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The Determination of the Optimal Dose of Milnacipran in the Olfactory Bulbectomized Rat Model of Depression

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REDMOND, A. M., J. P. KELLY AND B. E. LEONARD. *The determination of the optimal dose of milnacipran in the olfactory bulbectomized rat model of depression.* PHARMACOL BIOCHEM BEHAV **62**(4) 619–623, 1999.—Olfactory bulbectomy (OB) is associated with a variety of behavioral abnormalities such as hyperactivity in the "open-field" test. Previous studies have shown that chronic administration of antidepressants can reverse this behavioral deficit. The activity of milnacipran (20, 30, and 40 mg/kg, PO bid) administered in two equally divided doses twice daily was assessed in the olfactory bulbectomized rat model of depression. It was found that chronic treatment with milnacipran at the doses of 30 and 40 mg/kg, but not 20 mg/kg, attenuated the lesion-induced hyperactivity of the OB rat in the "open-field" test following 14 days of treatment. In the step-through passive avoidance test, administration of milnacipran at doses of 20, 30, and 40 mg/kg had no effect on the performance deficit associated with olfactory bulbectomy. Olfactory bulbectomy reduced the concentration of noradrenaline (NA) in the frontal cortex. However, chronic milnacipran treatment did not significantly alter this deficit. It is concluded that milnacipran, when administered chronically at doses of 30 and 40 mg/kg, are effective at reversing the "open-field" deficit associated with olfactory bulbectomy, and that a dose of 30 mg/kg is an optimal dose. © 1999 Elsevier Science Inc.

Milnacipran Bulbectomized rat "Open field" Brain monoamines

MILNACIPRAN is a new antidepressant drug, which has been demonstrated both in vivo and in vitro to be without any effect at postsynaptic receptors, but which inhibits with equal potency the reuptake of both noradrenaline and serotonin (3,13). In double-blind clinical trials, milnacipran has been shown to be equipotent with amitriptyline, and is tolerated by patients significantly better than tricyclic antidepressants (2, 23). In animal models of depression, milnacipran has been shown to be active in the forced-swim test (17), learned-helplessness test (11), in antagonizing tetrabenazine and apomorphine-induced hypothermia, and *p*-chloroamphetamine-induced hyperthermia (20); acute tests widely used to screen compounds for antidepressant-like activity.

Removal of the olfactory bulbs in rats is associated with a variety of behavioral abnormalities such as hyperactivity in the "open-field" apparatus. Antidepressant administration can only reverse this behavioral deficit following chronic (2 weeks) treatments and thus simulates the clinical situation, where mood elevation is normally only seen following 2–4 weeks of treatment (21). Previous studies have shown that a once-daily oral administration of milnacipran (14 days) attenuated the hyperactivity of the olfactory bulbectomized (OB) rat in the "open-field" test at a dose of 30 mg/kg but not at the lower doses of 1, 3, or 10 mg/kg (17).

Because milnacipran has a plasma elimination half-life of 7 h in rats (16), and clinical studies indicate that two daily doses of the compound are required to achieve antidepressant activity (1,2), the aim of the present study was to administer the compound twice daily in two equally divided doses at doses of 20, 30, and 40 mg/kg, PO and to determine the antidepressant activity of the compound following chronic treatment by examining the hyperactive response of the OB rat in the "open-field" test, and on the performance deficit of OB rats in the step-through passive avoidance test. In addition, the effects of milnacipran on monoamine levels in the frontal cortex of sham-operated and OB rats following chronic treatment was also examined.

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METHOD

Animals

Male Sprague–Dawley rats were obtained from Harlan Olac, UK (weight on arrival: 230–250 g). The animals were housed four per cage (two sham-operated and two olfactory bulbectomized) and maintained in a temperature (20–22°C) and light (light period 0800–2000 h)-controlled room. Food and water were given ad lib apart from the period of behavioral observations.

Olfactory Bulbectomy

After a 1 week acclimatization period, bilateral olfactory bulbectomy was performed in rats anaesthetized with 2.5% w/v 2-2-2 tribromoethanol (10 ml/kg IP) essentially as described by Cairncross et al. (5). The head was shaved, and a midline sagittal incision was made extending at least 1 cm rostral to the bregma. Sufficient pressure was applied to ensure that the periostium on the underlying bone had been penetrated. Two drill holes of 2-mm diameter were made in the skull 5 mm rostral to the bregma and 2 mm lateral to the midline. For sham animals, the dura was carefully pierced and the wound closed. For OB animals, the olfactory bulbs were aspirated using a water suction pump. Care was taken not to damage the frontal cortex. After the operation, bleeding was controlled by plugging the holes with hemostatic sponge (Haemofibrine®, Specialites Septodont, France). Oxytetracycline dusting powder was sprinkled on the wound prior to closure. The animals were allowed to recover for 14 days following surgery; they were handled daily throughout the recovery period to eliminate any aggressiveness that would otherwise arise (12).

