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Chronic treatment with fluoxetine decreases seizure threshold in naïve but not in rats exposed to the learned helplessness paradigm: Correlation with the hippocampal glutamate release

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Abstract

The proconvulsive effect of the new generation of antidepressants remains controversial. The authors investigated in naïve rats the effect of chronic treatment with fluoxetine (FLX) on the convulsive threshold and on two parameters of the hippocampal glutamatergic neurotransmission: the in vitro glutamate release and the binding of [^{3}H] MK801 to NMDA receptors. While the acute treatment with FLX provoked no change either in seizure susceptibility or in the glutamate release, the chronic treatment decreased the convulsive threshold in coincidence with an increment in the in vitro glutamate release. No significant effects on the binding of [^{3}H] MK801 to NMDA receptors were found to be attributable to the FLX treatment. We also assessed the effect of the chronic treatment with FLX on the seizure threshold in rats exposed to an experimental model of depression, the learned helplessness paradigm (LH). While a decrease in the K^+-stimulated glutamate release was observed in non treated LH animals, when they were chronically injected with FLX, no changes in the epileptic susceptibility and no increments in the glutamate release were found. Our results indicate that chronic treatment with FLX decreases the epileptic threshold in naïve but not in LH rats and that this effect correlates with the levels of the hippocampal glutamate release.

Keywords: Chronic treatment; Fluoxetine; Glutamate release; Hippocampus; Learned helplessness; Seizure threshold

1. Introduction

Antidepressants are psychotropic drugs frequently prescribed since they are used not only for the treatment of depression but also for the symptomatic relief of anxiety disorders. One of their reported adverse effects is the decrement in seizure threshold, which was originally observed with tricyclic antidepressants. Whether the new generations of antidepressants (i.e., selective serotonin reuptake inhibitors, SSRI) exert this effect remains controversial: some authors reported a proconvulsive action of the SSRI fluoxetine (FLX) (Hernandez et al., 2002; Raju et al., 1999) while others reported the opposite (Wada et al., 1995).

Most of the studies done in this matter examined the effect of acute treatments, a methodological approach that proved to be useful for analyzing the participation of serotonin in the seizure threshold. However, acute treatments seem not to be the best strategy to predict the pro-epileptic potential of these compounds in the clinical practice, since they are prescribed on chronic basis. This

Abbreviations: BDNF, brain-derived neurotrophic factor; CREB, cAMP responsive element binding protein; FLX, fluoxetine; i.p., intraperitoneal; LH, learned helplessness; MK801, dizocilpine; NLH, non learned helplessness; NMDA, *N*-methyl-D-aspartate; PTZ, pentylenetetrazole; SSRI, selective serotonin reuptake inhibitors.

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distinction is really important, considering that many of the mechanisms by which the antidepressants exert their therapeutic effects are only seen after a prolonged administration and depend on the induction of plastic changes in the central nervous system, in particular, in the hippocampus and related areas (Manji et al., 2003).

So, the first goal of our work was to investigate the potentiality of chronic treatment with FLX for decreasing the seizure threshold in two different conditions: in naïve rats and in rats exposed to an experimental model of depression, the learned helplessness (LH) paradigm.

As we found that chronic treatment with FLX decreases the convulsive threshold in naïve animals but not in LH ones, we also investigated two parameters of the hippocampal glutamatergic neurotransmission which have been related to convulsive threshold: the in vitro glutamate release in hippocampal slices and the binding of [^{3}H] MK801 to NMDA receptors.

2. Methods

2.1. Animals

Male adult Wistar rats, weighing 180 to 200 g at the beginning of the treatment, were housed in groups of 4–5 in a room with constant temperature and a 12:12 light–dark cycle. They received food and water ad libitum. The animals were treated according to the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 1996). All efforts were made to reduce the number and the unnecessary suffering of animals employed.

2.2. Drugs

All the reagents were analytical grade and purchased from Sigma–Aldrich Inc. (St Louis, Missouri). [^{3}H] MK801 was purchased from New England Nuclear.

2.3. Learned helplessness paradigm

We applied the method described by Reinés et al. (2004a) which consists of an induction and a test session.

2.3.1. Learned helplessness induction

Rats were randomly assigned to shocked and control groups. Animals were individually placed in the treatment chamber that consisted in a $28 \times 21 \times 25$ cm^3 box with black Plexiglas walls and equipped with a stainless-steel grid floor. A constant-current shocking device was used to deliver 60 inescapable foot shocks (0.6 mA) for 15 s every min (i.e., for 1 h; shocked group). Control rats (NLH) were placed on the grid in identical chambers for 1 h, without receiving the uncontrollable electric shocks. After this session, animals were replaced in their own cages. All the helplessness induction trials were performed during the morning.

