Chronic Administration of Fluoxetine or Venlafaxine Induces Memory Deterioration in an Inhibitory Avoidance Task in Rats

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ABSTRACT Antidepressants inhibiting the reuptake of both serotonin and norepinephrine may exhibit efficacy superior to that of selective serotonin reuptake inhibitors (SSRIs). Since the clinical effects of antidepressants appear gradually following weeks of treatment, the present study was designed to investigate if there is a benefit to long-term administration of the SSRI, fluoxetine, in comparison with venlafaxine, a 5-HT/norepinephrine (5-HT/NE) reuptake inhibitor (SNRI; both 20 mg/kg ip, once a day for 14 days) in a one-trial step-through passive avoidance task in rats. Locomotor activity was evaluated to assess the effects of the drugs on the motility of the animals. The comparison of training latencies versus test latencies showed inhibition of passive avoidance learning in fluoxetine- or venlafaxine-treated rats (e.g., no significant difference between training and test latencies) in a step-through test. There was no significant difference between fluoxetine- and venlafaxine-induced reduction of latency in rats in this test.

INTRODUCTION Several classes of antidepressants are used for treatment of depressive disorder, each with different benefits and limitations depending on the pharmacological profile. Tricyclic antidepressants (TCAs) and monoaminooxidase inhibitors (MAOIs) are effective in a large group of depressed patients, but their use is often limited by safety/tolerability problems. Selective serotonin reuptake inhibitors (SSRIs) are as potent as antidepressants as the tricyclics and are devoid of many of the secondary effects of the latter drugs. However, SSRIs do not address all the problems inherent to the treatment of depression; since certain patients do not respond to these drugs, their antidepressant effect only becomes significant after 3–4 weeks of treatment and their use may be limited by significant drug interactions [Benkert et al., 1999]. Venlafaxine is a representative of a new class of antidepressants with unique pharmacological properties enhancing its efficacy as well as its safety profile. It selectively inhibits the uptake of both serotonin and norepinephrine (SNRI) but shows no affinity for neurotransmitter receptors [Montgomery, 1995]. It is also devoid of the drug interactions characteristic of SSRIs [Benkert et al., 1999]. Cognitive problems such as a diminished capacity for thinking and concentration and loss of memory, as a
consequence, is usually encountered in depressed patients. A close relation between depression and memory impairment has been observed [Burt et al., 1995]. Several studies have suggested the involvement of the cholinergic system in learning and memory [Fornari et al., 2000; Levey, 1996] suggesting that the memory impairment of antidepressants may be attributed to anticholinergic actions [Amado-Boccara et al., 1985; Everitt and Robbins, 1997]. Anticholinergic drugs impair the performance of animals in a wide variety of memory tasks [Fibiger et al., 1991]. Many antidepressant drugs used in clinical practice have anticholinergic properties, in addition to memory deficits encountered in depression. Thus, it is important to know the cognitive profile of a drug in order to choose the most appropriate therapy in depressed patients.

Many drugs used in psychiatry may improve, reduce, or alter cognitive functions. Fluoxetine has been widely used over the last decade due to a profile with fewer adverse side effects compared to classical antidepressants. It has minimal affinity for muscarinic receptors and, as a result, its effect on memory appears less harmful compared to TCAs [Kumar and Kulkarni, 1996; Ridel and Van Praag, 1995]. Venlafaxine is another antidepressant distinguished by marked clinical efficacy and, possibly, more rapid onset of action. Because of its lack of affinity for muscarinic, cholinergic, α1-adrenergic, and histaminergic receptors, its safety profile is superior to that of TCAs [Benkert et al., 1998]. A large number of contradictory findings exist regarding the effects of these drugs on memory processes. Since clinical effects of antidepressants appear gradually during the weeks of treatment, the present study was conducted in order to determine the effects of chronic fluoxetine or venlafaxine treatment on memory tasks in rats in a step-through passive avoidance test and to investigate if there is an advantage between these two drugs in the performance of this task.

