Fluoxetine effects on retention of inhibitory avoidance: Enhancement by systemic but not intra-amygdala injections

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Male Sprague-Dawley rats were trained in an inhibitory avoidance task and injected with saline or fluoxetine (15.0 mg/kg i.p.) immediately after training. Fluoxetine significantly facilitated retention assessed on a retention test 48 h later. In contrast, posttraining intra-amygdala injections of fluoxetine (1.0, 3.0, or 10.0 μ g) did not modify retention of the inhibitory avoidance task. The findings suggest that the amygdala serotonergic system is not involved in the modulation of memory in this task.

It is now well established that memory storage in rats and mice can be enhanced or impaired by posttraining treatments affecting several neurotransmitter or neuromodulatory systems (McGaugh, 1973, 1983, 1989). For example, when administered shortly after training, systemic injection of either peripheral or centrally acting adrenergic agonists produces dose-dependent effects on subsequent retention (Introini-Collison & Baratti, 1986; Introini-Collison & McGaugh, 1986; McGaugh & Gold, 1989). Opioid peptide receptor antagonists enhance retention (Baratti, Introini, & Huygens, 1984; Gallagher & Kapp, 1978; Izquierdo, 1979; Messing et al., 1979), and opioid receptor agonists impair retention (Castellano, 1975; Introini & Baratti, 1984). GABA receptor antagonists enhance retention (Breen & McGaugh, 1961; Brioni & McGaugh, 1988; Castellano, Introini-Collison, Pavone, & McGaugh, 1989), and GABAergic agonists impair retention (Brioni, Nagahara, & McGaugh, 1989; Castellano et al., 1989).

Previous results from our laboratory, as well as other laboratories, suggest that adrenergic, opioid peptidergic, and GABAergic drugs modulate retention through influences involving the amygdaloid complex. Posttraining intra-amygdala injections of drugs affecting these neuromodulatory systems produce effects on memory that are comparable to those found with systemic injections (Brioni et al., 1989; Castellano et al., 1989; Ellis & Kesner, 1981; Gallagher, Kapp, McNall, & Pascoe, 1981; Introini-Collison, Nagahara, & McGaugh, 1989; Liang, Juler, & McGaugh, 1986; McGaugh, Introini-Collison, & Nagahara,

1988). Furthermore, the effects of these drugs are blocked by lesions of the amygdala or stria terminalis (a major amygdala pathway) (Ammassari-Teule, Pavone, Castellano, & McGaugh, 1991; Introini-Collison, Arai, & McGaugh, 1989; Liang & McGaugh, 1983; Liang et al., 1982). These findings suggest that the amygdala may play a general role in integrating neuromodulatory influences on memory storage (McGaugh, 1989; McGaugh et al., 1988; McGaugh, Introini-Collison, Nagahara, & Cahill, 1990).

In recent years, several studies have also reported that pharmacological manipulations of the serotonergic system affect memory processes (Altman & Normile, 1988; Altman, Ogren, Berman, & Normile, 1989; Flood & Cherkin, 1987; Richter-Levin & Segal, 1989). Flood and Cherkin (1987) reported that either systemic or central (i.c.v.) injections of the serotonin uptake inhibitor fluoxetine administered to mice before or after training facilitated retention in an active avoidance task as well as in an inhibitory avoidance task. Although the amygdala receives rich widespread serotonergic innervation from the raphe nuclei through the ventral serotonergic pathway (Nieuwenhuys, 1985), it is not yet known whether the memory-modulating effects of fluoxetine involve the amygdala. To address this issue, the present experiments examined the effects of systemic and intra-amygdala injections of fluoxetine on the retention of rats trained in an inhibitory avoidance task.

METHOD

Subjects

Male Sprague-Dawley rats (Charles River) weighing 200-250 g were individually housed and maintained on a 12:12-h light:dark cycle (lights on at 7:00 a.m.). Behavioral training and testing procedures were conducted during the light portion of the cycle. Food and water were available ad lib.