2.3.2. Learned helplessness behavior test

In our laboratory, we found that 49% of normal animals exposed to the inescapable shocks fail to develop the learned helplessness behavior. To control for this, 4 days after the exposure to inescapable shocks, a test session was performed. Animals were subjected to an avoidance task in a shuttle box which consisted of two equal-sized compartments divided by a Plexiglas partition fitted out with an opening (7×7 cm) and with a grid floor consisting of stainless-steel rods placed 1 cm apart. Animals were placed individually into the shuttle box and allowed to habituate to the environment for 5 min. Following this exploration time, 15 stimulus-shock trials (0.6 mA) were presented for a period of 15 min, i.e., one trial per min. In each trial, the shock duration was 20 s with an intertrial time of 40 s. In the first five trials, the door was opened immediately after starting the shock, and in the rest 10 trials, there was a 4 s delay period after starting the shock. The number of failures to change from the shuttled side to the safe side (the failure to respond during the 20 s shock on) was recorded as the way to evaluate the learned helplessness behavior acquisition. Only animals in which learned helplessness behavior was observed on day 4 were included in the LH group. In a separated set of experiments, we demonstrated that this behavior persists for at least 25 days from the induction session (i.e., 21 days after the test session). The values of escape latency (mean ± SEM) on day 25 were as follows: 17 ± 0.58 and 3.91 ± 0.65 s for LH and NLH animals respectively ($p < 0.0001$ versus NLH, Student's t test).

2.4. Drug treatments

The effect of the treatment with FLX was explored in two different conditions: in naïve rats (i.e., animals not exposed to the learned helplessness model) and in rats in which learned helplessness behavior (LH) was induced.

Naïve animals were randomly assigned to one of the treatment groups. For the acute experiments, animals received one injection of saline or FLX (10 mg/kg) (i.p.). For the chronic schedule, naïve rats were treated for 21 days with a daily injection (i.p.) of saline or FLX (10 mg/kg).

LH rats were randomly divided into two groups and chronically injected (i.p.) with FLX (10 mg/kg) or saline. The treatment started after the test session (day 4) and continued for 21 days (Fig. 1) since after this period of time in a different set of experiments, we observed that the antidepressant reverses the LH behavior (Reinés et al., 2004b) (mean ± SEM of escape latencies 5.4 ± 2.6 and 18.6 ± 2.1 s for the FLX and the saline-treated animals respectively, $p < 0.0001$, Student's t test).

2.5. Pentylenetetrazole-induced convulsions

These experiments were performed in the following groups of animals: naïve animals acutely ($n = 4$) or chroni-

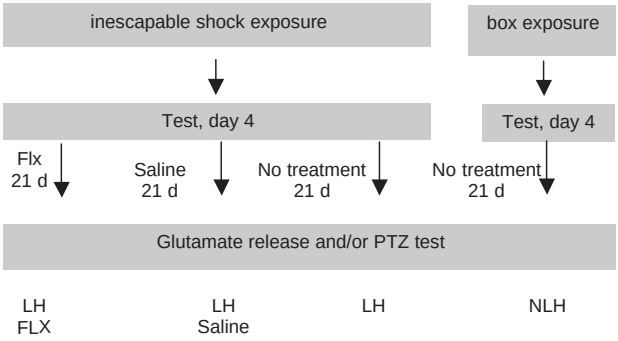

Fig. 1. LH experimental groups and their corresponding controls (NLH). LH animals were individually placed in the shuttle box and 60 inescapable foot shocks (0.6 mA) for 15 s every min were delivered. NLH rats were placed on the box for 1 h, but no electric shocks were delivered. Four days after, the escape deficit (test, day 4) was evaluated; animals in which learned helplessness behavior was observed (LH) were randomly assigned to receive either saline (LH saline), fluoxetine (LH FLX) or no treatment (LH). NLH animals were not treated.

cally treated with saline or FLX ($n=7$) and LH animals chronically treated with saline or FLX ($n=5$).

The scale described by Bazyan et al. (2001) was used to assess seizure threshold. Scores were as follows: 1=a twitch of the head, 2=occasional clonic seizures, 3=a series of clonic seizures, 4=clonic-tonic seizures with kangaroo posture, 5=clonic-tonic seizures and falling on one side.

