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Effects of Antidepressant Treatment on Inhibitory Avoidance Behavior and Amygdaloid β-Adrenoceptors in Rats

Lynette C. Daws, Ph.D., Ray Lopez, Ph.D., and Alan Frazer, Ph.D.

Chronic treatment of rats with a variety of antidepressants results in the down-regulation of β_1-adrenoceptors in the amygdaloid nuclei. The present study sought to determine if this specific neurochemical effect caused an alteration in inhibitory avoidance conditioning, a behavior considered to be mediated by β-adrenoceptors in the amygdala. Rats treated chronically with either desipramine (DMI) or phenelzine (PHEN), which down-regulate β_1-adrenoceptors in the amygdala, or fluoxetine (FLUOX), which does not do this, did not exhibit a deficit in the retention of the inhibitory avoidance task. However, when scopolamine was given prior to acquisition of the task in a dose that, by itself, did not affect retention, DMI- and PHEN-treated rats showed a marked deficit in retention. This effect was also observed after acute administration of these drugs, although they did not down-regulate amygdaloid β_1-adrenoceptors at this time. It seems that the ability of these antidepressants to potentiate the amnesic effect of scopolamine is unrelated to their effect on β_1-adrenoceptor number in the amygdala and that the extent of antidepressant-induced amygdaloid β_1-adrenoceptor down-regulation is not sufficient, by itself, to cause a deficit in an inhibitory avoidance task.

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KEY WORDS: *Inhibitory avoidance; Desipramine; Phenelzine; Fluoxetine; β_1-Adrenoceptors; Amygdala*

Chronic treatment of rats with a wide range of antidepressants produces a significant reduction in the number of β-adrenoceptors in brain (Banerjee et al. 1977; see Heninger and Charney 1987). In particular, quantitative autoradiographic studies have shown that different types of antidepressants most consistently down-regulate β_1-adrenoceptors in the amygdala (Ordway et al. 1988, 1991). This finding is of interest in light of previous reports emphasizing the putative role of the amygdala in mediating the behavioral actions of antidepressants (Horovitz 1966; Gorka et al. 1979; Broekkamp and Lloyd 1981; Sarter and Markowitsch 1985; Duncan et al. 1986; Beck and Fibiger 1995). It should be noted, however, that the behavioral effects observed in these studies followed acute administration of antidepressants; whereas, the down-regulation of β_1-adrenoceptors takes time (ca. 1 week) to occur (Ordway et al. 1991). Thus, both acute and chronic effects of antidepressants implicate the amygdala as a primary target of the pharmacological actions of this class of drugs. The purpose of this study was to attempt to identify a behavioral alteration that results from the down-regulation of amygdaloid β-adrenoceptors.

β-adrenoceptors in the amygdala have been implicated in mediating inhibitory (passive) avoidance behavior (e.g., Gallagher et al. 1977; Liang et al. 1986, 1990; Campeau and Davis 1995; Introini-Collison et al. 1996).

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Gallagher et al. (1977) observed that injection of β-adrenoceptor antagonists in the amygdala of rats produced retrograde amnesia on an inhibitory avoidance task that was both time- and dose-dependent and stereospecific. This work has been confirmed and extended by McGaugh and his colleagues (Liang et al. 1986; McGaugh 1989). Although there is a learning component to inhibitory avoidance behavior, there is also an emotional component attributable to its pairing of a nonaversive stimulus with an aversive one. The retention of memory caused by this pairing is manifest as an increased latency for rats to enter the dark compartment of a two-compartment chamber after being shocked there in a training trial. It is possible to view the increased latency as a type of "freezing" behavior, a behavior considered indicative of a state of fear or anxiety (Leaton and Borszcz 1985). This behavior may, therefore, be particularly relevant to study, because some antidepressants are efficacious in panic disorder (Sheehan et al. 1980; Murphy et al. 1985; Kahn et al. 1986), and some view anxiety as a central component of the depressive syndrome (Katz et al. 1984). The present study aims to characterize the effect of acute and chronic treatment with antidepressant drugs on inhibitory avoidance behavior in rats and to correlate this with changes in β_1-adrenoceptor number in the amygdala.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (270–350 g, Harlan, Indianapolis, IN) were housed either in groups of three, or individually (postsurgery). The colony was maintained on a 14:10 light/dark cycle with the lights on at 7:00 a.m. All training was conducted between 0800 and 1300 hours. Room temperature was maintained at 23 ± 2°C, and rats were provided with food and water *ad libitum*. All animal procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize both the number of rats used and discomfort to the rats during experimental procedures.

Inhibitory Avoidance Task

Rats were trained on a one trial step-through inhibitory avoidance task. The inhibitory avoidance chamber was made from Plexiglas and was comprised of two compartments separated by a guillotine door. The smaller compartment (20 × 16 × 24 cm) was illuminated by a 60-watt light, mounted 50 cm above the Plexiglas base. The base of the larger, dark compartment (30 × 20 × 20 cm), consisted of a series of metal rods separated by 1 cm, through which an electric shock could be delivered. On the day of acquisition training, each rat was placed into the illuminated compartment facing away from the closed door. The rat was allowed a 10-second habituation period before the door was opened, allowing free passage of the rat into the dark compartment. Once the rat had entered the dark compartment (i.e., all four paws being within the dark compartment), the door was closed behind the rat and a 0.5 mA shock delivered for a 3-second duration. Approximately 5 seconds later, the rat was removed from the chamber and placed into a holding container until all cage mates has been processed. All rats were then returned to their home cage. The latency for the rat to enter the dark compartment, i.e. acquisition latency, was recorded. Twenty-four hours later, retention was tested. This was done by placing each rat into the illuminated compartment, as described above, with the exception that the guillotine door was already open. The latency to enter the dark compartment was recorded as a measure of retention of the task. Rats that did not enter the dark compartment within 10 minutes were given a ceiling score of 600 seconds. To verify the involvement of amygdaloid β-adrenoceptors in mediating inhibitory avoidance behavior, a separate group of rats received intra-amygdala administration of propranolol or vehicle.

Surgery. Rats were anesthetized with sodium pentobarbital (65 mg/kg intraperitoneally [IP]), placed into a stereotaxic frame, and bilaterally implanted with 22-gauge stainless steel guide cannulae into the amygdala. The tips were directed at the dorsal surface of the amygdala complex (A.P. −2.3 mm, M.L. +4.6 mm, D.V. −7.5 mm; Paxinos and Watson 1986). Two skull screws served as anchors for the cranioplastic cement used to hold the cannulae in place. The patency of the cannulae was maintained by inserting a 28-gauge stylet.

Intra-Amygdala Drug Administration. Propranolol (34 or 68 nmol) or phosphate buffered saline (PBS, pH 7.4) was administered through a 28-gauge injector connected to a 10 μl Hamilton microsyringe by polyethylene tubing (PE-20). The injector was designed so that, when inserted into the cannula, the top protruded 1 mm in the brain tissue. Both microsyringe and tubing were filled with fluid. Twenty minutes prior to acquisition training, one microliter of the drug solution was delivered sequentially into each amygdala. Each injection was given over a 60 s period. The injector was left in the cannula for an additional 15 s to allow diffusion of the drug away from the injector tip. Stylets were replaced immediately after termination of the injection. Retention was tested 24 hours later.

