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Increased Shock-Induced Fighting With Supersensitive β-Adrenergic Receptors[1]

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HEGSTRAND, L. R. AND B. EICHELMAN. *Increased shock-induced fighting with supersensitive β-adrenergic receptors.* PHARMACOL BIOCHEM BEHAV **19**(2) 313–320, 1983.—Adult male rats were treated chronically with haloperidol (1 mg/kg) daily or propranolol (10 mg/kg bid) and evaluated for changes in shock-induced fighting. Haloperidol suppressed fighting. Chronic propranolol facilitated fighting when rats were tested eight hours after injection. Acutely, either 5 or 10 mg/kg of d,l-propranolol suppressed shock-induced fighting. Chronic pindolol (10 mg/kg bid) and chronic l-propranolol (5 mg/kg bid) administration increased fighting. Chronic d,l-propranolol, l-propranolol or pindolol administration was associated with an increase in B_{max} for β-adrenergic receptors. No change in fighting or B_{max} was observed with the chronic administration of d-propranolol (5 mg/kg bid) or metoprolol (10 mg/kg bid). This increase in shock-induced fighting appears to be a behavioral response developing as a consequence of increased β-adrenergic receptors responding to endogenously released norepinephrine.

Shock-induced fighting Aggressive behavior β-Adrenergic receptors Propranolol Pindolol Metoprolol Haloperidol

CATECHOLAMINES have been well-implicated in laboratory studies of aggressive behavior [5], particularly in the affective aggression of cats [16] and rats [6]. Of the catecholamines, norepinephrine (NE) has been postulated to be a primary facilitator of affective aggression [8]. Various pharmacological and environmental manipulations in the rat which enhance noradrenergic function or turnover increase defensive, shock-induced, aggression in the rat [8]. Also using the paradigm of shock-induced fighting in rats, Stolk *et al.* [22] demonstrated a correlation between the number of attacks and the disappearance of centrally labeled NE from rat brainstem. In the cat, Reis and Fuxe [17] demonstrated a depletion of brainstem NE which was proportional to the frequency of sham rage attacks induced in cats by brainstem transection. However, there have been experimental observations inconsistent with a noradrenergic facilitatory hypothesis of affective aggression. Selective depletion of brain NE with 6-hydroxydopa increases shock-induced fighting in the rat after three to four days [24]. Infusion of NE into rat ventricles acutely suppresses shock-induced fighting [10].

Acute pharmacological studies have attempted to clarify this issue using relatively specific catecholaminergic agonists and antagonists. Acute treatment with dopamine (DA) antagonists generally suppresses affective aggression [25]. Acute treatment with DA agonists such as apomorphine fails to enhance shock-induced fighting [20,23]. Acute treatment with the α-adrenergic antagonist piperoxane which increases locus coeruleus firing and central noradrenergic activity [3] facilitates shock-induced fighting at a dose of 2.5 mg/kg [20]. Conversely, d,l-propranolol suppresses shock-elicited fighting [20,26]. The present study examines in greater detail the effects of chronic treatment of rats with DA or NE (β-adrenergic) antagonists on defensive aggressive behavior in conjunction with alterations in the density of neuronal receptors.

EXPERIMENT 1

The initial experiment examined the effect of chronic DA or NE antagonist treatment on the level of shock-induced fighting and examined whether chronic antagonist treatment altered receptor number.

METHOD

Animals

Forty-eight adult male Sprague-Dawley derived rats, 350–400 g, obtained from Holtzman in Madison, WI were individually housed at least one week prior to behavioral testing. All rats had free access to Purina Laboratory Chow and water. A continuous 12 hr light–12 hr dark cycle with constant humidity and temperature was maintained throughout the experiment.

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Apparatus

The testing chamber for shock-induced fighting was a 32×25×30 cm Coulbourn Instruments (Lehigh Valley, PA) Model E10-10 small animal test cage. A Coulbourn Instruments solid state shock-distributor and power supply were programmed to deliver scrambled footshock of 2 mA for a duration of 0.5 sec which was presented every 7.5 sec for 50 shocks to paired rats.