MATERIALS AND METHODS

Animals and laboratory

Wistar male rats obtained from Istanbul University Medical Sciences Research Center (DETAM) were used in the experiments (weight on arrival: 230–250 g). The animals were housed four per cage and maintained in a temperature (23±2°C) and 12:12-h light-dark cycle (light period between 06:00 and 18:00 h) controlled environment. Animals were adapted to laboratory conditions for at least 1 week prior to testing. Food and water were given ad libitum apart from the period of behavioral observations.

All animals used for the experiments were naïve to the passive avoidance apparatus. The experiments were conducted between 09:00 and 12:00 h in a soundproof and semi-dark laboratory. Each rat was tested only once. All procedures for the treatment of animals were in compliance with the European Communities Council Directive of 24 November 1986 and approved by the Kocaeli University Ethical Committee.

Drugs

The following drugs were used: Venlafaxine (a generous gift from Dr. Uzay, Psychopharmacology Unit of Gulhane Military Medical Academy, Ankara, Turkey) and fluoxetine (Deva, Pharmaceutical Company Istanbul, Turkey). The drugs were dissolved in 0.9% physiological saline and given ip at a volume of 0.01 ml/g body weight. Doses refer to the free base of a given drug. Animals were treated once daily for 14 days with physiological saline, fluoxetine, or venlafaxine at doses of 20 mg/kg. Drug treatment ceased 24 h prior to test.

Apparatus

Animals were trained in a one-trial, step-through, light-dark passive avoidance apparatus for evaluating memory task (Ugo Basile model 7551, Italy). The training apparatus consisted of two compartments, each measuring 22 × 21 × 22 cm. The illuminated white chamber was connected to the dark chamber, which was equipped with an electrifiable grid floor and the shock was delivered to the animal’s feet via a shock generator. The two chambers were separated by a flat-box partition, including an automatically operated sliding door at floor level.

Training Trial

This was carried out as described by Hiramatsu et al. [1998], and Monleón et al. [2002]. Animals were randomly divided into three groups and were subjected to daily treatment with saline, 20 mg/kg of fluoxetine, or 20 mg/kg of venlafaxine for 14 days. Rats were subjected to a one-trial step-through inhibitory avoidance task 24 h after the last injection. On the first day of training, rats were placed individually into the light compartment and allowed to explore the boxes. After 30 sec, the door between these two boxes was opened and the animal moved into the dark compartment freely (preacquisition trial).

The acquisition (training) trial was carried out 15 min after the preacquisition trial. Rats were again placed in the light compartment of the passive avoidance apparatus. After a 30-sec adaptation period in the safe chamber, the door between the compartments was opened. Having completely entered the dark compartment, the sliding door was closed...
automatically and an electric foot-shock (0.5 mA) of 3-sec duration was delivered through the grid floor. The time taken to enter the dark compartment was recorded. Any animal failing to cross from the illuminated to the dark compartment within 300 sec was discarded from the experiment. Animals were then removed from the dark chamber and returned to their home cages. Between each training session, both compartments of the chamber were cleaned to remove any confounding olfactory cues.

**Retention Trial**

Recall of this inhibitory stimulus was evaluated 24 h post-training by returning the animals into the light compartment and recording their latency to enter the dark compartment (four paws in). No foot shock was applied in this trial. If the animal had not entered the dark compartment within 300 sec, it was returned to its cage and a maximum latency of 300 sec was recorded. This latency served as a measure of retention performance of the step-through avoidance response (test latency).

**Locomotor Activity**

To examine spontaneous locomotor activity, rats receiving the drugs or saline treatment for 14 days were placed in the activity cage (May 9803 Activity Monitor, Commat iletişim Ltd. May Pentium Computer). The total distance traveled by rats and the total number of rearings (vertical movements) were evaluated for a 5-min period.