"Open Field"

On day 15 of milnacipran treatment, and before milnacipran administration on that day, each rat was placed singly into the center of the "open-field" apparatus.

This apparatus is essentially as described by Gray and Lalljee (8). The open field consisted of a circular base, 90 cm in diameter, which was divided into 10-cm squares by faint yellow lines. The wall surrounding the base consisted of a 75-cm high aluminum sheet. Illumination was provided by a 60-W bulb, positioned 90 cm above the floor of the apparatus. All measurements were carried out in a darkened room to create a stressful environment, between 0700–0900 h. Each animal was placed in the center of the "open-field" apparatus, and the ambulation scores (number of squares crossed) were measured over a 3-min period for each animal.

Step-Through Passive Avoidance

On days 17, 18, and 19 of treatment animals were placed into the step-through passive avoidance test. The apparatus consisted of a two-compartment box (24 × 16 × 20 cm for each box) and was similar to that described by Venault et al. (22). The front illuminated (40-W bulb) chamber (white box) was connected to the rear dark chamber, which was equipped with a grid floor. The two chambers were separated by a guillotine door.

On day 1 of the test rats were placed individually into the illuminated compartment and allowed to explore the boxes. The latency to enter the dark chamber was recorded.

On day 2 the animals were again placed in the light chamber, and upon entry into the dark chamber, received a mild foot shock (1 mA) of a 3 s duration. On day 1 and 2 animals did not receive milnacipran treatment, so as to allow for a drug withdrawal interval.

On day 3 the animals were again placed in the light chamber, and the latency to enter the dark chamber was recorded. If the animal had not entered the dark side after 180 s, it was returned to its cage and the latency recorded as greater than 180 s.

Drug Administration

The following compounds were used: milnacipran HCl (Centre de Developpement Pierre Fabre, Castres, France) and 2-2-2-tribromoethanol (Aldrich Chemical Co., Gillingham, UK).

Two weeks following surgery, drug treatment began. Milnacipran was dissolved in distilled water and administered at doses of 10, 15, and 20 mg/kg, PO twice daily (morning and evening) for 22 days in an injection volume of 1 ml/kg. However, animals received no drug treatment on days 17 and 18 of the experiment, when the passive avoidance test was carried out, so as to allow for drug washout. Controls received injections of vehicle alone.

Animal Sacrifice and Brain Dissection

At the termination of the study, the rats were sacrificed by decapitation, and the brains rapidly removed. In the case of bulbectomized and sham-operated rats, the brains were checked for signs of cortical damage or incomplete removal of the olfactory bulbs during the surgical procedure. If damage was observed, these animals were excluded from the data analysis. The frontal cortex was selected for neurotransmitter determination and was rapidly dissected on an ice-cold plate, using a modified method by Popov et al. (15). The frontal cortex was removed using a sharp dissection blade, weighed, and placed in a tube for analysis.

Determination of Brain Biogenic Amine Concentrations

On the last day of the study, 24 h after their last treatment, rats were killed by decapitation, the brains removed, and the frontal cortex dissected (15). Concentrations of serotonin, noradrenaline, dopamine, and DOPAC were measured by high-performance liquid chromatography with electrochemical detection (18). The frontal cortex was homogenized by sonication in 1.0-ml elution buffer (pH 2.8), containing 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 1.4 mM octane-1-sulphonic acid, and 0.1 mM EDTA. This differed from the mobile phase in that it was "spiked" with 20 ng/50 μl *N*-methyl-dopamine as an internal standard. Homogenates were centrifuged at 15,000 rpm in a Hettich Mikro/K refrigerated centrifuge for 15 min. A 20-μl sample of the sample was injected directly into a reverse-phase column (RP 18, 25 cm × 4 mm internal diameter, particle size 5 μm) for separation of indoles and catecholamines (flow rate 1 ml/min). The neurotransmitters were quantified using a Merck-Hitachi D-2000 integrator.