Behavioral Procedure

After one week of housing in the laboratory, the rats were ear punched, numbered, and randomly paired. They received three days of testing for shock-induced fighting as described by Eichelman [4]. Attacks were defined as a "directed movement toward the opponent which resulted in contact, including at least one additional response of the following: biting, sparring, upright attack posturing, or supine submissive posturing, adopted by the attacked rat." All pairs were maintained throughout the experiment. A baseline number of attacks were determined, being the average of three test sessions on the three consecutive days prior to drug treatment. The rats were then injected daily for 14 days. There were three experimental groups. The first group (8 pair) received one ml (IP) of isosaline daily. The second group (8 pair) received haloperidol (McNeil Laboratories, Inc., Fort Washington, PA) given daily at a dose of 1 mg/kg. The third group (8 pair) received d,l-propranolol (Ayerst, Montreal, Canada) given in a bid dose of 10 mg/kg. Drugs were given in a one ml volume. On days 12 through 14 the rats were again tested for shock-induced fighting. Each group was compared with itself by a 2-tailed matched pairs t-test.

Biochemical Procedures

Preparation of brain membranes. At the completion of the experiment the rats were decapitated. Control and propranolol-treated rats were killed 48 hours after the last injection. Because haloperidol has a long half-life, haloperidol-treated rats were killed one week after their last injection. Following decapitation, rat brains were dissected on ice to obtain cortical (average weight 500 mg) and caudate (80 mg) tissue. To measure [^{125}I]-iodohydroxybenzylpindolol (IHYP) binding, tissues were homogenized with a Brinkman Polytron (setting 2–3) for 10 to 15 sec in 20 volumes of ice cold isotonic NaCl (0.9%) containing 20 mM Tris-Cl, pH 7.5 (Tris-isosaline, 4°C). Homogenates were centrifuged at 20,000 × g for 10 min at 4°C. The supernatants were discarded and the pellets were resuspended in 200 volumes of Tris-isosaline per gram wet weight of tissue and stored frozen at −80°C until assayed. Since propranolol has a similar effect on neuronal β-adrenergic receptors throughout the brain [29], β-adrenergic receptors were only measured in cerebral cortex which is a large tissue high in neuronal adrenergic receptors and which has high specific binding for IHYP. To measure [^{3}H]-spiroperidol binding to DA receptors in caudate membranes, homogenization of caudate nuclei was in 200 volumes at 0.32 M sucrose/20 mM Tris-Cl (pH 7.5) with a Brinkmann Polytron (setting 2–3) for 10 to 15 sec. Homogenates were also centrifuged at 20,000 × g for 10 min at 4°C. Membranes were resuspended to contain about 100 μg protein in 0.9 ml of Tris-isosaline. Binding for DA receptors was performed on freshly prepared membranes.

DA receptor binding assay. [^{3}H]-Spiroperidol was purchased from New England Nuclear, Boston, MA. An aliquot (0.9 ml) of resuspended caudate membranes in Tris-isosaline was incubated with [^{3}H]-spiroperidol in the absence or presence of 2 μM d-butaclamol (Ayerst). The binding assays were performed in new polypropylene tubes in a total volume of 1.0 ml containing 139 mM NaCl; 18 mM Tris-Cl, pH 7.5; 1 μg BSA; and 50 μg ascorbic acid.

To determine the density of DA binding sites, the amount of specifically-bound [^{3}H]-spiroperidol was determined at nine concentrations. The data were analyzed by the method of Scatchard [19] to determine the density of DA receptors and the K_d of [^{3}H]-spiroperidol. The concentrations of [^{3}H]-spiroperidol ranged from 40 to 800 pmol (1000–20,000 cpm).

Samples were incubated at 37°C for 15 minutes. The reaction was then terminated by addition of 10 ml of ice-cold buffered saline, and the samples were immediately filtered through Gelman type AE glass fiber filters. Each filter was washed with an additional 10 ml of buffered isosaline. Radioactivity was determined by liquid scintillation spectrometry in 3 ml of a 2:1 toluene: Triton-X-100-based fluor.

Specific binding of [^{3}H]-spiroperidol was considered to be the amount of [^{3}H]-spiroperidol bound in the absence of competing ligand minus the amount bound in the presence of 2 μM d-butaclamol.

β-Adrenergic receptor binding assay. Hydroxybenzylpindolol [1] was iodinated and IHYP was purified to theoretical specific activity (2.2 Ci/μmol) as described previously [11,14]. An aliquot (0.15 mg) of resuspended membranes in Tris-isosaline was incubated with IHYP in the absence or presence of 50 μM l-isoproterenol. The binding assays were carried out in new disposable polypropylene tubes (Sarstedt) in a total assay volume of 0.25 ml containing 92.4 mM NaCl; 12 mM Tris-Cl, pH 7.5; 1 μg BSA; 50 μg ascorbic acid; 100 μM phentolamine; and 100 μM GTP.