Histology. At the conclusion of the experiment, one microliter of Chicago blue dye was injected into the amygdala over a 60 s period to mark the site of the injector tip. Rats were then decapitated, and the brain was rapidly removed and stored in 10% formalin for 24

hours. The brain was frozen (−15°C) prior to sectioning (40 μm thick slices) through the cannula tracts. Sections were thaw mounted onto gelatin-coated microscope slides, and placement of the injector tip was verified. Behavioral data from rats in which the injector tip had missed the anygdala complex were omitted from the analysis.

Experiments were also undertaken to corroborate the existence of an interaction between muscarinic cholinergic and β-adrenergic systems in the mediation of inhibitory avoidance behavior (Decker et al. 1990; Decker and McGaugh 1991). Rats were administered either scopolamine (SCOP, 0.15 mg/kg or 0.3 mg/kg), propranolol (PROP, 10 mg/kg), a combination of SCOP (0.15 mg/kg or 0.3 mg/kg) and PROP (10 mg/kg) or vehicle, 20 minutes prior to acquisition training. All drugs were given IP. Retention was recorded 24 hours later.

Effect of Antidepressant Treatment and Acute Muscarinic Receptor Blockade on Inhibitory Avoidance Behavior

Experiment I—Effect of Chronic Antidepressant Treatment (60-Hour Washout). In a preliminary experiment, dose-dependent effects of acute scopolamine prior to acquisition training were explored. Rats were allowed 10 days to acclimate to the animal facility before chronic drug treatment was initiated. Desipramine (DMI; 10 mg/kg, IP, BID) or saline was administered intraperitoneally for 8 days. Body weights were monitored daily. After rats had remained drug free for 60 hours, 0.15, 0.3, or 0.45 mg/kg scopolamine, or saline was injected IP 20 minutes prior to acquisition training. Retention of the task was tested 24 hours later. In the subsequent experiment, separate groups of rats were injected IP twice daily with either DMI (10 mg/kg), phenelzine (PHEN; 5 mg/kg), fluoxetine (FLUOX; 10 mg/kg), or saline for a period of 8 days. Body weights were monitored daily. After a 60 hour drug free period, rats were administered IP either 0.3 mg/kg scopolamine or saline, 20 minutes prior to acquisition training. Retention was measured 24 hours later. Two hours after the completion of the inhibitory avoidance task, rats were decapitated. The brains were rapidly removed and frozen on powdered dry ice before being stored at −70°C for autoradiographic analysis of amygdaloid β_1-adrenoceptor number.

Experiment II—Effect of Acute Antidepressant Treatment (No Washout Period). Rats received IP either DMI (10 mg/kg), PHEN (5 mg/kg), or saline 20 minutes prior to acquisition training. Scopolamine (0.3 mg/kg) or saline were injected 20 minutes prior to acquisition of the inhibitory avoidance task. Retention was tested 24 hours later, after which the rats were sacrificed and brains stored as described above.

Experiment III—Effect of Acute Antidepressant Treatment (60 Hour Washout). Rats were injected IP with either DMI (10 mg/kg), PHEN (5 mg/kg), or saline. Sixty hours later, they were trained in the inhibitory avoidance task. Twenty minutes prior to acquisition training, rats were injected IP with scopolamine (0.3 mg/kg) or saline. Retention of the task was tested 24 hours later. Two hours later, the rats were sacrificed and the brains stored as described above.

Quantitative Autoradiography

The procedure used for quantitative autoradiography was based on the method of Rainbow et al. (1984), as modified by Ordway et al. (1988). Briefly, coronal sections (20 μm) were cut at the level of plate 30 according to the Stereotaxic Atlas of the Rat Brain (Paxinos and Watson 1986). Sections were thaw mounted onto gelatin-coated microscope slides and dried under a vacuum for 12 hours at 4°C. Binding of [^{125}I]-iodopindolol ([^{125}I]-IPIN, 250 pM) (Dupont-NEN, MA) to β_1 receptors was determined by incubating duplicate sections in the presence or absence of the β_2 antagonist, ICI 118 551 (50 nm) for 120 min at 22°C in buffer (20 mm Tris, 125 mM NaC1, pH 7.4). Nonspecific binding of [^{125}I]-IPIN was determined in the presence of l-isoproterenol (100 μm). The reaction was terminated by washing the slides in ice-cold buffer for 60 min; they were then dipped into ultrapure water to remove buffer salts, and dried. Sections and calibrated standards were exposed against LKB [^{3}H] Ultrofilm for 20 hours to generate autoradiograms. Optical densities of brain images were converted to femtomoles per milligram protein using a computer image analysis program (NIH-IMAGE), which compared brain image optical densities to images of [^{3}H] standards (American Radiolabeled Chemicals, St. Louis, MO) on the same piece of film. The [^{3}H] standards were calibrated with brain mash sections containing a known amount of [^{125}I]-IPIN and a known amount of protein, according to the method of Artymyshyn and Wolfe (1987). The density of β_1 adrenoceptors were quantified for the amygdaloid complex, where the amygdaloid complex included the lateral, basolateral, basomedial, and central amygdaloid nuclei.

Drugs

Scopolamine HBr, propranolol HCI (RBI, Natick, MA), desipramine HCl, phenelzine sulfate, and fluoxetine HCl (Sigma, St. Louis, MO) were administered IP in a volume of 1.0 ml/kg, unless otherwise indicated. Doses were calculated using the salt weight, and all drugs were dissolved in distilled water.

Statistical Analyses

Kruskall-Wallis one-way analysis of variance was used to determine the significance of dose and/or drug effects. Mann-Whitney U-tests or Fisher Exact tests were used to compare differences in the acquisition and retention latencies between treatment groups. Differences in binding of [^{125}I]-IPIN between treatment groups were assessed using *t*-tests for independent samples. A two-tailed probability level of $p < .05$ was accepted as statistically significant for all tests.

RESULTS

Effect of Amygdaloid β-Adrenoceptor Blockade on Inhibitory Avoidance Behavior

The overall goal of these experiments was to examine if down-regulation of amygdaloid β-adrenoceptors caused by certain antidepressants produced a behavioral deficit in an inhibitory avoidance task. It seemed important to verify, therefore, that acute blockade of such receptors would, in our hands, produce an amnesic effect in such a task. In addition, in much of the research on adrenergic influences on memory storage, β-adrenoceptor antagonists were injected into the amygdala post-training (see McGaugh 1989). However, the antidepressant-induced down-regulation of β-adrenoceptors would be present at the time of training. Consequently, in our experiments with propranolol, the drug was administered 20 min prior to acquisition training.

Acquisition latency did not differ significantly between rats receiving intra-amygdala administration of PBS or either dose of propanol. By contrast, there was a marked amnesic effect of propranolol when administered into the amygdala (Figure 1). Rats administered either 34 or 68 nmol propanolol exhibited significantly shorter retention latencies than those measured in control animals ($p < .003$, Kruskal-Wallis). The amnesic effect of propranolol followed a dose-dependent trend, although the difference between the two concentrations did not reach statistical significance. In cases where the injector tip was not located in the amygdala complex, neither dose of propranolol elicited an amnesic effect in the retention of the inhibitory avoidance task; in these instances ($n = 4$), neither the acquisition nor retention latencies of these rats were different from those injected with PBS (data not shown). In all cases, maximal retention latencies were exhibited.