To identify a subconvulsive dose, a separated group of rats was injected (i.p.) with different doses of pentylenetetrazole (PTZ), dissolved in saline solution, ranging from 15 to 50 mg/kg. A subconvulsive dose of PTZ (30 mg/kg) that failed to induce seizures in 85% of the animals and produced a score not higher than 2 in the remaining 15% (data not shown) was used.

This acute subconvulsive dose was injected 1 h after the single (acute treatment) or the last (chronic treatment) injection of FLX or saline. The convulsive behavior was observed for 20 min. Results are expressed as maximum score (mean±SEM) obtained in 20 min.

2.6. In vitro glutamate release

These experiments were performed in the following groups of animals: in naïve rats acutely ($n=5$) or chronically ($n=4$) injected with saline or FLX, and in LH animals chronically (21 days) treated with saline or FLX (4 to 6 animals per group). In order to know whether the exposure to the learned helplessness paradigm induces any change in this parameter, in a separated set of experiments, the hippocampal glutamate release was analyzed in LH and NLH animals 21 days after the test session (day 4).

The experimental procedure was performed according to that previously described by Bonavita et al. (2002). Animals were killed by decapitation 60 min after the single dose (acute treatment), and after the last dose of chronic treatments. The hippocampi were removed and immediately placed on a Petri dish containing ice-cold Krebs buffer (5 mM KCl, 127 mM NaCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, 0.8 mM Na_2HPO_4, 25 mM $NaHCO_3$, 11 mM glucose) pregassed with a mixture of O_2 (95%) and CO_2 (5%). The structure was cut in longitudinal slices (250 μm) by means of a Sorvall tissue sectioner, and these placed in a superfusion chamber maintained at 37 °C, perfused at a constant rate of 0.4 ml/min. After an initial washout period of 60 min following slicing to allow tissue stabilization, one sample was collected to quantify basal release levels. To elicit glutamate release, the perfusing Krebs buffer was switched for 5 min to one containing 60 mM KCl (NaCl adjusted to 72 mM to maintain osmolarity), and then switched back to the original buffer. Samples were stored at −70 °C and the glutamate content was assayed in a 250-μl aliquot by HPLC with electrochemical detection following the method by Durkin et al. (1988). Results are expressed as pmol of glutamate/mg of tissue/ml of perfusate.

2.7. [^{3}H] MK801 binding

Binding experiments were performed in hippocampal membranes obtained from naïve rats acutely ($n=6$) and chronically ($n=8$) treated with saline or FLX. Sixty minutes after the single dose (acute treatment) or the last dose (chronic treatment) of saline or FLX, animals were killed by decapitation and hippocampi were rapidly removed and frozen at −70 °C. Membranes were prepared according to the method described by Weiland et al. (1997). Briefly, tissue was thawed and homogenized (1:20 w/v) in 0.32 M sucrose containing 5 mM EDTA, and was then centrifuged for 10 min at 1000×g. The supernatant was centrifuged at 30,000×g and the pellet resuspended (1:100 w/v) in 5 mM EDTA and incubated for 30 min at 30 °C. Membranes were centrifuged and washed twice by resuspension in 5 mM EDTA and incubated at 30 °C for 30 min. An aliquot was assayed for total proteins using the method of Bradford. The final pellet was frozen at −70 °C overnight. On the day of the experiment, membranes were resuspended in 20 mM HEPES/5 mM EDTA, pH 7, at an approximate protein concentration of 0.5 mg/ml, and saturation binding assays were performed (50 μg of protein per tube) using 1–50 nM [^{3}H] MK801 (specific activity 28.9 Ci/mmol). All tubes also contained 100 μM glutamate, 20 μM glycine and 16 μM spermidine. Non-specific binding was determined in the presence of 8 μM MK801. Samples were incubated at 30 °C for 3 h. The reaction was terminated by the addition of 4 ml of ice-cold 5 mM Tris–chloride buffer (pH 7.4) and vacuum filtration through Whatman GF/B glass-fiber filters. The filters were rinsed 3 more times with the same volume of buffer and the radioactivity retained was measured with a liquid scintillation spectrometer. The parameters B_{max} and K_D were estimated with the GraphPad Prism software (version 3.0).