**Statistical Evaluation of Results**

Data from the passive avoidance and locomotor activity studies were expressed as the means ± SEM. One-way analysis of variance (ANOVA) followed by the Tukey-Kramer post hoc test was used for the comparison of groups. Comparisons between training and test sessions within the same drug treatment were performed using the “paired t-test.” Values of $P < 0.05$ were deemed to be significant.

**RESULTS**

The comparison of latencies of long-term fluoxetine- or venlafaxine- (20 mg/kg/day ip for 14 days) treated animals versus controls, on day 1 of step-through passive avoidance, was not statistically significant ($F = 2.970$, $P = 0.0689$). Conversely, the comparison of training latencies versus test latencies revealed longer latencies in the test phase than in the training phase in the control group ($t = 8.339$, $P < 0.001$) whereas performance deterioration was observed in groups of fluoxetine- or venlafaxine-treated rats (i.e., no significant difference between training and test latencies) ($t = 2.009$, $P = 0.0607$ for fluoxetine and $t = 2.256$, $P = 0.0587$ for venlafaxine).

The post-hoc test showed that chronic administration of fluoxetine and venlafaxine significantly impaired the acquisition of learning, as shown by the reduction of retention latency during the passive avoidance task when administered for 14 days ($F = 32.064$, $P < 0.001$). Retention latencies of the rats that received saline (control), fluoxetine, or venlafaxine were $258.86 ± 24.24$, $59.22 ± 15.94$, and $78.67 ± 15.30$, respectively. Although the retention test of the inhibitory avoidance task was slightly higher in the venlafaxine-treated group, there was no significant difference between the decreased latency of groups treated with fluoxetine or venlafaxine in rats in the step-through passive avoidance test (Fig. 1).

An increase in locomotor activity may produce a behavioral disinhibition, which could lead to an impairment of inhibitory avoidance responding. To clarify this possibility, the locomotor activity of the animals was also tested before a change in performance could be attributed to the learning and memory process. Statistical analysis of the present (ANOVA, Table 1) and accompanying studies [Monleon et al., 2002; Nowakowska et al., 2002, 2003] showed that both fluoxetine and venlafaxine did not significantly modify locomotor activity of rats after multiple administration for 14 days at the doses studied.

**DISCUSSION**

In the present study, effects of chronic treatment of fluoxetine, a selective serotonin reuptake inhibitor,
or venlafaxine, a 5-HT/norepinephrine reuptake inhibitor, on memory task were investigated on a one-trial step-through passive avoidance task. Our results suggested that long-term treatment with fluoxetine or venlafaxine significantly impaired the acquisition of learning in a step-through, passive avoidance task in rats. Moreover, there was no significant difference between the drug-induced impairment in the performance of memory task.

Passive avoidance is commonly used in studying the cognitive alterations following drug administration, lesions, or behavioural manipulations [Sahgal, 1993]. Impairment of memory by antidepressant drugs with strong anticholinergic properties such as amitriptyline [Everss et al., 1999] and maprotiline [Parra et al., 2000] was observed by this method. Besides having some advantage in being simple to carry out and requiring little equipment and time, the most important point is that it is not clear what the technique precisely measures. Certainly memory is involved, but since motivational and other factors may influence the results, it may be considered as a preliminary test for evaluating the effects of drugs on memory. So it is important to evaluate whether the drug affects the passive avoidance performance before using other tests.