Effect of Muscarinic and β-Adrenergic Receptor Blockade on Inhibitory Avoidance Behavior

β-Adrenergic antagonists administered systemically have also been reported to potentiate the amnesic ef-

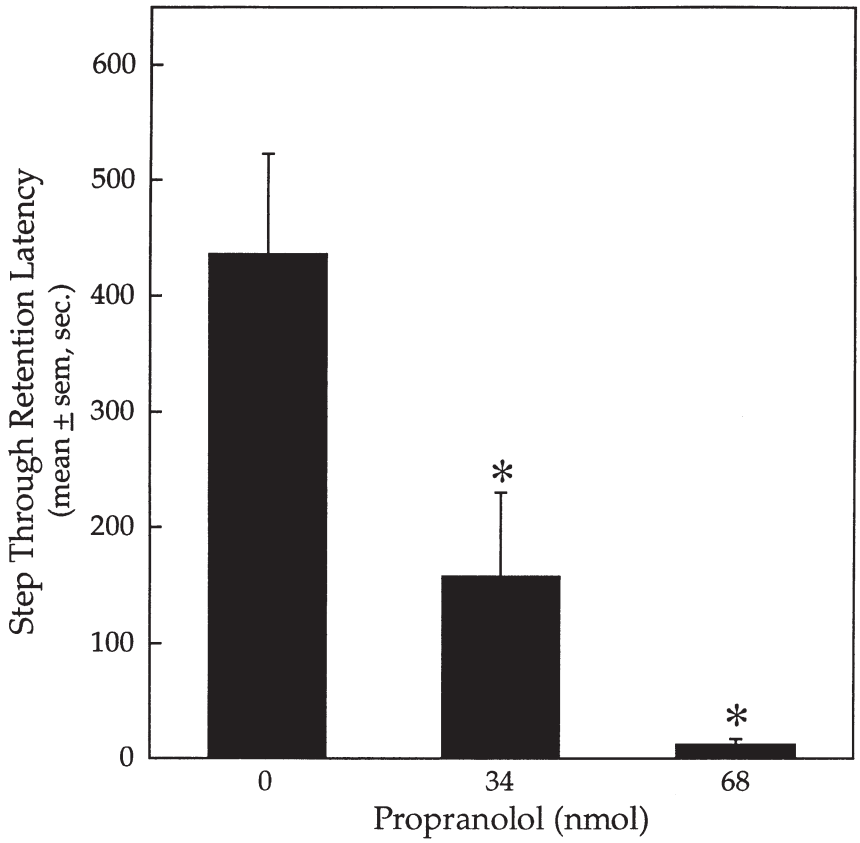

Figure 1. Retention performance (mean ± SEM) of rats receiving intra-amygdala injections of PBS or propranolol 20 minutes prior to the acquisition of the inhibitory avoidance task. The number of rats per group ranged from 7 to 8. *$p < .01$ from PBS control, Mann-Whitney U.

fects of muscarinic antagonists such as scopolamine (Decker et al. 1990). Although it is unclear if the locus of action of the β-adrenoceptor antagonists is peripheral and/or central (see McGaugh 1989), drug administration in this paradigm is effective when given prior to training (Decker et al.). Because, as mentioned previously, antidepressant-induced down-regulation of β-adrenoceptors would be present during training, we investigated if we could reproduce this result.

Neither systemic administration of scopolamine, propranolol, nor the combination of these drugs altered the latency of the rats to enter the dark compartment on the day of acquisition training. Latencies to enter the dark compartment during the retention test are illustrated in Figure 2. There was a significant effect of treatment ($p < .01$, Kruskal-Wallis). Rats receiving either saline, propranolol alone, or scopolamine (either 0.15 or 0.3 mg/kg) alone showed maximal or near maximal retention of the inhibitory avoidance task; the retention latencies exhibited by these groups did not differ significantly from each other. By contrast, administration of propranolol in combination with either dose of scopolamine produced a marked amnesic effect, as evidenced by the significantly shorter retention latencies displayed by these rats as compared to their counterparts receiving scopolamine or propranolol alone. Although not significant, rats receiving 0.3 mg/kg scopolamine in combination with propranolol generally exhibited shorter retention latencies than those receiving the lower dose of scopolamine with propranolol.

These results, then, confirm those of Gallagher et al. (1977) and Decker et al. (1990). They show that injection of propranolol directly into the amygdala prior to training caused decreased retention of an inhibitory avoidance task, but it did not do this when given systemically. However, systemic administration of propranolol prior to acquisition training did potentiate the retention deficit induced by systemically administered scopolamine. Because of these data, it seemed appropriate to test if drug treatments expected to down-regulate amygdaloid β-adrenoceptors would reproduce the effects caused by administration of propranolol and acute muscarinic receptor blockade on inhibitory avoidance behavior. The tricyclic antidepressant, desipramine, and the irreversible monoamine oxidase inhibitor (MAOI), phenelzine, were selected for study, because they have been reported to down-regulate amygdaloid β_1-adrenoceptors by around 30 and 50%, respectively, after

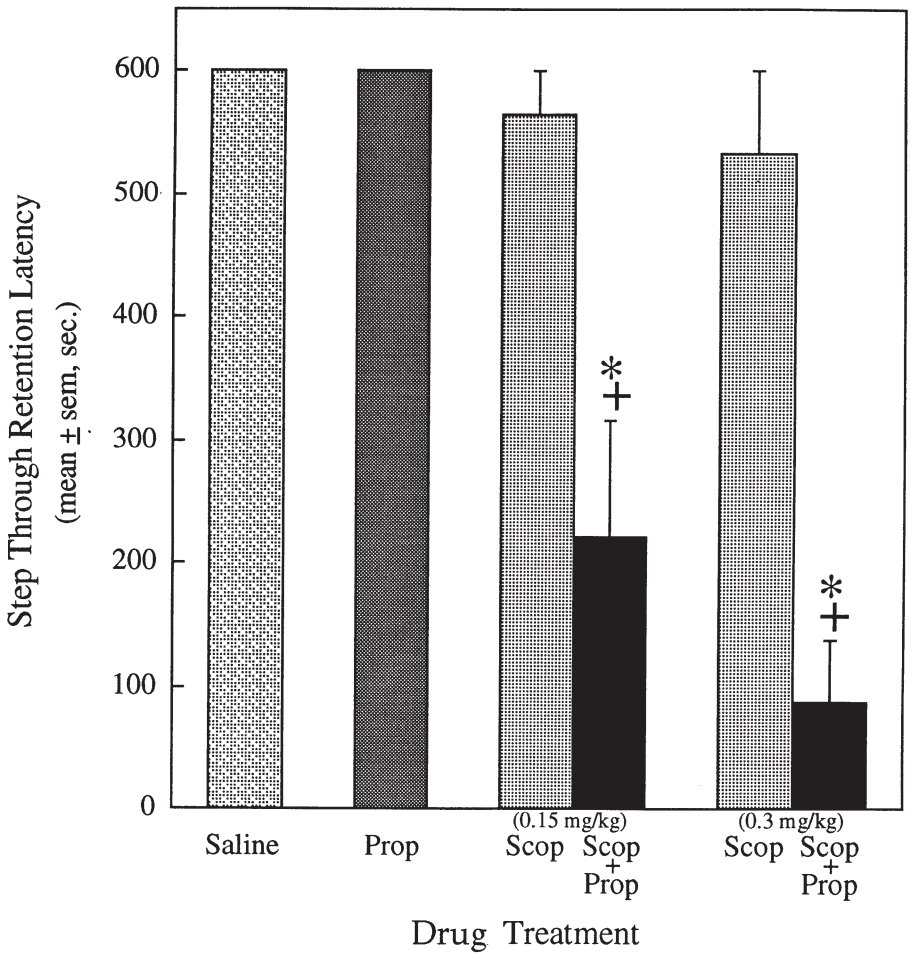

Figure 2. Retention performance (mean ± SEM) of rats receiving intraperitoneal injection of saline, propranolol (10 mg/kg), scopolamine (0.15 or 0.30 mg/kg), or a combination of scopolamine (0.15 or 0.30 mg/kg) and propranolol (10 mg/kg), 20 minutes prior to acquisition of the inhibitory avoidance task. The number of rats in each group ranged from 6 to 16. $^{*}p < .025$ from same dose scopolamine counterpart, Mann-Whitney U. $^{+}p < .01$ from propranolol, Mann-Whitney U.

chronic treatment (Ordway et al. 1988, 1991). In addition to studying whether these treatments alone caused an amnesic effect, drug-treated rats were also given scopolamine. It was predicted that if the expected down-regulation of amygdaloid β_1-adrenoceptors caused by the antidepressants alone was insufficient to produce a behavioral deficit in the inhibitory avoidance task, then the combination of scopolamine with the antidepressant-induced reduction in the number of β_1-adrenoceptors may reveal the retention deficit. The selective serotonin reuptake inhibitor (SSRI), fluoxetine, was studied also, because previous work from this laboratory did not find it to down-regulate amygdaloid β_1-adrenoceptors (Ordway et al. 1991).