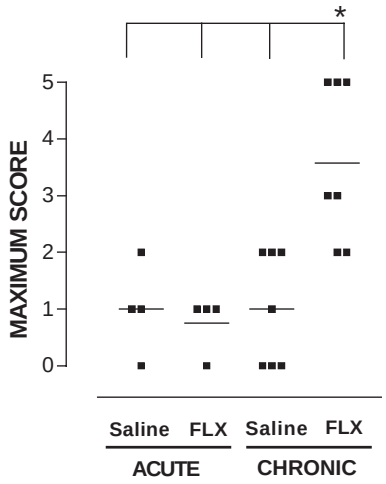

Fig. 2. Effect of acute or chronic treatment with FLX (10 mg/kg, i.p.) or saline on the convulsive threshold in naïve rats. A subconvulsive dose (30 mg/kg) of pentylenetetrazole was injected (i.p.) 1 h after the single dose (acute treatment) or the last dose of the chronic treatment (21 daily injections). Animals were observed for 20 min and the maximum score according to the method described by Bazyan et al. (2001) was recorded. Results are expressed as mean ± SEM of 4–7 animals per group. *$P<0.05$ versus the other three conditions, Kruskal–Wallis followed by Dunn test.

2.8. Statistical analysis

Results from pentylenetetrazole experiments were compared by Kruskal–Wallis test followed by Dunn test for naïve animals or by Mann Whitney test for LH animals treated with FLX or saline. Results from glutamate release and binding experiments were compared by two way-analysis of variance (ANOVA) (treatment × chronicity) followed by Bonferroni test for naïve animals or by Student's *t* test for either LH/NLH animals or LH animals treated either with saline or FLX.

Table 1
[^{3}H] MK801 binding to hippocampal membranes obtained from naïve rats acute or chronically treated (21 day) with FLX (10 mg/kg) or saline (i.p.)

Treatment	K_D (nM)	B_{max} (fmol/mg protein)
Acute saline	4.83 ± 0.41	3668 ± 356
Acute FLX	5.07 ± 0.38	3217 ± 386
Chronic saline	5.31 ± 0.34	2753 ± 169*
Chronic FLX	5.59 ± 0.66	2729 ± 205*

Animals were killed by decapitation 60 min after the single or the last dose of the 21-day treatment. Hippocampal membranes were prepared and kept at −70 °C until used. On the experimental day, [^{3}H] MK801saturation curves were performed with concentrations ranging from 1 to 50 nM. Binding parameters were estimated from the experimental hyperbola using the GraphPad Prism Software. Results are expressed as mean ± SEM of 6–8 animals per group.
* $F_{(1,23)}=6.70$ versus acute treatments, two-way ANOVA followed by Bonferroni test.

3. Results

3.1. Neurochemical and behavioral experiments in naïve rats

3.1.1. Pentylenetetrazole-induced convulsions

When administered to naïve animals, chronic treatment with FLX induced a significant decrease in the convulsive threshold, as assessed by the convulsive response to a subconvulsive dose of pentylenetetrazole (257%, $P<0.05$ versus acute FLX, Kruskal–Wallis followed by Dunn test). On the contrary, acute treatment did not induce any change in this parameter (Fig. 2).