Statistical Analysis

Initially, a two-way analysis of variance was performed. If any statistically significant change was found, the data was further analyzed using the post hoc Student–Newman–Keuls test. Results are tabulated as group mean and standard error of the means.

RESULTS

Neither olfactory bulbectomy nor milnacipran treatment significantly altered bodyweight gain in any of the groups. In the "open-field" test, a two-way ANOVA indicated that there was a typical increase in ambulation scores in the OB control group compared to the sham-operated control group, $F(1, 56) = 39.17$, $p < 0.05$, and that there was an interaction between the lesioned group and the milnacipran treated group, $F(3, 35) = 3.35$, $p < 0.05$ (Fig. 1). Post hoc analysis indicated that this hyperactivity was significantly attenuated by the 30 and 40-mg/kg, but not the 20-mg/kg dose.

On day 1 $F(1, 56) = 5.83$, $p < 0.05$, day 2, $F(1, 56) = 4.45$, $p < 0.05$, and day 3, $F(1, 56) = 12.5$, $p < 0.05$, of step-through passive avoidance, the latency to enter in the OB control group was decreased compared to the sham controls (Fig. 2). However, chronic milnacipran treatment did not alter this response on any of the days.

Following olfactory bulbectomy there was a significant decrease in noradrenaline content in the frontal cortex, $F(1, 56) = 15.7$, $p < 0.05$ (Table 1). However, milnacipran treatment did not alter this OB response, $F(1, 56) = 0.53$, $p < 0.05$. OB caused an increase in DA and DOPAC and decreases in 5-HT and 5-HIAA levels, but these changes were not statistically significant.

DISCUSSION

Previous experiments in this laboratory have found that a single daily dose of milnacipran at a dose of 30 mg/kg (but not lower doses) attenuated the behavioral hyperactivity of the olfactory bulbectomized rat in the "open-field" test following chronic administration (17).

In the present study, an increase in ambulation scores was seen in the OB control group compared to the sham control group in the "open-field" test. These results are consistent with previous studies (17,21). Chronic administration of 30 and 40 mg/kg (but not 20 mg/kg) of milnacipran attenuated the hyperactivity of OB rats in this apparatus. This attenuation of hyperactivity is similar to that seen following chronic (but not acute) treatment with both typical and atypical antidepressants (9).

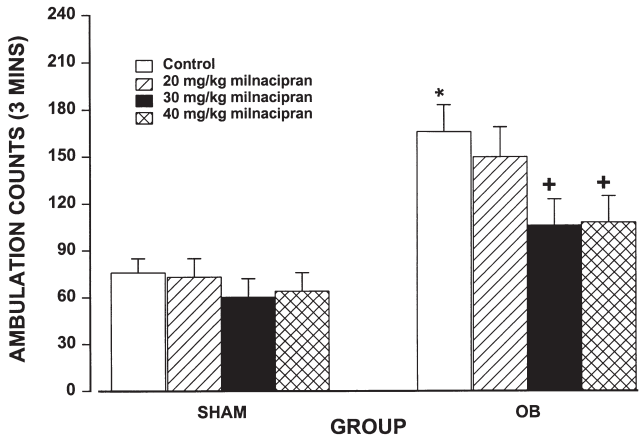

FIG. 1 Effect of milnacipran administration (PO, bid) on the ambulation scores of the OB rat in the "open-field" test. Data expressed as means ± SEM ($n = 8$). $^{*}p < 0.05$ vs. sham + vehicle. $^{+}p < 0.05$ vs. OB + control (Student–Newman–Keuls test).

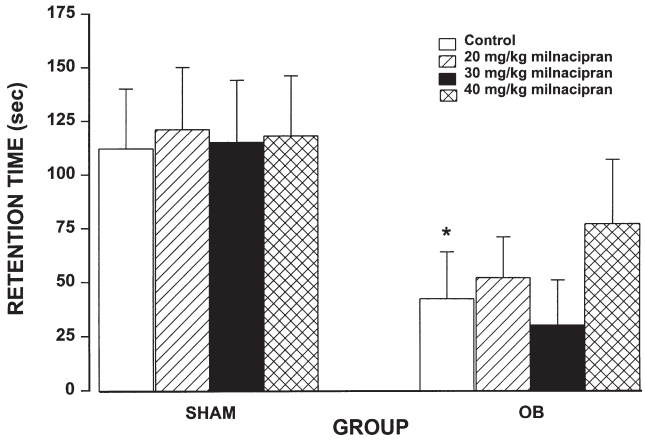

FIG. 2. Effect of milnacipran administration (PO, bid) on the retention time of the OB rat on day 3 of passive avoidance. Data expressed as means ± SEM ($n = 8$). $^{*}p < 0.05$ vs. sham + vehicle (Student–Newman–Keuls test).