Effect of Chronic Antidepressant Treatment. The purpose of the initial experiment in this series was to determine if chronic treatment of rats with DMI would by itself produce an amnesic effect or potentiate the amnesic effect of scopolamine. To do this, rats were treated chronically with DMI before being challenged with one of three doses of scopolamine. Acquisition latencies did not differ between groups, regardless of chronic treatment or dose of scopolamine. Retention latencies differed as a function of treatment ($p < .001$, Kruskal-Wallis). As illustrated in Figure 3, chronic administration of saline did not significantly reduce the retention latency of rats injected acutely with either saline or the two lower doses of scopolamine (0.15 or 0.3 mg/kg). In saline-treated rats, an amnesic effect of scopolamine was caused by the higher dose of scopolamine (0.45 mg/kg), as reflected by the significantly reduced retention latency of these rats as compared to saline controls. Rats treated chronically with DMI exhibited maximal retention latencies when given an acute injection of saline; that is, DMI alone caused no amnesic effect (Figure 3). By contrast, DMI-treated rats given scopolamine (regardless of dose) demonstrated a marked retention deficit ($p < .001$, Kruskal-Wallis). All DMI-treated rats receiving scopolamine exhibited significantly reduced retention latencies as compared to DMI-treated rats receiving saline. However, treatment of rats with DMI potentiated the amnesic effect of the two lower doses of scopolamine but not that caused by the highest dose (0.45 mg/kg); an amnesic effect was produced by this dose alone.

Based on these results, a dose of scopolamine of 0.3 mg/kg was selected for use in subsequent experiments, which tested the effect of the combination of scopolamine with DMI or two other antidepressants; namely,

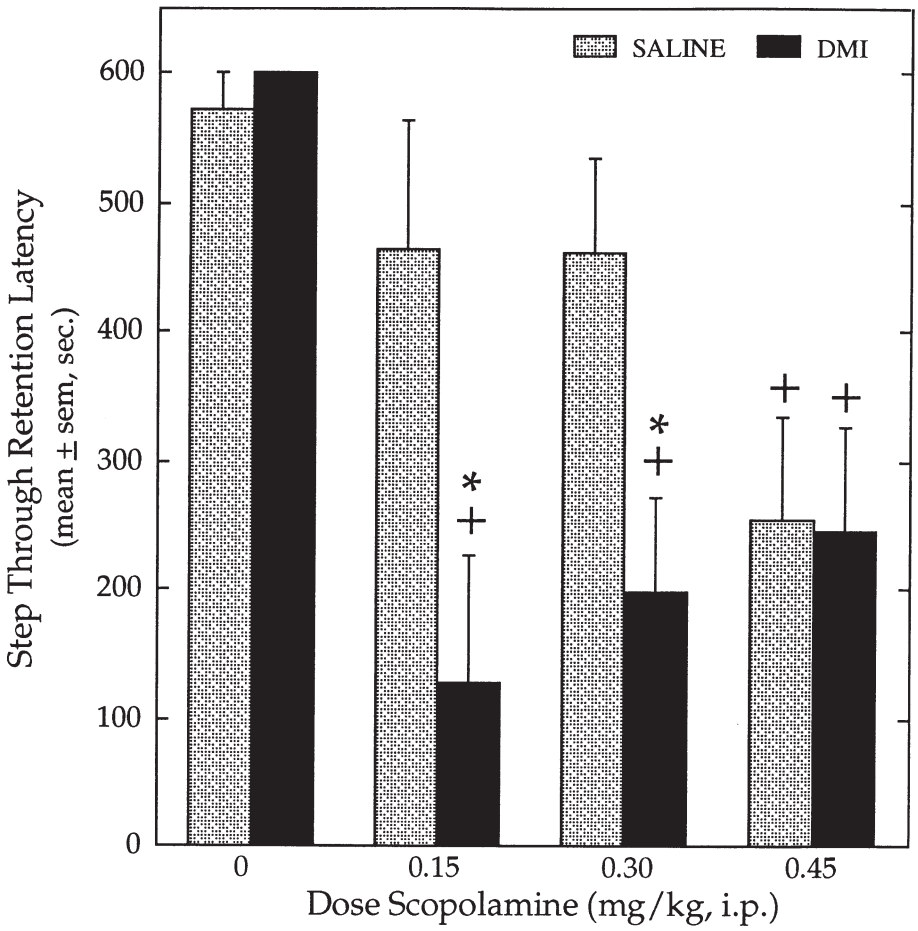

Figure 3. Retention performance (mean ± SEM) of rats receiving saline or scopolamine after 8 days of saline or DMI (10 mg/kg, IP, BID). Rats were kept drug free for 60 hours prior to the administration of scopolamine. Scopolamine was given, at the doses indicated, 20 minutes prior to the acquisition of the task. There were 6 to 13 rats per group. $^*p < .05$ from same dose SCOP counterpart, Mann-Whitney U. $^+p < .005$ from SAL–SAL and DMI–SAL, Mann-Whitney U.

Table 1. Effect of DMI, PHEN, FLUOX or SALINE on Latency To Enter the Dark Compartment During the Acquisition Phase of Inhibitory Avoidance Conditioning

	Acquisition Latency (s)							
	Saline		DMI		Phenelzine		Fluoxetine	
Treatment	SAL	SCOP	SAL	SCOP	SAL	SCOP	SAL	SCOP
Chronic	12.3 ± 2.1	10.3 ± 2.1	13.6 ± 1.6	12.7 ± 2.2	10.2 ± 1.4	18.2 ± 3.3	13.6 ± 4.0	14.1 ± 3.0
(60-h washout)	(15)	(15)	(15)	(15)	(6)	(10)	(9)	(10)
Acute	9.5 ± 1.5	10.0 ± 2.0	70 ± 30*	58 ± 17*	10.0 ± 2.6	14.0 ± 4.4	——	——
(No washout)	(2)	(4)	(4)	(4)	(4)	(4)		
Acute	24.0 ± 7.5	9.0 ± 3.6	15.0 ± 7.2	20 ± 11.4	16.4 ± 5.7	9.3 ± 2.8	——	——
(60-h washout)	(4)	(4)	(5)	(7)	(5)	(7)		

Rats received either (1) a single injection of SAL, DMI or PHEN 40 minutes or 60 hours prior to acquisition of the inhibitory avoidance task, or (2) repeated administration (8 days, BID) or SAL, DMI, PHEN, or FLUOX until 60 hours prior to acquisition of the inhibitory avoidance task. SAL or SCOP (0.3 mg/kg) was given 20 minutes prior to acquisition of the task. The number of rats per group is shown in parentheses. Data are expressed as mean ± SEM.