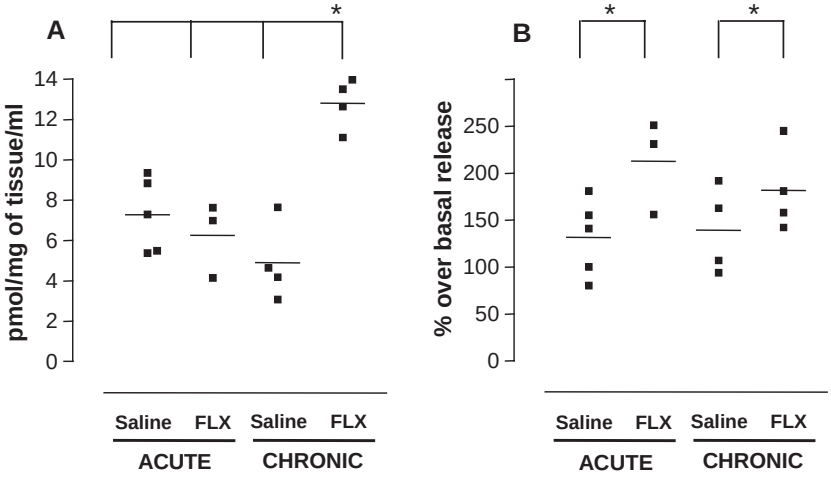

Fig. 3. Glutamate release from hippocampal slices of naïve rats acutely or chronically treated with FLX (10 mg/kg, i.p.) or saline. Animals were killed by decapitation 60 min after the single dose (acute treatment) or the last dose of the chronic treatment (21 days). After an initial washout period of 60 min, a sample was collected to assess basal release levels. The perfusing Krebs buffer was switched for 5 min to one containing 60 mM KCl, and then switched back to the original buffer. Results are expressed as mean ± SEM of 3–5 animals per group. (A) Basal glutamate levels, *$F_{(1,12)}=5.499$ versus the other three conditions, two-way ANOVA followed by Bonferroni test. (B) Potassium-stimulated glutamate release expressed as percentage over basal values. *$F_{(1,12)}=7.3$, acute and chronic FLX versus acute and chronic saline respectively, two-way ANOVA followed by Bonferroni test.

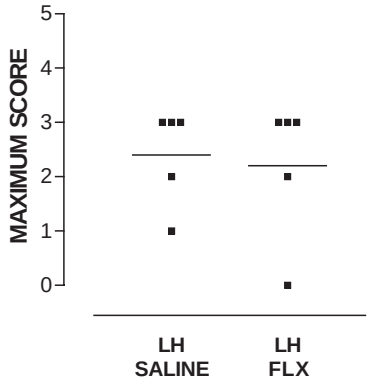

Fig. 4. Effect of the chronic treatment with FLX (10 mg/kg, i.p.) or saline (21 daily injections) on the convulsive threshold in animals exposed to the learned helplessness paradigm (LH FLX and LH saline rats, respectively). Sixty minutes after the last injection, a subconvulsive dose (30 mg/kg) of pentylenetetrazole was injected (i.p.). Animals were observed for 20 min and the maximum score according to the method described by Bazyan et al. (2001) was recorded. Results are expressed as mean ± SEM of the maximum scores observed in 5 animals per group. No differences were observed between groups (Mann Whitney test).

3.1.2. In vitro glutamate release

In naïve animals chronically treated with FLX, a significant increment in the basal glutamate release was observed (261%, $F_{(1,12)}=5.499$ versus chronic administration of saline) (Fig. 3A). On the other hand, both acute and chronic treatment with FLX induced a significant increase in the K^+-evoked glutamate release (76% for acute treatment, 61% for chronic one; $F_{(1,12)}=7.3$) and the extent of the increment was similar for both kind of treatments (Fig. 3B).

In order to estimate in each condition (acute or chronic treatment with saline or FLX), the amount of glutamate exocitotically released after the K^+-stimulus, experiments with or without Ca^{++} in the perfusing solution were performed. In Ca^{++}-free experiments, the levels of glutamate released by the stimulus were significantly lower compared with the values obtained in the presence of Ca^{++} (data not shown), but no differences among the treatments were observed. Alanine concentration was also quantified in the samples as a non-transmitter amino acid control and no effect of 60 mM K^+ on its release profile was found (data not shown). These results indicate a major contribution of exocitotic release in the K^+-evoked glutamate response observed in each group, and therefore, the participation of calcium-independent mechanisms in the glutamate release changes produced by the different treatments can be ruled out.

3.1.3. [^{3}H] MK801 binding experiments

Experiments of [^{3}H] MK801 binding to NMDA receptors were performed employing hippocampal membranes obtained from naïve rats acutely and chronically treated with saline or FLX. When acute treatments were compared with chronic ones, saline and FLX chronically treated animals exhibited a significant diminution (by 75%, $F_{(1,23)}=6.70$ versus acute treatment) in B_{max} values, but differences between them were not statistically significant (Table 1).

3.2. Neurochemical and behavioral experiments in LH rats

3.2.1. Pentylenetetrazole-induced convulsions

No changes were observed in the convulsive threshold of LH animals chronically treated with FLX since seizure scores were similar to those observed in LH animals chronically injected with saline (Mann Whitney test) (Fig. 4).

