficit (immobility) in the forced swim test and are sub-sensitivity to serotoninergic agonist [11]. Taken together, the escape behavioral deficit and swim stress-induced hyperalgesia may result from a deficit in the central serotoninergic transmission which produces both depression and a sensitization of central pain relay pathways. Stress swim induced hyperalgesia appears to result from changes in the central serotonin system, which is an important biological link between stress, depression and pain.

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