*$p < .05$, from SAL–SAL, SAL–SCOP counterparts, Mann-Whitney U.

phenelzine and fluoxetine, on inhibitory avoidance behavior. In the next experiment, rats were treated chronically with these antidepressants. In this experiment, acquisition latencies did not differ between the various treatment groups whether or not scopolamine was administered (Table 1). When rats treated chronically with these antidepressants were given saline, retention latencies were maximal or near maximal (Figure 4). Scopolamine caused no amnesic effect in rats treated chronically with saline or FLUOX. However, scopolamine did

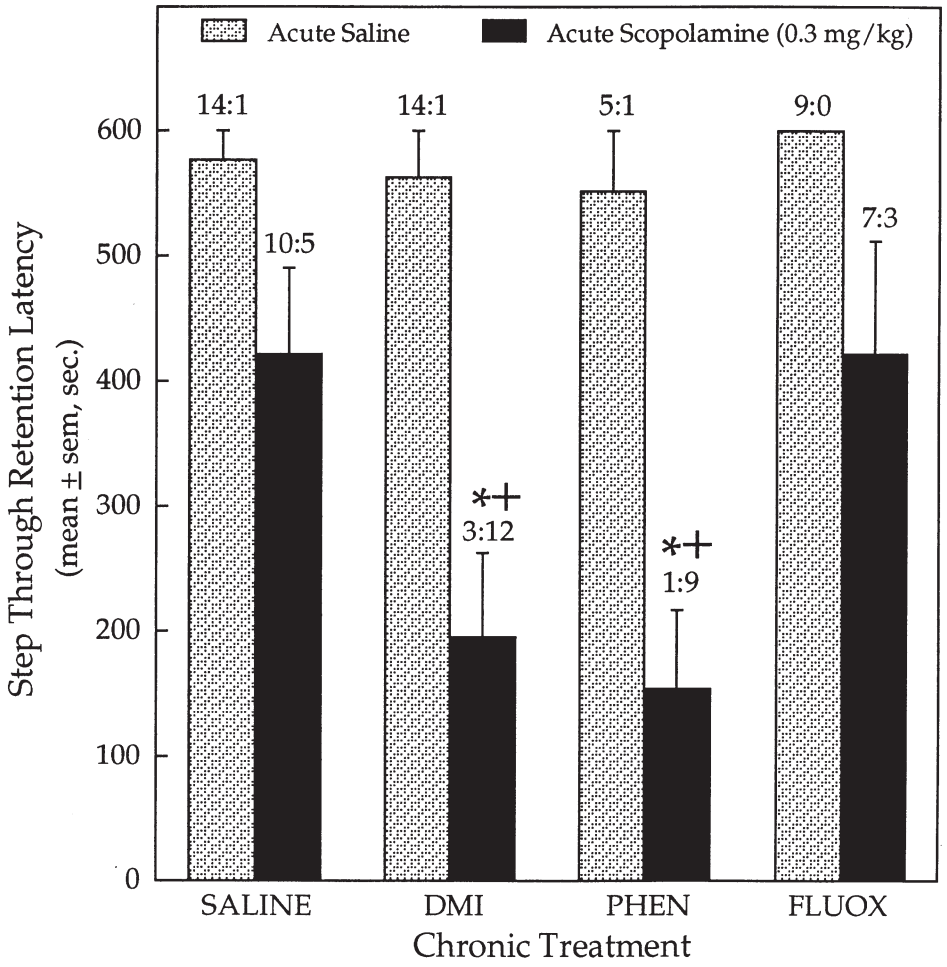

Figure 4. Retention performance (mean ± SEM) of rats given SAL or SCOP (0.3 mg/kg) after 8 days of saline, DMI (10 mg/kg, IP, BID), PHEN (5 mg/kg, IP, BID) or FLUOX (10 mg/kg, IP, BID). Rats were kept *drug free for 60 hours* prior to the administration of SAL or SCOP. SAL or SCOP was given 20 minutes prior to the acquisition of the task. There were 6 to 15 rats per group. The numbers above the bars represent the number of rats attaining maximal retention of the task (i.e., remaining in the illuminated compartment for 600 s) to the number that failed to attain the 600 s ceiling. *$p < .005$ from SAL–DRUG counterpart, Mann-Whitney U. $^{+}p < .025$ from SAL–SCOP, Mann-Whitney U.

cause a significant reduction in retention latency in rats chronically treated with either DMI or PHEN ($p < .01$, Kruskal-Wallis). These rats exhibited significantly shorter retention latencies than either SAL–SCOP and FLUOX–SCOP treated rats ($p < .05$, Fisher Exact tests). These data are consistent with the view that those antidepressants that down-regulate β_1-adrenoceptors in the amygdala, although not causing an amnesic effect on their own, are capable of potentiating the amnesic effect of scopolamine. The next experiment was designed to determine if these drugs caused the same effect after acute treatment as such treatment does not down-regulate β_1-adrenoceptors (Sarai et al. 1978; Ordway et al. 1988, 1991).

Effect of Acute Antidepressant Treatment (No Washout). Rats were given a single injection of either saline, DMI or PHEN 40 minutes prior to acquisition training. Saline or scopolamine (0.3 mg/kg) were then given 20 minutes prior to acquisition. Acquisition latencies did not differ between saline or PHEN treatment groups. However, rats given DMI–SAL or DMI–SCOP demonstrated a significant increase in acquisition latency as compared to either their saline or PHEN counterparts ($p < .05$, Kruskal-Wallis) (Table 1). With respect to retention latencies, neither drug treatment alone had any amnesic effect (Figure 5). However, acute treatment with DMI, but not PHEN, potentiated the amnesic effect of scopolamine ($p < .001$, Kruskal-Wallis). The ability of a single injection of DMI to potentiate the amnesic effect of scopolamine was unexpected. It was hypothesized that this may have been attributable to the ability of DMI to block muscarinic cholinergic receptors (see Frazer 1997). Consequently, in the next experiment, rats were given a single injection of DMI, as well as PHEN, but the training was carried out after a sufficient time for DMI to be eliminated from the rats.

Effect of Acute Antidepressant Treatment (60-Hour Washout). To determine whether either chronic treatment and/or drug being present are essential to produce a deficit in the retention of the inhibitory avoidance task, rats were given a single injection of either saline, DMI, or PHEN 60 hours prior to acquisition training. This is the same washout time as that used when these drugs were given repeatedly. Saline or sco-