To determine the density of binding sites, the amount of specifically bound IHYP was determined at nine concentrations. The data were analyzed by the method of Scatchard [19] to provide a value for the density of receptors and the dissociation constant (K_d) of IHYP. The concentration of IHYP was varied over a range of 15 to 240 pmol (10,000–250,000 cpm).

Samples were incubated 30 minutes at 37°C. Reactions were then stopped by adding 10 ml of buffered isosaline at 37°C to each assay tube. The samples were rapidly filtered through Gelman type AE glass fiber filters. Each filter was washed with an additional 10 ml of buffered isosaline following which the radioactive filters were counted in a gamma counter.

Specific binding of IHYP was defined as the amount of IHYP bound in the absence of competing ligand minus the amount bound in the presence of 50 μM l-isoproterenol. All binding assays were conducted such that bound ligand was less than 10% of the total ligand. Average protein concentration was 100 μg/tube.

RESULTS

Rats injected IP for two weeks with haloperidol (1 mg/kg daily) showed a small, but significant decrease ($p<0.05$, 2-tailed matched pairs t-test) in the number of attacks in the fighting paradigm when tested six to eight hours following drug injection and compared with pre-drug baseline fighting levels (Table 1). Conversely, propranolol injections (10 mg/kg bid) increased attacks by an average of 8.3 above

TABLE 1

EFFECT OF CHRONIC HALOPERIDOL AND PROPRANOLOL INJECTIONS ON SHOCK-INDUCED FIGHTING IN RATS*

Group	N (Pairs)	Pre-Injection Attacks/50 Shocks	Post-Injection Days 12–14 Attacks/50 Shocks	Difference In No. of Attacks
Saline	8	18.1 ± 3.0	18.8 ± 3.0	+0.7 ± 1.7
Haldol (1 mg/kg daily)	8	21.8 ± 1.3	18.8 ± 2.1	−3.0 ± 1.4†
Propranolol (10 mg/kg bid)	8	22.9 ± 2.3	31.7 ± 3.1	+8.6 ± 1.9‡

*Values are expressed as the mean ± S.E.M.
†$p<0.05$, 2-tailed matched pairs t-test.
‡$p<0.01$, 2-tailed matched pairs t-test.

TABLE 2

EFFECT OF CHRONIC HALOPERIDOL INJECTIONS* ON DA-RECEPTORS IN RAT CAUDATE NUCLEUS

Group	$K_d \times 10^{10}$ (M)	B_{max} (fmoles/mg prot)
Saline	1.65 ± 0.16	500 ± 29
Haloperidol (1 mg/kg daily)	1.56 ± 0.17	685 ± 42†

*Rats were sacrificed one week after the last injection. ^{3}H-spiroperidol was used as the ligand. Values are expressed as mean ± S.E.M.
†$p<0.01$, 2-tailed, t-test.

baseline levels, testing after 12 to 14 days of drug treatment six to eight hours after the a.m. dose ($p<0.01$, 2-tailed matched pairs t-test; Table 1). DA receptor density increased 37% in the caudate nucleus membranes of the chronically injected haloperidol rats as compared with the saline injected controls ($p<0.01$, see Table 2), suggesting effective dopaminergic blockade during the period of behavioral testing. No difference was observed in the affinity of [^{3}H]-spiroperidol for the DA receptors. β-Adrenergic receptors in rat cerebral cortical membranes measured 48 hours following the last propranolol injection showed no differences in number or affinity compared to control. Failure to demonstrate increased β-adrenergic receptor number at 48 hours left open the hypothesis that increased receptor number had not been induced by the chronic regimen or alternatively that receptor number had returned to control levels more rapidly than seen with DA receptors.

EXPERIMENT 2

The decrease in shock-induced fighting following a DA antagonist is consistent with prior reports [25]. However, since both Vassout and Delini-Stula [26] and Sheard [20] had reported a decrease in rat shock-induced fighting following an acute dose of propranolol, whereas we had observed an increase with chronic propranolol administration, we examined this discrepancy in a second experiment measuring the effect of propranolol injections on shock-induced fighting over a 15 day time course.