Apparently contradictory findings exist regarding the effects of SSRI s or SNRI s on memory. Fluoxetine improved the performance of memory task in the response passive avoidance test and labyrinth test [Nowakowska et al., 1996]; it caused a very marked improvement of reference memory in the reference spatial memory test [Nowakowska et al., 2000; Benkert et al., 1999]; showed no effect on learning and memory but significantly reversed scopolamine-induced memory impairment on elevated-plus maze and passive avoidance tests [Kumar et al., 1996]. Citalopram and fluoxetine impaired spatial learning in the Morris water maze in rats [Majlessi and Nagndi, 2002]. Monleon et al. [2001] demonstrated that acute treatment with fluoxetine did not impair inhibitory avoidance learning while chronic administration showed memory deterioration on inhibitory avoidance in male mice [Monleon et al., 2002]. Venlafaxine is an effective antidepressant and has also been approved for the treatment of generalized anxiety disorders. It is characterized as a "dual uptake inhibitor" since it selectively inhibits 5-HT reuptake at low doses, whereas at high doses, it inhibits both 5-HT and NE reuptake [Harvey et al., 2000; Redrobe et al., 1998; Rénéréc and Lucki, 1998; Roseboom and Kalin, 2000]. Both single and multiple administration of venlafaxine improved the working memory [Nowakowska et al., 2002, 2003] and spatial memory [Nowakowska et al., 2002] processes in the labyrinth test and in the Morris water maze test in rats. The discrepancies between these studies may be attributable to the kind of the task, the gender differences [Frackiewicz et al., 2000], and the sex differences [Heinsbroek et al., 1988] in the drug effects on behaviour.

The antidepressant mechanism of fluoxetine or venlafaxine, resulting in the improvement of the synaptic availability of norepinephrine (NE), serotonin (5-HT), and dopamine (DA) in the CNS [Kumar and Kulkarni, 1996; Luo and Tan, 2001], corresponds with the hypothesis of depression pathogenesis, which assumes NE, 5-HT, and DA deficiency as the underlying cause of the disease [Kumar and Kulkarni, 1996; Luo and Tan, 2001; Stahl, 1998]. It has been claimed that the anticholinergic actions of antidepressants are responsible for memory impairment [Amado-Boccara et al., 1995; Everitt and Robbins, 1997; Riedel and Van Praag, 1995; Monleon et al., 2002; Everss et al., 1999; Parra et al., 2000]. Since both fluoxetine and venlafaxine have weak to minimal anticholinergic effects, additional cerebral mechanisms must play a role in their impairing effects on inhibitory avoidance tasks, besides their anticholinergic actions.

Although the role of 5-HT receptors in learning and memory formation remains equivocal, pre- and post-synaptic 5-HT receptors appear to be involved [Meneses, 1999]. The cognition-enhancing potential of 5-HT₆ receptor antagonists may, in part, relate to the facilitation of cholinergic systems [Foley et al., 2004; Rogers and Hagan, 2001] since it has been suggested that 5-HT₆ receptor antagonists produce a modest, albeit nonsignificant, increase in extracellular acetylcholine levels [Shirazi-Southall et al., 2002].

Fluoxetine, pharmacokinetically, elevates the concentration of 5-HT at the synapses a few hours after administration. However, the onset of the antidepressant activity requires a period of chronic treatment, usually 2–3 weeks, before this activity can be observed. So the symptoms of depression do not disappear rapidly upon the elevation of 5-HT levels. Thus, besides the update knowledge regarding the
antidepressant mechanism of fluoxetine, it is claimed that fluoxetine inhibits the nitric oxide synthase (NOS) catalyzing the over-production of nitric oxide (NO) in rat brain [Luo and Tan, 2001], whereas another SSRI paroxetine was also reported to act as a novel NOS inhibitor [Finkel et al., 1996]. Luo and Tan [2001] demonstrated that fluoxetine renormalized atrophied hippocampal neurons in the rat chronic mild stress model and thus explained the delayed efficacy of most antidepressants since it takes time to “reconstruct” the injured neuronal connections. Moreover, it was suggested that the deformation of neurons in some brain areas is one of the indicators of depression development. Previous investigations have revealed that NO participates in the acquisition of learning and the execution of memory tasks [Chapman et al., 1992; Estall et al., 1993; Holscher and Rose, 1992]. Moreover, it has also reported that NO plays a facilitatory role in the memory consolidation process of an inhibitory avoidance learning task in rats [Chapman et al., 1992; Huang and Lee, 1995; Telegdy and Kokavszky, 1997]. Future investigation of these compounds is required to determine if the supplemental addition of NO to determine if the supplemental addition of NO.

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REFERENCES