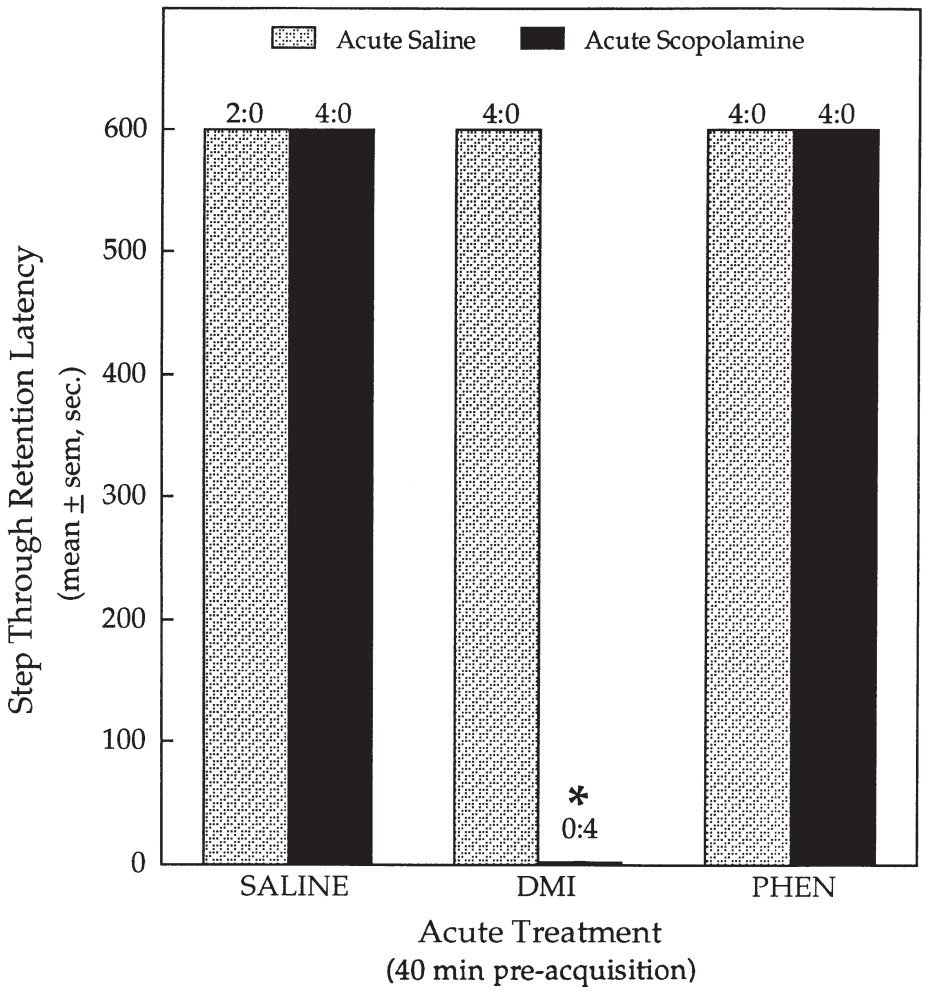

Figure 5. Retention performance (mean ± SEM) of rats given SAL or SCOP (0.3 mg/kg) after a *single injection* of SAL, DMI (10 mg/kg, IP) or PHEN (5 mg/kg, IP). A period of *40 minutes* was permitted to elapse between the SAL, DMI, or PHEN injection and the subsequent SAL or SCOP injection. The latter were given 20 minutes prior to the acquisition of the task. There were 2 to 4 rats per group. The numbers above the bars represent the ratio of the number of rats attaining maximal retention of the task (i.e., remaining in the illuminated compartment for 600 s) to the number that failed to attain the 600 s ceiling. *$p < .01$ from SAL–DMI and from SAL–SCOP, Mann-Whitney U.

polamine (0.3 mg/kg) were then given 20 minutes prior to acquisition. Acquisition latencies did not differ between groups, regardless of treatment (Table 1). Figure 6 illustrates the retention latencies of rats given either a single injection of saline, DMI, or PHEN, in combination with saline or scopolamine. In all cases, when rats were given saline 20 minutes prior to acquisition, retention latencies were maximal. As expected from the previous dose-response experiment (Figure 2), this dose of scopolamine given to rats injected with saline caused no amnesic effect (Figure 6). However, both DMI and PHEN potentiated the amnesic effect of scopolamine, as evidenced by a significant reduction in retention latency ($p < .02$, Kruskal-Wallis), the effect being comparable to that observed after 8 days of treatment with the same drugs (compare Figure 6 with Figure 4).

Quantitation of β_1-Adrenoceptors in the Amygdala Complex

The binding of [^{125}I]-IPIN to the lateral, basolateral, basomedial, and central amygdaloid nuclei were analyzed as a group, and are referred to throughout as the *amygdaloid complex*. Because there was no significant difference in the number of [^{125}I]-IPIN binding sites between drug-treated rats given either SAL or SCOP, these data were pooled within each treatment group. Repeated treatment of rats with either DMI or PHEN significantly reduced the number of β_1-adrenoceptors in the amygdala complex ($p < .025$ and $p < .001$, respectively, independent *t*-tests). DMI and PHEN both produced a 36% decrease in the number of amygdaloid β_1-adrenoceptors relative to saline-treated controls. By contrast, chronic treatment with FLUOX did not alter the number of β_1-adrenoceptors in the amygdala as compared to saline-treated controls (Figure 7). The number of amygdaloid β_1-adrenoceptors did not differ significantly between rats treated acutely with either DMI, PHEN or SAL administered either 60 h or 40 min prior to acquisition.

DISCUSSION

The purpose of this study was to determine if there was a behavioral sequelae that resulted from the ability of

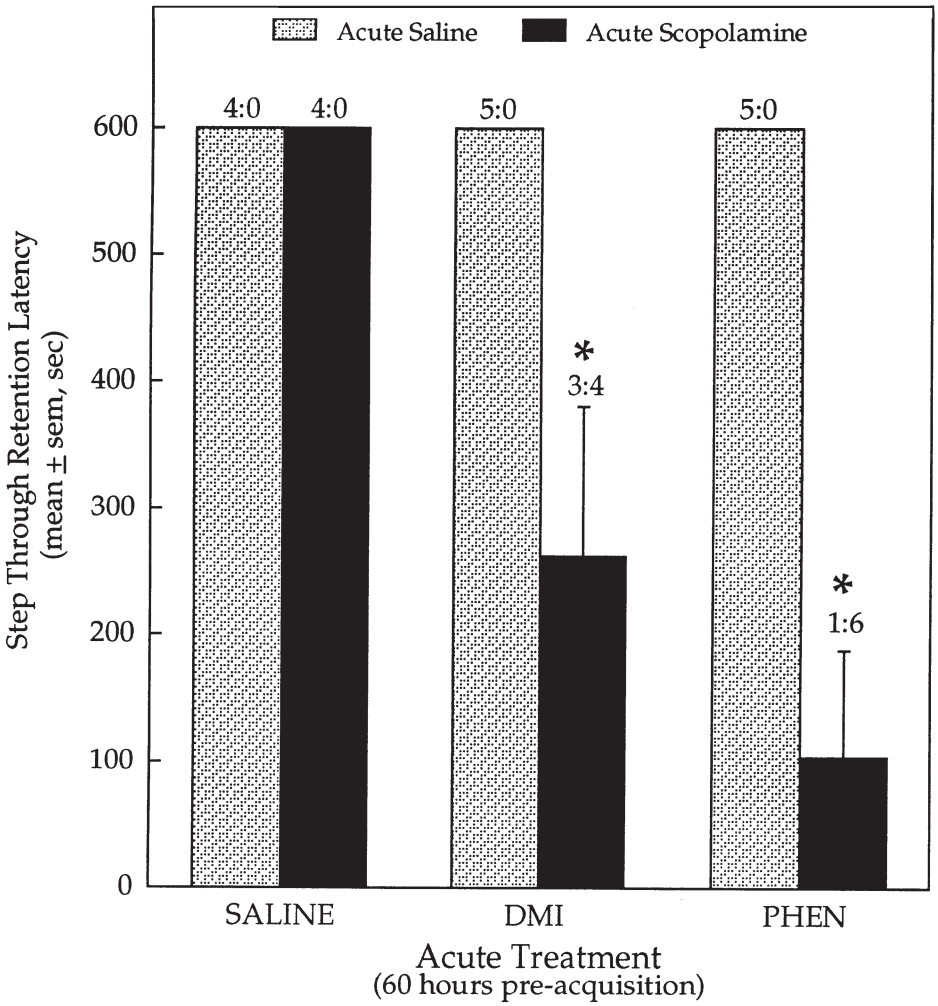

Figure 6. Retention performance (mean ± SEM) of rats given SAL or SCOP (0.3 mg/kg) after a *single injection* of SAL, DMI (10 mg/kg, IP), or PHEN (5 mg/kg, IP). A period of *60 hours* was permitted to elapse between the SAL, DMI, or PHEN injection and the subsequent SAL or SCOP injection. The latter were given 20 minutes prior to the acquisition of the task. There were 4 to 7 rats per group. The numbers above the bars represent the ratio of the number of rats attaining maximal retention of the task (i.e., remaining in the illuminated compartment for 600 s) to the number that failed to attain the 600 s ceiling. *$p < .05$ from SAL–DRUG counterpart and from SAL–SCOP, Mann-Whitney U.

