

DIFFERENT BEHAVIORAL EFFECTS OF MAPROTILINE AND FLUXILAN IN RATS

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Abstract — Serotonin and noradrenaline are involved in the mechanisms of action of most antidepressant drugs. We examined the effects of chronic treatment with maprotiline, a selective inhibitor of noradrenaline reuptake, and fluxilan, a selective inhibitor of serotonin reuptake, on the behavior of unstressed controls and chronic unpredictable mild stress (CUMS) model rats in the forced swim test (FST) and elevated plus maze test. Both selective reuptake inhibitors resulted in a significant reduction of time spent in immobility. Climbing was significantly increased in maprotiline- and swimming was exclusively elicited in the fluxilan-treated unstressed control and CUMS rats. Maprotiline-treated animals displayed decreased anxiety and fluxilan-treated rats enhanced anxiety. The obtained results suggest that central noradrenergic and serotonergic systems might be affected differently during FST. The results also demonstrate that the anxiogenic effects of chronic fluxilan treatment are similar to those reported by many other studies. These differences observed for the effects of fluxilan in relation to those reported for maprotiline and probably due to the different pharmacological profiles of these drugs.

Key words: Behavior, rats, antidepressants, monoamine reuptake inhibitors

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INTRODUCTION

Stress has been implicated as a causative factor in the development of depression. It is known that noradrenergic and serotonergic neurons originating from cell bodies in the brain stem ascend to many brain regions thought to be involved in some of the symptoms associated with depression. Serotonin and noradrenaline are involved in the mechanisms of action of most antidepressant drugs and both mediate the antidepressant response, their effects being to some extent independent. Noradrenergic and serotonergic antidepressants have been associated with somewhat different clinical effects. Noradrenergic antidepressants are thought to have prominent effects on motivation and drive, while antidepressants acting on serotonin are believed to have beneficial effects on anxiety and mood in depressed patients (Montgomery, 1995, 1997). The neurochemical and behavioral effects of reduced central neurotransmitter function and subsequent influence of antidepressants are difficult to study in humans for ethical reasons. Valuable tools for researching affec-

tive disorders are methods inducing depressive-like states in experimental animals. One of the frequently used approaches is the chronic unpredictable mild stress (CUMS) model of depression. Several animal models have been developed to evaluate putative antidepressants. Among these, the forced swim test (FST) proposed by Porsolt and co-workers (1978) is a conventional model in which many antidepressants reduce immobility, indicating that this is an index of antidepressant activity. However, there are few reports of differences between noradrenergic and serotonergic antidepressants in this test. In a number of studies, sertraline modified behavioral activity in the model (Cervo et al., 1991; Kelly and Leonard 1994), while in another study sertraline, fluoxetine, and paroxetine were all reported to attenuate immobility times at moderate doses (Detke et al., 1995). Weiss and co-workers (1981) were the first to discriminate different forms of active behavior in the FST. They distinguished climbing and swimming. It was postulated that noradrenergic reuptake inhibitors promote strug-

gling or climbing behavior, and that horizontal swimming is the behavioral manifestation of selective treatment with serotonin reuptake inhibitors. A number of studies of chronic antidepressant treatment in the FST have been undertaken with variable outcome, e.g., either unchanged or augmented behavioral responses relative to the standard acute treatment (Borsini, 1995). The effects of antidepressants following chronic treatment have received little attention. Antidepressants acting on serotonin are thought to have beneficial effects on anxiety in depressed patients (Montgomery, 1995). This view was supported by the observation that such antidepressants appear to be effective across the spectrum of anxiety disorders, while in some anxiety disorders, noradrenaline antidepressants were not effective (Dubovsky, 1994). However, two studies reported that fluoxetine-treated animals displayed enhanced anxiety (Silva et al., 1999; Shishkina et al., 2006), while Rodgers and co-workers (1997) recorded an anxiolytic-like effect with a low dose of maprotiline.

The purpose of the present study was to examine the effects of chronic treatment with maprotiline, a selective inhibitor of noradrenaline reuptake, and fluxilan, a selective inhibitor of serotonin reuptake, in unstressed controls and CUMS rats, including detailed analyses of behavior to determine if reuptake inhibitors selective for distinct monoaminergic systems produce exclusive behavioral responses.

METHODS

Animals

Adult Wistar rat males weighing 280 - 320 g at the onset of experiments and maintained in a temperature-controlled room ($21 \pm 1.0^\circ\text{C}$) under conditions of a 12 h/12 h light/dark cycle were used.