Surgery and Histology

One week after arrival, the rats were implanted bilaterally with 15-mm, 23-ga stainless steel cannulas. They were anesthetized with

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ketamine hydrochloride (up to 100 mg/kg i.p.) and given atropine (0.4 mg/kg i.p.) and xylazine (5.0 mg/kg i.p.) as preanesthetics. The tips of the cannulas were aimed at the ventral caudate so that the injection needle tips (which protrude 2 mm from the tips of the cannulas) would be placed dorsal to the central nucleus of the amygdala according to Paxinos and Watson (1986) (AP -2.3 mm; ML $\pm$ 4.4 mm; DV -5.2 mm). Two surgical screws served as anchors and were placed in the left frontal (2 mm anterior to bregma and 2 mm lateral) and right posterior (7 mm posterior to bregma and 2 mm lateral) cortices. The cannulas were affixed to the skull with dental cement. Stylets made of 00 insect pins were inserted into the cannulas and remained there at all times except during injections. Immediately after surgery, the animals received penicillin G benzathine/G procaine (30,000 U in 0.1 ml) and were maintained in temperature-controlled chambers for at least 2 h or until they recovered from the anesthesia.

All implanted rats were sacrificed within 1 week after the completion of the behavioral testing. The rats were sacrificed by an overdose of pentobarbital (100 mg/kg i.p.) and perfused with physiologic saline solution (0.9%) followed by formalin (10%). The brains were then removed, stored in 10% formalin at least 48 h, and sliced (40 μ m). The slices were stained with cresyl violet. The position of the cannulas and the needle tips were examined through the projection and examination of the slides. The projections were made blindly with respect to both the treatment given to the animals and the behavioral data. The position of the needle tips is shown in Figure 1. The behavioral data of approximately 5% of the animals were excluded from the analysis because at least one of the needle tips in these animals was not located in amygdaloid nuclei.

Behavioral Procedures

One week after either arrival (unoperated rats) or surgery, the rats were trained on a trough-shaped inhibitory avoidance apparatus constructed of a stainless steel floor and walls (McGaugh et al., 1988). On the training trial, each rat was placed in the lighted com-

partment facing the door leading to the dark compartment. When the rat turned around, the door leading to the dark compartment was opened; after the rat stepped through the door into the dark compartment, the door was closed and a footshock (0.35 mA; 60 Hz during 0.7 sec) was delivered through the floor plates. The rat was immediately removed from the apparatus and injected systemically (i.p.) or intra-amygdally as described below. On the retention test 48 h later, the rat was placed in the lighted compartment as in the training session and the step-through latency (maximum of 600 sec) was recorded.

Intra-Amygdala Injection Procedure

Immediately after training, the cannula stylets were removed and the drugs were administered through 30-ga injection needles connected to a 10- μ l Hamilton syringe by 1.0-m polyethylene tubing (PE-20). The needles protruded 2 mm beyond the tips of the cannulas. A 0.5- μ l injection of a drug was delivered (during 40 sec) to each of the cannulas simultaneously by an automated syringe pump. The needles were retained in the cannulas for 30 sec after the injections were completed, and the stylets were replaced in the cannulas as soon as the injection needles were removed.

Drugs

The drug used for these experiments was the serotonergic uptake inhibitor fluoxetine (Eli Lilly). For the intraperitoneal injections, fluoxetine was dissolved in a saline solution and controls received the saline solution (1.0 ml/kg). The solution for the intracannula injection consisted of a phosphate buffer solution prepared by dissolving 0.105 g of $\text{PO}_4\text{NaH}_2\cdot\text{H}_2\text{O}$ and 0.029 g of $\text{PO}_4\text{Na}_2\text{H}$ in the 100 ml of saline solution. Doses are expressed as the salt. Control animals were injected with the buffer (0.5 μ l/40 sec).

Statistical Analyses

Data are expressed as median step-through latencies and interquartile ranges and were analyzed by Mann-Whitney *U* tests. In

Figure 1. Position of the needle tips in the brains of the rats used in the intra-amygdala experiment.

Figure 2. Effect of fluoxetine (15.0 mg/kg i.p.) given immediately after training on retention of an inhibitory avoidance task in rats ($n = 9$ rats per group). Retention was tested 48 h after training. Data are expressed as medians (interquartile ranges). ** $p < .01$ compared with the saline-injected control group.

all cases, p values of less than .05 were considered significant. All experiments were conducted blind with respect to the drug treatments. There were 9-10 rats per group.

RESULTS

Effects of Systemically Administered Fluoxetine

The purpose of the first experiment was to determine whether the memory-enhancing effects of systemically

administered fluoxetine seen in mice (Flood & Cherkov, 1987) could be obtained with rats. The rats were trained in the inhibitory avoidance task and given immediate posttraining i.p. injections of either saline or fluoxetine (15.0 mg/kg). This dose was selected in view of the findings of Flood and Cherkov (1987) indicating that when it was injected systemically after training, this dose enhanced the retention of mice in active as well as inhibitory avoidance tasks. Retention was assessed 48 h after the training session. As is shown in Figure 2, the retention latencies of rats given posttraining administration of fluoxetine were significantly greater than those of controls ($p < .002$). These findings are comparable to those reported by Flood and Cherkov (1987).