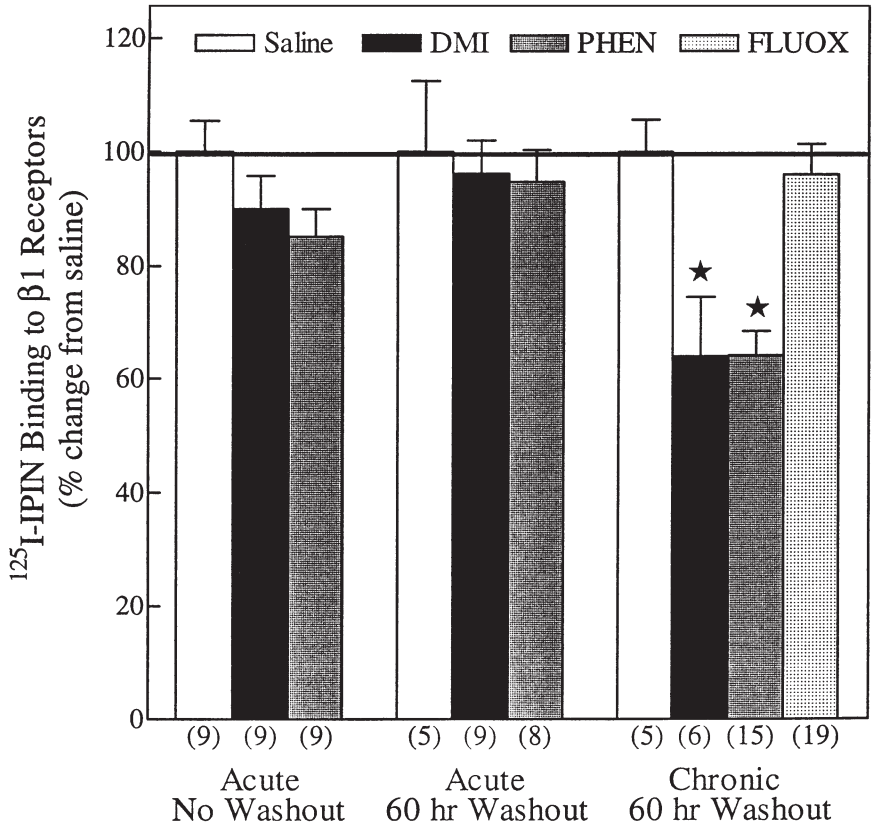

Figure 7. Effect of DMI (10 mg/kg), PHEN (5 mg/kg) or FLUOX (10 mg/kg) treatment on the binding of [^{125}I]-IPIN to β_1-adrenoceptors in the amygdala of the rat brain. Rats received either (1) a single injection of SAL, DMI, or PHEN 40 minutes or 60 hours prior to acquisition of the inhibitory avoidance task, or (2) repeated administration (8 days, BID) of SAL, DMI, PHEN, or FLUOX until 60 hours prior to the acquisition of the inhibitory avoidance task. Data are expressed as the mean ± SEM percentage change from saline controls. Values for saline-treated rats were 21.6 ± 1.8 fmol/mgpr (acute, no washout), 20.1 ± 2.5 fmol/mgpr (acute, 60 h washout) and 16.3 ± 1.9 fmol/mgpr (chronic, 60 h washout). *$p < .025$ from SAL counterpart, *t*-test for independent samples.

some antidepressants to down-regulate β_1-adrenoceptors in the amygdala. Behavior in an inhibitory avoidance task was selected for investigation as: (1) administration of β-adrenoceptors directly into the amygdala produces a deficit in the retention of the task (Gallagher et al. 1977); and (2) systemic administration of β-adrenoceptor antagonists potentiates the retention deficits induced by the muscarinic cholinergic antagonist scopolamine on such behavior (Decker et al. 1990; Decker and McGaugh 1991). It is, however, unclear if the effect of systemically administered propranolol is attributable to an action at peripheral and/or central β-adrenoceptors; evidence supporting catecholamine effects on memory at both peripheral and central loci has been obtained (see McGaugh 1989). The results are clear in demonstrating that rats treated chronically with antidepressants such as DMI or PHEN, which down-regulate β_1-adrenoceptors in the amygdala, do not exhibit retention deficits in an inhibitory avoidance task (Figure 4). Antidepressant-induced down-regulation of amygdaloid β_1-adrenoceptors does not, then, reproduce the retention deficit caused by injection of propranolol directly into the amygdala. However, administration of such antidepressants does potentiate the retention deficit induced by scopolamine in this task, similar to that seen upon systemic administration of propranolol. The potentiating effect of these antidepressants can be observed after their acute administration, although acute administration of DMI or PHEN did not down-regulate amygdaloid β_1-adrenoceptors. It seems, then, that the ability of these antidepressants to potentiate the retention deficit induced by scopolamine is unrelated to their effect on β_1-adrenoceptors in the amygdala. Our conclusion is that antidepressant-induced down-regulation of amygdaloid β_1 adrenoceptors does not cause impaired learning in an inhibitory avoidance task.

In the experiments involving chronic administration of antidepressants, the rats were kept drug free for 60 hours following chronic treatment in order to reduce the confound of high central levels of drug being present at the time of inhibitory avoidance training. In the rat, the half life of DMI is 9 h (Dingell et al. 1964), and for FLUOX and its active metabolite, norfluoxetine, 8–13 h and 15–16 h, respectively (Caccia et al. 1990). Based on this, it was calculated that the uptake inhibitors would essentially be eliminated from the body after the 60 hour washout period and, thus, reduce the likelihood that any observed effects were attributable to state-dependent factors (e.g., presence of drug-altering level of perceived pain). In addition, there are several reports that antidepressants, by themselves, do not have appreciable antinociceptive activity (Lee and Spencer, 1980; Testa et al., 1987; Tura and Tura 1990). Because phenelzine is essentially an irreversible inhibitor of MAO, this enzyme would still be inhibited 60 h after the drug was given. Thus, under the present conditions,

the possibility that effects of phenelzine on inhibitory avoidance conditioning are attributable to state-dependent factors cannot be excluded. However, there is some evidence that phenelzine given chronically can exert anxiogenic effects (Johnston and File 1988) and can reverse conditioned analgesia (Murua and Molina 1991). Based on these reports, it might be predicted that if state-dependency is induced by phenelzine in the inhibitory avoidance task, then retention would be enhanced rather than inhibited. Each of the chronic drug treatments alone failed to impair retention of inhibitory avoidance conditioning. However, when a non-amnesia-producing dose of scopolamine (0.3 mg/kg) was administered prior to acquisition, rats that had received either DMI or PHEN exhibited a marked impairment in the retention of the task. By contrast, when scopolamine was given to rats that had received FLUOX chronically, there was no significant disruption of retention of inhibitory avoidance conditioning. These results are interesting in view of reports that certain antidepressants can impair intellectual function in humans (Vassiliou and Himwich 1961; Di Mascio et al. 1964). Furthermore, these data suggest that treatment of depression, particularly in the elderly where cognitive measurement may already be a consideration, may best be treated with SSRIs.