3.2.2. In vitro glutamate release

To assess whether the animal exposure to the LH paradigm induces by itself any change in this parameter, the hippocampal glutamate release was compared for LH

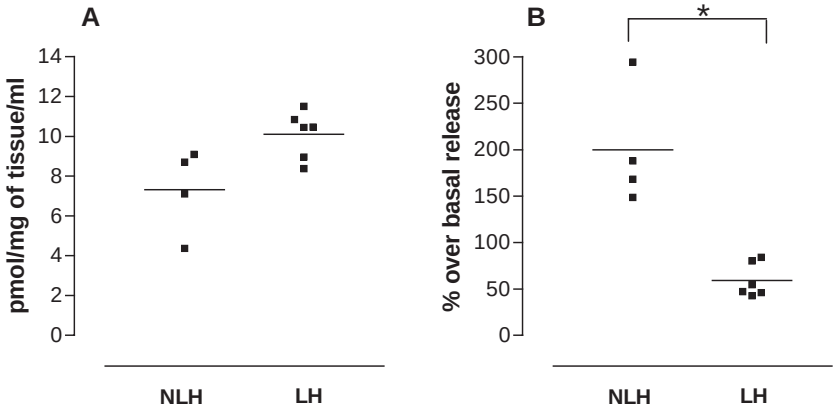

Fig. 5. Glutamate release from hippocampal slices of animals exposed to the learned helplessness paradigm (LH) and their respective controls (NLH). Animals were killed by decapitation 21 days after the test session performed on day 4 (see Fig. 1). Hippocampal slices were put in a superfusion chamber. After an initial washout period of 60 min, a sample was collected to assess basal release levels. The perfusing Krebs buffer was switched for 5 min to one containing 60 mM KCl, and then switched back to the original buffer. Results are expressed as mean ± SEM of 4-6 animals per group. (A) Basal glutamate levels, no differences were found between groups (Student's t test). (B) Potassium-stimulated glutamate release expressed as percentage over basal values. *$P<0.005$ versus NLH animals (Student's t test).

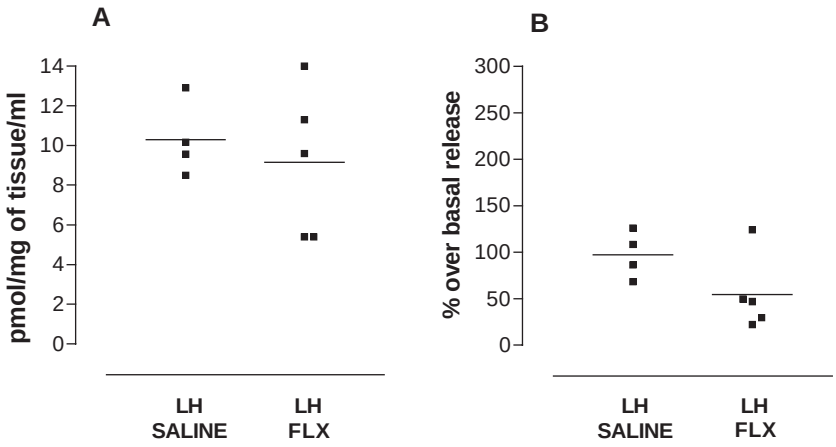

Fig. 6. Glutamate release from hippocampal slices of animals that developed the learned helplessness behavior and were chronically treated with FLX (10 mg/kg, i.p.) (LH FLX) or saline (LH saline). Animals were killed by decapitation 60 min after the last injection. Hippocampi were removed and slices were placed in a perfusing chamber. One sample was collected to assess basal release levels after an initial washout period of 60 min. The perfusing Krebs buffer was switched for 5 min to one containing 60 mM KCl (K^+-stimulus), and then switched back to the original buffer. Results are expressed as mean ± SEM of 4–6 animals per group. No statistical differences were observed between groups, Student's *t* test.

and NLH animals (Fig. 5). Although it is observed that there is a trend toward an increment in the basal glutamate release in the LH group, it does not reach statistical significance. On the other hand, we found a significant diminution in the potassium-stimulated glutamate release of LH animals (percent over basal release NLH: 200 ± 45; LH: 59 ± 12. $P < 0.005$ versus NLH animals, Mann Whitney test).

The hippocampal glutamate release was also evaluated in LH animals chronically treated with FLX or saline (Fig. 6). The chronic treatment with FLX of LH animals did not induce any change in the basal glutamate release and failed to increase the stimulated glutamate release (P = n.s. versus LH rats chronically treated with saline, Student's *t* test) (Fig. 6A and B).

4. Discussion

In the present work, we investigated the effect of the prolonged treatment with FLX on the seizure threshold in two different conditions: in naïve animals and in animals exposed to an experimental model of depression, the learned helplessness paradigm (LH rats).

When naïve animals were chronically treated with FLX, a decrease in the seizure threshold, assessed by the response to a subconvulsive dose of pentylenetetrazole, was observed. On the contrary, the chronic treatment with FLX did not exert any effect on the convulsive threshold of LH animals. We also determined in naïve and LH rats the effect of the chronic treatment with FLX on the hippocampal glutamate release. In naïve rats, it was found that the decrement in the seizure threshold was coincident with an increase in the hippocampal glutamate release. In LH animals, the glutamate release was found to be lower than that observed in NLH rats and the chronic treatment with FLX did not produce any change on this parameter.