Effects of Intra-amygdala Injections of Fluoxetine

Rats in this experiment were trained in the inhibitory avoidance task as described above and given intra-amygdala injections of buffer or fluoxetine (1.0, 3.0, or 10.0 μ g) immediately after training. This range of doses was selected on the basis of the findings of Flood and Cherkov (1987) indicating that doses as low as 8 μ g were effective in enhancing retention when administered intracerebroventricularly after training. As Figure 3 shows, there were no significant differences among the groups in performance on the retention test 48 h later ($p > .05$ in all cases).

DISCUSSION

These findings confirm and extend those of Flood and Cherkov (1987) indicating that when fluoxetine is administered systemically to mice, it significantly facilitates retention in an inhibitory avoidance task. The aim of the present experiment using systemic injections was to confirm the memory-enhancing effect of fluoxetine. It is of

Figure 3. Effect of posttraining intra-amygdala injections of fluoxetine (1.0, 3.0, or 10.0 μ g) on retention of an inhibitory avoidance task in rats ($n = 10$ rats per group). Retention test took place 48 h after training. Data are expressed as medians (interquartile ranges).

interest that the same dose used by Flood and Cherkin (1987) in experiments examining the effects of fluoxetine on retention of active avoidance and inhibitory avoidance by mice also facilitated rats' retention in an inhibitory avoidance task.

The findings of Flood and Cherkin (1987), indicating that comparable memory-enhancing effects were obtained with systemic (s.c.) and central (i.c.v.) injections, strongly suggest that the effects are due to central actions of the drug. Our previous findings based on experiments examining the effects of adrenergic, opioid peptidergic, and GABAergic drugs (McGaugh et al., 1990) suggested the possibility that the effects might be mediated by influences involving the amygdaloid complex. The present findings clearly indicate that retention was not influenced by intra-amygdala injections of fluoxetine. With the intra-amygdala site used, the range of doses injected (1.0, 3.0, and 10.0 μ g), fluoxetine did not influence retention when administered after training. Flood and Cherkin (1987) reported that when fluoxetine was administered i.c.v., it was effective in influencing memory in doses as low as 8.0 μ g. If such effects were due to actions on serotonergic function within the amygdala, fluoxetine should have been effective in doses of 1.0 to 10.0 μ g administered directly into the amygdala. Clearly, it was not. It should be noted that the amygdala injection site was the same as that used in our previous studies examining the effects of drugs affecting other neuromodulatory systems.

Thus, the present findings provide no evidence suggesting the direct involvement of the amygdala serotonergic system (Cheetham, Yamaguchi, & Horton, 1989; Kilpatrick, Jones, & Tyers, 1988, 1989; Nieuwenhuys, 1985; Sakurai-Yamashita et al., 1989) in the effects of fluoxetine on memory. Relatively few experiments have investigated the brain system (or systems) mediating the effects of serotonergic influences on memory. Several studies have pointed to the hippocampus as a possible site mediating serotonergic influences on memory (Baker & Reynolds, 1989; Richter-Levin & Segal, 1989). Furthermore, the findings of Pellemounter et al. (1986) suggest that nigral serotonergic activity might also influence memory processes. Although the present findings provide no evidence that memory storage is modulated by direct activation of serotonergic receptors within the amygdala, they do not rule out the possibility that amygdala processes involved in modulating memory storage are influenced by activation of serotonin receptors in other brain regions.

In contrast with the findings reported in this paper along with those from other laboratories (Flood & Cherkin, 1987), other investigators have reported that memory is enhanced by serotonin antagonists (Altman & Normile, 1988; Altman et al., 1989). These conflicting results may be a consequence of the difference in specificity for serotonergic receptor agonists and antagonists. Fluoxetine is a serotonin uptake inhibitor and induces its pharmacological effects through an increase in the concentration of serotonin in the synaptic cleft. Serotonin has a greater

affinity for 5-HT₁ receptors than for 5-HT₂ receptors, while the opposite is observed with serotonin receptor antagonists (Altman & Normile, 1988). Thus, the different results obtained with experiments examining the effects of serotonergic agonists and antagonists may be due to activation of different serotonin receptors.

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