[^{125}I]-IPIN binding to amygdaloid β_1-adrenoceptors was significantly reduced in rats treated chronically with DMI or PHEN; whereas, FLUOX was without effect. These data corroborate those of Ordway and coworkers (1988, 1991). Because amygdaloid β_1-adrenoceptors were down-regulated by around 36%, it may be argued that this represents a "modest" deficit, which in and of itself, was insufficient to produce a behavioral deficit in inhibitory avoidance conditioning. That is to say, the inhibition of β-adrenergic-mediated transmission in the amygdala caused by this degree of down-regulation may be less than that attributed to administration of propranolol directly into the amygdala. The interaction between the noradrenergic system and scopolamine on memory also seems to require extensive interference with noradrenergic transmission (Decker and Gallagher 1987; Decker and McGaugh 1991). In view of this, it seems unlikely that the modest 36% reduction in amygdaloid β_1-adrenoceptors caused by either DMI or PHEN treatment accounts for their ability to potentiate the amnesic effect of scopolamine.

Further support for this conclusion comes from the report that antidepressant drugs must be administered chronically to down-regulate β_1-adrenoceptors (Ordway et al. 1988, 1991, and the present study). However, acute administration of either DMI or PHEN still potentiated the amnesic effect of scopolamine when the training was carried out after a 60 h washout period. At this time, neither DMI or PHEN altered the number of amygdaloid β_1-adrenoceptors. It seems, then, that neither acute DMI nor PHEN, when given in combination with SCOP, exert their effect as a direct consequence β_1-adrenoceptor down-regulation. Because DMI and PHEN caused a comparable potentiation of the amnesic effect of scopolamine, whether or not they produced down-regulation of amygdaloid β_1-adrenoceptors, provides further evidence to support the conclusion that mechanisms other than β_1-adrenoceptor down-regulation are responsible for the ability of DMI and PHEN to potentiate the amnesic effect of scopolamine. That a single dose of DMI caused an impairment in the retention of the inhibitory avoidance task 60 h after its administration is somewhat surprising. This result suggests that DMI is capable of disrupting inhibitory avoidance behavior for substantive periods after its administration, even when it is no longer present in the body or has not produced down-regulation of β_1-adrenoceptors. The mechanism underlying this effect of DMI is unclear; however, it was not in the scope of the present study to pursue this phenomenon.

When either DMI or PHEN were given 40 minutes prior to acquisition, only rats receiving DMI in combination with scopolamine exhibited the retention deficit. These data may best be explained by the anticholinergic effects of DMI (Rehavi et al. 1977; Snyder and Yamamura 1977; Golds et al. 1980; Hall and Ogren 1981; Johnson 1991). It is well known that anticholinergic mechanisms impair memory processes (Sitaram et al. 1978; Decker and McGaugh 1991). Moreover, in the present study, rats given DMI acutely took significantly longer to enter the dark compartment during acquisition training. This is likely caused by the anticholinergic effect of DMI (Mancinelli et al. 1988). Thus, although DMI by itself did not impair retention, when given in combination with a dose of scopolamine, which by itself also did not impair retention, blockade of muscarinic cholinergic receptors may have been sufficient to produce the retention deficit.

Our experiments corroborate previous reports of amygdaloid β-adrenoceptor and muscarinic cholinergic involvement in mediating the retention of an inhibitory avoidance task. As reported originally by Gallagher et al. (1977), the present study also demonstrated that propranolol injected into the amygdala of rats trained on an inhibitory avoidance task, produced decreases in the retention of inhibitory avoidance conditioning. In addition, propranolol injected into the caudate putamen, just dorsal to the amygdala complex did not disrupt retention of inhibitory avoidance conditioning. This finding is consistent with earlier reports that injection of propranolol into other brain regions does not alter retention of an inhibitory avoidance task (see Gallagher et al. 1977; Ellis and Kesner 1983; Liang et al., 1986). These results support the interpretation that β-adrenergic blockade in the amygdala of rats disrupts long-term memory formation in inhibitory avoidance learning.

The present study also confirmed the results of Decker et al. (1990), that systemic administration of propranolol did not produce a deficit in the retention of inhibitory avoidance conditioning. However, when given in combination with a nonamnesic dose of scopolamine, propranolol produced a profound deficit in the retention of the task (Decker et al. 1990).

In the present study, all drugs were administered pretraining. This strategy was employed, because in the studies investigating the effect of chronic treatment with antidepressants on inhibitory avoidance behavior, the antidepressant-induced down-regulation of β-adrenoceptors would be present at the time of training. Thus, whereas Gallagher et al. (1977) administered intra-amygdala propranolol immediately post-training, in the present study, propranolol was given 20 minutes prior to training. The subsequent impairment in retention of the task was not different between the two studies, suggesting that the effect of propranolol on the retention of an inhibitory avoidance task is not dependent upon whether propranolol is given pre- vs. post-training, at least in the time frames compared here. With respect to the administration of scopolamine prior to training, the present results are in good agreement with others (Decker et al. 1990). Although it is well established that scopolamine given prior to training produces a dose-dependent impairment in retention (see McGaugh 1989), studies where scopolamine has been administered post-training have yielded conflicting results. Some have reported no impairment in retention (Gower 1987; Hagan et al. 1986); whereas, others have observed significant impairments (Kameyama et al. 1986; Introini-Collison and McGaugh 1988). Interestingly, those who have reported impairments in retention of inhibitory avoidance tasks after post-training administration of cholinergic drugs have used higher doses than those required for producing impairment with pre-training injections. For example, in the present study, amnesic effects were observed at 0.45 mg/kg scopolamine, although doses as high as 4.0 mg/kg have not impaired retention when given post-training (Duran-Arevalo et al. 1990). Whether these conflicting findings can be attributed to the influence of cholinergic antagonists on processes other than memory storage remain unresolved.

Likewise, if down-regulation of β_1-adrenoceptors does not contribute to the behavioral effects caused by either DMI or PHEN, it is important to consider the mechanism(s) through which these drugs exert this effect. As discussed earlier, it is unlikely that the effects of DMI or FLUOX in the 60 h washout experiments were attributable to state-dependent changes in learning the task. However, certainly in other experiments, effects on nonassociative performance factors cannot be excluded when drugs are administered before training. Under the conditions used in the present study, scopolamine by itself is unlikely to have produced any state-dependent effects (Elrod and Buccafusco 1988; Quirarte et al. 1994). However, antidepressants are known to modify reactions to stress (Weiss and Simson 1985; Adell et al. 1989) and noxious stimuli (Lund et al. 1989, 1991). Thus, drug- and/or stress-induced analgesia, hyperanalgesia, as well as altered levels of anxiety (Amit and Galina 1986; Johnston and File 1988; Jørum 1988a,b) may contribute to alterations in performance in inhibitory avoidance conditioning. Despite this, the results presented here seem clear inasmuch as the ability of these antidepressants to disrupt inhibitory avoidance conditioning is unrelated to whether down-regulation of β_1-adrenoceptors has occurred.

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