4.1. Pentylenetetrazole-induced convulsions in naïve rats treated with FLX

The effect of the new generation of antidepressants on the convulsive threshold remains controversial, and when assessed in experimental settings, the results seem to depend on the treatment schedule (i.e., acute versus chronic) and the methodology employed for seizure induction.

As shown in our experiments, and in coincidence with the results obtained by other authors (Wada et al., 1999; Raju et al., 1999), a single dose of FLX administered to naïve animals does not have any effect on seizure threshold. On the other hand, the chronic administration induces a significant diminution in this parameter. This latter observation is similar to that reported by Raju et al. (1999), who observed an increment in the ED_{50} of phenytoin to prevent the electroshock-provoked seizures in animals chronically injected with FLX. Previous works examining chronic effect of fluoxetine on seizure threshold used genetically epilepsy-prone rats (Dailey et al., 1992), and in this condition, an increment in the seizure threshold was reported. Our results confirm that chronic treatment with this compound decreases, rather than increases, the convulsive threshold in normal animals. Although genetically epilepsy-prone rats, electrically-provoked seizures and chemically-induced convulsions are all suitable methods for screening anticonvulsant properties of drugs, no direct comparisons among their neurobiological substrates are currently available, and therefore, similarities or differences among the effect of different treatments on them should be cautiously interpreted. In fact, for example, tryciclic antidepressants, drugs with a well-known proconvulsive effect in depressed patients and in experimental animals, exhibit anticonvulsant

properties in genetically epilepsy-prone rats (Dailey and Jobe, 1985; Reigel et al., 1986).

4.2. In vitro glutamate release and [^{3}H] MK801 binding in naïve rats treated with FLX

Seizures are often considered to be the result of an excitatory/inhibitory imbalance subsequent to increased glutamatergic excitation and/or to reduced GABAergic inhibition. Microinfusion of glutamate (200 μM) in the hippocampus has been shown to induce a decrease in the convulsive threshold (Sierra-Paredes et al., 2001) and conversely a significant increment in basal extracellular glutamate levels was detected in hippocampi of animals exposed to an experimental model of epilepsy (Richards et al., 2000). In this study, we observed that chronic treatment with FLX induces an increment in the hippocampal basal glutamate release. Stimulated glutamate release, calculated as percentage over basal values, was incremented both by acute and chronic treatment with FLX, but evidently, as a consequence of the higher basal levels observed after chronic administration, absolute values of stimulated glutamate release were higher in the chronically treated group. We have also observed that chronic treatment decreases the number of NMDA hippocampal receptors. As this modification was also seen in saline-treated animals, it seems to be the result of the chronic handling rather than to the chronic treatment with the drug, an observation previously reported by our laboratory and by other authors (Bonavita et al., 2002; Warenycia et al., 1990). Therefore, as no specific effect of FLX on NMDA receptors could be informed, this change seems not to contribute to the diminution in the convulsive threshold observed.

Thus, our in vitro results suggest that the chronic treatment with FLX generates a condition similar to that experimentally induced by glutamate microinfusion in hippocampus, consequently leading to the diminution of the convulsive threshold. As the increment in glutamate release is observed after multiple dosing but not after acute treatment, it is possible to speculate that it could be due to trophic or plastic changes induced by prolonged administration of FLX. In fact, among others, increased CREB, BDNF and cell proliferation have been reported as a consequence of prolonged administration of antidepressants (Malberg et al., 2000; Nibuya et al., 1995, 1996) therefore probably leading to a greater number of neurotransmitter releasing neurons. Whether this process affects predominantly the glutamatergic neurons is not known. On the other hand, while a diminution in GABAergic neurotransmission could eventually contribute to the decrease in the convulsive threshold, it is unlikely that this phenomenon could underlie the increment in the basal glutamate release observed after chronic treatment with FLX. However, it should be noted that our in vitro experimental approach has some limitations, as it does not allow to examine the effect of the treatment on some important factors affecting extracellular glutamate concentration (i.e., astrocytic glutamate release and astrocytic and neuronal glutamate transporters). In vivo experiments using the microdyalisis technique could definitively confirm our results, provide data about the above mentioned parameters and also allow to evaluate the participation of some other neurotransmitters, as for example GABA, in the proconvulsive status observed.