METHOD

Animals

Animals were 48 adult male Sprague-Dawley derived rats, 350–400 g obtained from Holtzman in Madison, WI and housed as described in Experiment 1.

Apparatus

The same as in Experiment 1.

Behavioral Procedure

After one week of housing in the laboratory, the rats were ear punched, numbered, and randomly paired. They received three days of baseline testing for shock-induced fighting. There was a saline control group (8 pair), a 5 mg/kg propranolol group (8 pair) which received 5 mg/kg of d,l-propranolol bid for 15 days, and a 10 mg/kg propranolol group (8 pair) which received 10 mg/kg of d,l-propranolol bid for 15 days. The rats were then subsequently tested for shock-induced fighting on the first day, one hour after the initial injection of drug and then on the 3rd, 5th, 7th, 11th, 13th, and 15th days, six to eight hours after the a.m. injection. On Day 15 the rats were sacrificed by decapitation after fighting and eight hours after the last injection of drug. Statistically, the data were analyzed using an ANOVA for repeated measures with Dunnett's test used for specific comparisons with the control, comparing changes from baseline for all groups over the days of testing.

Biochemical Procedure

Cerebral cortical membranes were prepared and β-adrenergic receptors were measured for the control and the 10 mg/kg propranolol group using IHYP as described in the Method section of Experiment 1.

RESULTS

The ANOVA comparing the changes from baseline for the three experimental groups over 15 days of treatment was

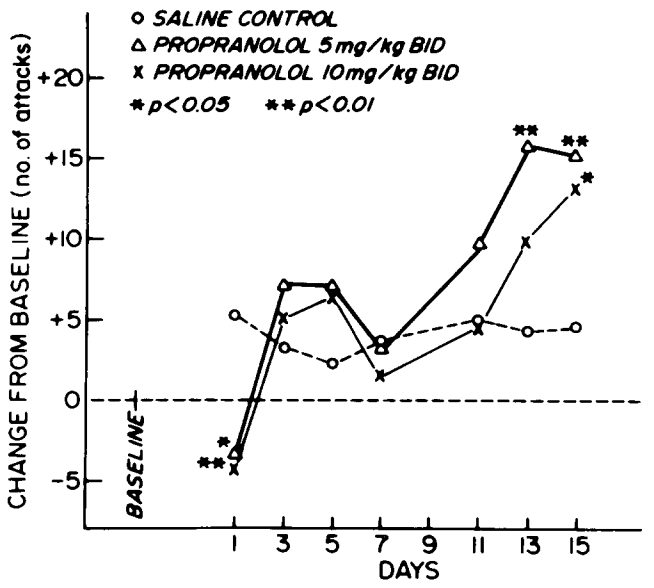

FIG. 1. Effect of propranolol with time on shock-induced fighting in rats. Before receiving either saline or propranolol bid, pairs of rats were tested for the number of attacks during 50 footshocks to establish baseline. The identical pairs of rats were fought again on the days indicated to determine the effect of propranolol with time. Each point is the difference in the means of the number of attacks after X days of injections for 8 pairs of rats per group. Statistical analysis is based on Dunnett's *t*-test comparing experimental attack level values with control.

statistically significant ($p<0.001$). Dunnett's test indicated a significant decrease in shock-induced fighting following the acute dose of propranolol for both doses of drug given when compared to control values ($p<0.05$ for 5 mg/kg; $p<0.01$ for 10 mg/kg). From Day 1 until Day 13 the level of fighting did not change from baseline or differ from the control. However, on Days 13 and 15 (see Fig. 1) the level of shock-induced fighting was markedly elevated from baseline levels. Fighting was significantly greater for the 5 mg/kg propranolol-treated group on both Days 13 and 15 ($p<0.01$). It was significantly greater for the 10 mg/kg group on Day 15 ($p<0.05$). Biochemically, there was a marked increase of approximately 80% in the number of β-adrenergic receptors within the cortices of rats which had received 10 mg/kg of propranolol bid for 15 days. Receptor affinity also increased in the propranolol-treated rats (see Table 3). One should note that had there been sufficient propranolol still present in the system to block the receptors, there would have been an apparent increase rather than a decrease in the K_d.

These results agreed with the findings of Vassout and Delini-Stula [26] and Sheard [20], regarding the acute effect of propranolol. The study also replicated our earlier observation that chronic propranolol administration enhances shock-induced fighting, at least in a period six to eight hours after the last propranolol injection. This study also demonstrated that chronic propranolol administration can induce an increase in β-adrenergic receptors. Taken in conjunction with Experiment 1, however, it appeared that the increase in receptor number was transitory and the number returned to baseline within 48 hours.