Drugs and chronic treatment protocols

The rats were randomly divided into control (unstressed) and CUMS groups. These two groups were further divided into three subgroups each, the animals receiving daily injections of: 1. vehicle (sterile water); 2. maprotiline (10 mg/kg); or 3. fluxilan (10 mg/kg) via the i.p. route. Exposure to CUMS

and the vehicle, i.e., drug administration started on the same day and were continued for 4 weeks. Maprotiline (Sigma-Aldrich Chemie, Germany) and fluxilan (Aeigis LTD, Cyprus) solutions in sterile water sonicated for approximately 10 min were prepared *ex tempore*.

Chronic unpredictable mild stress (CUMS)

The CUMS procedure, a slight modification of the method described by Grippo et al. (2002), was designed to maximize the unpredictable nature of the stressors. The CUMS groups were exposed to the following stressors in random order: continuous illumination (24 h), continuous darkness (24 h), 40° cage tilt along the vertical axis, crowding (eight rats per cage), soiled cage (300 ml water spilled onto the bedding), restraint in a small cage, cold room (4°C), individual housing (24 h), forced running (15 min), and food and water deprivation. Animals were also maintained under conditions of a reversed light/dark cycle from Friday evening to Monday morning.

Forced swim test procedure

This test is based on the original method of Porsolt et al. (1978). Rats were transferred to individual cages 24 h before the first day of the two-day FST. On the first day of the experiment, the rats were plunged individually into a glass cylinder (height, diameter: 35x24 cm) containing 20 cm of water at 25°C . Clean water was used for each behavioral trial, as use of water previously swum in by another rat has been shown to alter behavior. The animals were left to swim in the water for 15 min before being removed, dried with paper towels, and returned to their home cage. Twenty-four hours later the procedure was repeated in a 5-min test session. The total times spent in each of the three behavioral classifications during the 5-min test session were recorded. Instead of measuring the duration of the presence or absence of only immobility, the sampling procedure measures the frequency of behaviors over 5-s intervals during the test session. The behaviors measured were: immobility (i.e., floating and only making the movements necessary to keep the head above water); swimming (i.e., making deliberate horizontal movements around the cyl-

under across the top of the water); and climbing (i.e., making intense movements with all four limbs, with the two forepaws either breaking the surface of the water or directed against the walls of the cylinder). Uncontrollable reflex movements during periods of immobility, such as shivering or wiping of water away from the eyes, were ignored.

Elevated plus-maze procedure

The plus-maze consisted of two open arms, 50 x 10 cm (length x width), and two enclosed arms, 50 x 10 x 50 cm (length x width x height), arranged so that the two arms of each type were opposite to each other. The maze was elevated to a height of 50 cm. The rats were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration. The behavior of each animal in the maze was analyzed, taking into account the standard measures recorded in each section of the maze (closed and open arms, central platform), comprising the percent of open arm entries (arm entry defined as all four paws into an arm), total arm entries, and the percent of time spent by the animals in open arms of the maze.

Statistics

Statistical analyses included the one-way ANOVA test. Data expressed as means \pm SEM represent an average of eight animals. Statistical significance was accepted at $p < 0.05$.

RESULTS

The results presented in Table 1 show that vehicle-treated CUMS rats were characterized by significantly decreased climbing and swimming and increased immobility in comparison with the vehicle-treated unstressed control. There were significant effects of treatment with both antidepressants maprotiline ($p < 0.001$) and fluxilan ($p < 0.01$) - on immobility time. Both selective reuptake inhibitors resulted in a significant reduction in time spent immobile in comparison with vehicle-treated unstressed control and CUMS rats. Climbing was significantly increased in maprotiline-treated unstressed control and CUMS rats compared with the vehicle-treated groups. Administration of fluxi-

lan in the unstressed control and CUMS variants had no significant effect on climbing compared to the vehicle-treated groups. Swimming as a form of active behavior was exclusively elicited by the serotonin reuptake inhibitor fluxilan; as a result, the maprotiline-treated groups were identical to those of the vehicle-administered controls.

CUMS rats treated with the vehicle showed significant ($p < 0.01$) decrease of total arm entries, percentage of entries into open arms and time spent in open arms compared to vehicle-treated unstressed control rats. Maprotiline significantly increased the percentage of open arm entries in unstressed control ($p < 0.05$) and CUMS ($p < 0.01$) rats and increased the percentage of time spent in open arms in the unstressed control ($p < 0.001$) and CUMS ($p < 0.05$) variants. Animals treated with fluxilan in both the unstressed control and CUMS variants also displayed increase in each of the two parameters, but it was not statistically significant (Table 2).