In the present study, chronic milnacipran treatment did not reverse the performance deficit associated with olfactory bulbectomy in the passive avoidance test. It is possible that the drug washout period of 42 h was not sufficient, as it has been found that chronic antidepressants reverse this deficit of OB rats following 48 and also 72 h after drug washout (14). Previous studies have emphasized the importance of serotonin in the mediation of the effects of certain antidepressants in attenuating the performance deficit of OB rats in the passive avoidance test (4,10). Destruction of the serotonergic terminals in the olfactory bulbs by intrabular injection of 5,7-dihydroxytryptamine produced a deficit in passive avoidance similar to that caused by bulbectomy, while the destruction of catecholaminergic neurons did not (6). Microinjection of serotonin not noradrenaline into the amygdala improved passive avoidance deficit of OB rats (7). The passive avoidance deficit of OB rats is improved by the systemic injection of imipramine, amitriptyline, and fluoxetine, and metergoline antagonizes the effect of imipramine (4). In addition, metergoline counteracts the effect of locally applied fluoxetine and imipramine in the amygdala in the passive avoidance in OB rats (7). Thus, the inability of milnacipran to reverse the OB-related deficit in the passive avoidance test may be due to its lack of effect in normalizing the serotonergic system. In fact, in the present study milnacipran treatment failed to reverse the decreased 5-HT and 5-HIAA concentrations in the frontal cortex of OB rats. Another study has found that chronic milnacipran produced no change to either 5-HT_1 or 5-HT_2 receptors; receptors that are modified by other antidepressants (3).

In the previous studies it was found that, following bulbectomy, there was a decrease in the concentration of noradrenaline and serotonin and an increase in DOPAC, in the frontal cortex of the OB rat (19). In the present study there was a decrease in noradrenaline, serotonin, and 5-HIAA, and an increase in dopamine and DOPAC. These findings are similar those of previous studies [for a review, see (19)]. In the present study, there was only a significant decrease in the concentration of noradrenaline; chronic milnacipran treatment did not significantly alter this response.

It can be concluded that administration of milnacipran, at the higher doses of 30 and 40 mg/kg were effective in reducing

TABLE 1

EFFECT OF CHRONIC MILNACIPRAN ADMINISTRATION (PO,BD) ON NEUROTRANSMITTER LEVELS IN THE FRONTAL CORTEX OF OB AND SHAM-OPERATED RATS

Treatment	NA	DOPAC	DA	5-HIAA	5-HT
Sham + Vehicle	462 ± 29	48 ± 14	228 ± 57	203 ± 16	725 ± 37
Sham + Mil (20)	478 ± 20	17 ± 11	127 ± 21	191 ± 18	697 ± 81
Sham + Mil (30)	409 ± 23	65 ± 25	380 ± 164	207 ± 20	701 ± 40
Sham + Mil (40)	437 ± 17	33 ± 24	258 ± 150	186 ± 12	631 ± 33
OB + Vehicle	363 ± 17	83 ± 20	408 ± 171	182 ± 13	636 ± 52
OB + Mil (20)	402 ± 34	69 ± 20	209 ± 49	173 ± 13	649 ± 64
OB + Mil (30)	367 ± 29	67 ± 21	411 ± 116	199 ± 35	663 ± 81
OB + Mil (40)	382 ± 17	118 ± 36	723 ± 265	199 ± 16	711 ± 45

Data expressed as means ± SEM, in units of ng/g ($n = 8$).

MIL = milnacipran, NA = noradrenaline, DA = dopamine, DOPAC = dihydroxyphenylacetic acid, 5-HIAA = 5-hydroxyindoleacetic acid, 5-HT = serotonin.

*$p < 0.05$ vs. sham + vehicle (Student–Newman–Keuls test).

the OB-induced hyperactivity in the "open-field" test. However, milancipran failed to reverse the performance deficit of OB rats in the passive avoidance test, possibly due to its lack of effect on the serotonergic system. It can be concluded that administration of milnacipran at a dose of 30 mg/kg is an optimal dose in the OB rat model of depression.

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