4.3. Pentylenetetrazole-induced convulsions in LH rats chronically treated with FLX

A very different picture was obtained when the same experiments were performed with animals exposed to a model of depression. LH rats chronically treated with FLX did not show the decrement in the seizure threshold, and as discussed below, overall glutamate release is lower than that observed in NLH animals. This observation seems to give further support to the possible causal role of the higher levels of glutamate in the proconvulsive state induced by chronic FLX in the naïve rats.

As far as we know, experiments exploring the effect of antidepressants on convulsive threshold have been performed in normal or epilepsy-prone animals, but not in animals that develop a depressive-like behavior. From our results, it seems likely that LH induction generates a neurobiological condition in which chronic treatment with FLX lacks proconvulsive action.

4.4. In vitro glutamate release in LH rats

In our experiments, we found that the potassium-evoked glutamate release was lower in LH rats compared with NLH, that FLX chronic treatment failed to increment the basal glutamate outflow as it was seen in naïve animals, and also that no increment in the potassium-stimulated glutamate release was observed as a consequence of the chronic treatment.

To our knowledge, hippocampal glutamate release has not been previously studied in experimental models of depression. It is possible to speculate that the decrease in stimulated glutamate release could, at least in part, be related to the well-known profound structural changes that have been observed in hippocampal neurons of animals exposed to depression paradigms. Among others, atrophy of apical dendrites of pyramidal neurons in the CA3 hippocampal region (Bisagno et al., 2000; Magariños and McEwen, 1995a,b; Sousa et al., 2000), atrophy in granule and CA1 pyramidal cells and synaptic loss (Sousa et al., 2000) as well as a significant diminution of light neurofilaments of the cytoskeleton in hippocampal neurons (Reinés et al., 2004a) have been reported. All these structural changes may compromise the normal function of hippocampal neurons, and in fact behavioral impairment in hippocampus-dependent memory and avoidance tasks have been observed. As glutamate is the main transmitter in all these hippocampal cell populations, we propose that the

decrease in the overall glutamate availability observed in our experiments could be additional evidence linking atrophy with behavior impairment.

4.5. In vitro glutamate release in LH rats chronically treated with FLX

In contrast to what was observed in naïve animals, chronic treatment with FLX to LH rats failed to increment either the basal or the stimulated glutamate release. Indeed, while no significant differences were observed in basal glutamate release between NLH and LH groups, the latter shows a significant lower stimulated release (about 50% compared with 200% of the NLH animals) which remains below NLH and naïve values even after FLX treatment. So, absolute values of extracellular glutamate in the hippocampi of LH rats even after the administration of FLX are lower than that observed in naïve animals chronically treated with the drug. In our opinion, the absence of the proconvulsant effect of FLX in LH rats could be, at least in part, attributable to the lack of effect of the drug on the extracellular glutamate concentration.

Antidepressants are thought to exert their effect through neuronal trophic stimulation, and so it remains unclear why FLX fails to restore glutamate levels to that seen in NLH or naïve rats. However, some authors (Magariños et al., 1999) observed that either fluoxetine or another SSRI, fluvoxamine, fail to block the dendritic atrophy induced by chronic restraint stress, a well-known model of depression. Additionally results from our laboratory suggest that chronic treatment with FLX fails also to reverse the diminution in the neurofilament subunit of 68 kDa (Reinés et al., 2004b). This cytoskeletal protein constitutes the core for the intermediate neurofilament assambly therefore playing a critical role for morphology and functionality maintenance (Reinés et al., 2004b) All these observations suggest some kind of incapability of hippocampal neurons of animals exposed to depression models to undergo plastic changes. Consistently with these findings, some other authors also reported that congenitally LH rats show a lack of adaptive responses, as could be considered that of chronic treatment with FLX (Vollmayr et al., 2001).

5. Conclusion

We have proved that chronic treatment with FLX, as previously observed with older antidepressants, exerts a proconvulsant action in normal animals and an increment in the basal and consecutively in the stimulated glutamate release. On the other hand, the same FLX treatment of animals exposed to an experimental model of depression fails to induce both the changes in the glutamate release and the decrement in the seizure threshold. Taken as a whole, our results seem to support the notion that proconvulsive effect of antidepressants are closely associated with increases in the hippocampal glutamate levels. Additionally, we observed that the exposure to the learned helplessness paradigm induces a decrement in the in vitro stimulated glutamate outflow in hippocampal slices, whose causes should be further clarified.

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