DISCUSSION

Chronic stress has been found to affect different behavioral variables. We found that long-term social isolation in rats produces anxiety (Spasojevic et al., 2007). Chronic unpredictable mild stress, which has been used as a model of depression, was found to evoke various behavioral alterations, including increased immobility, decreased climbing and swimming, and changes in anxiety-like behavior. In the present study, analysis of immobility revealed reductions caused by both selective reuptake inhibitors, maprotiline and fluxilan, following chronic treatment. Consistent with the present findings, Connor and co-workers (2000) did not find any changes in immobility after treatment with the serotonergic reuptake inhibitor paroxetine, but they observed that noradrenergic reuptake inhibitor desipramine significantly decreased immobility in the FST. Contradictory results can be attributed to different procedural approaches used in individual experiments. This is probably important in defining the role of brain monoamine neurotransmitters in depression. The results of Cryan and co-workers (2002) suggest that serotonin and noradrenaline are not ordinarily involved in mediating baseline stress-

Table 1. Effect of chronic maprotiline and fluxilan treatment on immobility, climbing, and swimming behaviour sampled every 5 sec during the 5-min FST. Data expressed as means \pm SEM of nine rats. Statistical significance: # $p < 0.05$; ### $p < 0.001$ unstressed vehicle-receiving control vs. CUMS group receiving vehicle; ** $p < 0.01$; *** $p < 0.001$ maprotiline vs. vehicle; + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$ fluxilan vs. vehicle.

| <i>plus-maze test Variable</i> | <i>Total arm entries</i> | <i>Percent open arm entries</i> | <i>Percent open arm time</i> |
|--------------------------------|--------------------------|---------------------------------|------------------------------|
| Unstressed control | | | |
| + vehicle | 6,50 \pm 0,76 | 18,67 \pm 2,49 | 6,33 \pm 1,05 |
| CUMS | ## | ## | ## |
| + vehicle | 2,00 \pm 0,52 | 5,50 \pm 1,18 | 1,16 \pm 0,20 |
| Unstressed control | | * | *** |
| + maprotiline | 7,50 \pm 0,76 | 30,83 \pm 4,65 | 35,83 \pm 4,41 |
| CUMS | | ** | * |
| + maprotiline | 2,67 \pm 0,42 | 17,16 \pm 2,15 | 4,52 \pm 1,08 |
| Unstressed control | | | |
| + fluxilan | 3,83 \pm 0,60 | 24,50 \pm 2,32 | 7,76 \pm 0,99 |
| CUMS | | | |
| + fluxilan | 4,33 \pm 0,49 | 7,67 \pm 0,88 | 2,80 \pm 0,66 |

Table 2. Effect of chronic maprotiline and fluxilan treatment on total arm entries, the percentage of entries into open arms, and percentage of time spent in open arms of the elevated plus-maze. Data expressed as means \pm SEM of nine rats. Statistical significance: ## $p < 0.01$ unstressed vehicle-receiving control vs. CUMS group receiving vehicle; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ maprotiline vs. vehicle.

| <i>FST Variable</i> | <i>climbing</i> | <i>immobility</i> | <i>swimming</i> |
|---------------------|------------------|-------------------|------------------|
| Unstressed control | | | |
| + vehicle | 15,28 \pm 1,30 | 20,87 \pm 1,20 | 23,86 \pm 1,60 |
| CUMS | ### | ### | # |
| + vehicle | 8,17 \pm 0,48 | 32,83 \pm 2,85 | 19,00 \pm 1,10 |
| Unstressed control | *** | *** | |
| + maprotiline | 35,17 \pm 1,72 | 5,00 \pm 1,79 | 19,83 \pm 2,43 |
| CUMS | *** | *** | ** |
| + maprotiline | 21,50 \pm 0,85 | 26,00 \pm 0,86 | 12,67 \pm 1,17 |
| Unstressed control | | ++ | +++ |
| + fluxilan | 10,60 \pm 0,87 | 15,60 \pm 0,51 | 33,80 \pm 0,73 |
| CUMS | | +++ | + |
| + fluxilan | 8,25 \pm 0,75 | 20,25 \pm 0,85 | 31,50 \pm 3,77 |

evoked performance, but do mediate changes in performance produced by antidepressant treatments. The given neurotransmitters could be involved in augmenting immobility caused by stress, but this hypothesis has not been tested. Espejo and Minano (1999) suggested dopaminergic mediation of the FST. They showed that prefronticortical dopamine lesions reduce overall immobility scores. The sequence of behaviors in the FST may be important for understanding the different functions of climbing and swimming. Climbing is ordinarily observed during the first minutes of the 5-min test, bouts of swimming occur throughout the interval, and immobility is most frequent at the end of interval. Climbing and swimming appear to be two separate responses, linked sequentially in series, that facilitate escape during the FST. Reneric and Lucki (1998) as well as Cryan and co-workers (2005) reported that climbing and swimming are exclusively evoked by selective noradrenergic and serotonergic reuptake inhibitors, respectively. In the present study, enhancement of climbing behavior appeared to be exclusively a product of noradrenergic reuptake inhibition, while augmentation of swimming was exclusively the product of serotonergic reuptake inhibition. Because three serotonergic reuptake inhibitors - fluoxetine, sertraline, and paroxetine similarly evoked increased swimming in the FST, a serotonergic mechanism was inferred to mediate their behavioral activity (Cryan et al., 2005). Subsequently, it was shown that the swimming behavior produced by fluoxetine was prevented by pretreatment with the tryptophan hydroxylase inhibitor parachlorophenylalanine, but not the climbing behavior produced by the noradrenaline reuptake inhibitor desipramine (Page et al., 1999).

The obtained results suggest that central noradrenergic and serotonergic systems might be affected differently during the FST. Izumi and co-workers (1996) reported that stress induced by either handling or saline injection increased the tissue content of noradrenaline as well as immobility in the tail suspension test in rats, whereas the tissue content of serotonin was not affected.

The elevated plus-maze is one of the most widely

used animal models in contemporary preclinical research on anxiety (Handley and McBlane, 1993; Hog, 1996; Rodgers and Cole, 1994). This model is based on the innate fear rodents have for open and elevated spaces. Stressed animals spent less time in open arms and more time in closed arms (Qi et al., 2006). Evidence derived from clinical studies suggests that antidepressant drugs can effectively treat anxiety disorders. Recent research has suggested that noradrenergic and serotonergic reuptake inhibitors are effective in this regard (Nowakowska et al., 2000; Kurt et al., 2000). However, preclinical investigations with serotonergic reuptake inhibitors in animal models of anxiety disorders reveal highly variable effects of these drugs (Silva et al., 1999). Our results show that chronic treatment with maprotiline increased the percentage of open arm entries and time spent in open arms, suggesting an anxiolytic effect. Chronic treatment with fluxilan did not change the percentage of open arm entries or time spent in open arms, suggesting an anxiogenic profile. These results could be connected with the data of Shishkina and co-workers (2006), who found that animals chronically treated with fluoxetine displayed enhanced anxiety and decreased locomotor activity. These differences observed for the effects of fluxilan in relation to those reported for maprotiline and probably due to the different distinct pharmacological profiles of these drugs.

In conclusion, our results indicate that the anxiogenic effects of chronic fluxilan treatment are similar to those reported in many other studies. The lack of anxiolytic effects of chronic fluxilan treatment also conforms with the poor efficacy of this drug in generalized anxiety disorders.

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РАЗЛИЧИТО ДЕЈСТВО МАПРОТИЛИНА И ФЛУКСИЛАНА НА ПОНАШАЊЕ ПАЦОВА

НАТАША СПАСОЈЕВИЋ, ЉУБИЦА ГАВРИЛОВИЋ и СЛАЂАНА ДРОЊАК

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Серотонин и норадреналин су укључени у механизам дејства већине антидепресива. Изучавано је дејство хроничног третмана са мапротилином, који је селективни инхибитор поновног преузимања норадреналина и флуксилана, селективног инхибитора поновног преузимања серотонина, на понашање код нестресираних контрола и пацова изложених хроничном непредвидивом благом стресу (CUMS), тестом пливања (FST) и тестом плус лавиринт платформе. Оба селективна инхибитора доводе до значајног смањења имобилности. Мапротилин доводи до повећаног пењања, док флуксилан изазива повећано плива-

ње, како код нестресираних контрола тако и код CUMS пацова током теста пливања. Животиње третиране са мапротилином показују смањење анксиозности, док пацови третирани са флуксиланом показују повећање анксиозности. Добијени резултати указују да тест пливања може утицати различито на централни норадренергички и серотонергички систем. Резултати такође показују да је анксиогено дејство хроничног третмана са флуксиланом слично са резултатима других студија. Уочене разлике између мапротилина и флуксилана су вероватно резултат различитог фармаколошког профила ова два лека.