TABLE 3

EFFECT OF CHRONIC PROPRANOLOL INJECTIONS* ON β-ADRENERGIC RECEPTORS IN RAT CEREBRAL CORTEX

Group	$K_d \times 10^{10}$ (M)	B_{max} (fmoles/mg prot)
Control	1.83 ± 0.08	38.6 ± 2.5
Propranolol (10 mg/kg bid)	1.20 ± 0.09†	70.5 ± 3.9†

*Rats were decapitated 8 hours after the last injection of propranolol. Values are expressed as mean ± S.E.M.
†$p<0.001$, *t*-test, 2-tailed.

EXPERIMENT 3

This experiment was designed to see whether the facilitating effect of propranolol generalized to other β-adrenergic antagonists and whether the effect was stereospecific for propranolol. We also wished to determine whether any of the experimental treatments would show an increase in shock-induced fighting without an increase in receptor number. The experiment was done in two parts.

METHOD

Animals

These were 80 adult male Sprague-Dawley derived rats, 350–400 g obtained from Holtzman in Madison, WI and housed as described in Experiment 1.

Apparatus

The same as in Experiment 1.

Behavioral Procedure

In the first portion of this experiment eight pairs of control rats and eight pairs of experimental rats received three days of baseline testing for shock-induced fighting. The control group then received one ml of saline bid for 14 days. The experimental group received pindolol (Sandoz, East Hanover, NJ) in a one ml dose of 10 mg/kg bid IP for 14 days. The rats were tested for shock-induced fighting again on days 12–14 six to eight hours after the a.m. dose. Change from baseline in the amount of fighting was compared between the two groups by *t*-test, two-tailed.

In the second portion of the experiment rats (6 pair per group) were divided into four groups. All received three days baseline testing for shock-induced fighting. The control group received one ml of normal saline bid IP for 14 days. The experimental groups received the following in one ml doses: metoprolol 10 mg/kg bid (CibaGeigy, Summit, NJ); d-propranolol 5 mg/kg bid (Ayerst); or l-propranolol 5 mg/kg (Ayerst). On days 12–14 the rat pairs were again tested for fighting behavior. Changes from baseline were analyzed by ANOVA across groups and, if significant, compared to the control with Dunnett's test.

Biochemical Procedure

The rats were sacrificed after testing on the 14th day approximately eight hours after the a.m. dose of propranolol.

TABLE 4

EFFECT OF CHRONIC PINDOLOL INJECTIONS

A. On Shock-Induced Fighting in Rats*†

Group	N (Pairs)	Pre-Injection Attacks/50 Shocks	Post-Injection Day 14 Attacks/50 Shocks	Change In Number of Attacks
Control	8	23.1 ± 1.6	29.3 ± 2.6	+ 6.2 ± 2.1
Pindolol (10 mg/kg bid)	8	18.7 ± 2.7	36.5 ± 2.5	+17.8 ± 3.8‡

B. On β-Adrenergic Receptors in Rat Cerebral Cortex

Group	$K_d \times 10^{10}$ (M)	B_{max} (fmoles/mg prot)
Control	1.96 ± 0.21	52.1 ± 2.6
Pindolol	1.89 ± 0.26	61.6 ± 4.2§

*Rats were fought 6–8 hours following the last injection. Values are expressed as the mean ± S.E.M.
†Rats were decapitated 8 hours after the last injection of pindolol.
‡$p<0.02$, 2-tailed, t-test.
§$p<0.05$, 2-tailed, t-test.

TABLE 5

EFFECT OF CHRONIC β-ADRENERGIC ANTAGONISTS ON SHOCK-INDUCED FIGHTING IN RATS

Group	N (Pairs)	Change in Number of Attacks* Day 14	Day 15
Saline	6	−4.8 ± 1.3	−3.3 ± 1.0
Metoprolol (10 mg/kg bid)	6	−5.6 ± 1.3	−1.9 ± 2.0
d-Propranolol (5 mg/kg bid)	6	−1.0 ± 2.1	−2.4 ± 2.2
l-Propranolol (5 mg/kg bid)	6	+4.4 ± 2.8†	+9.4 ± 1.3†

*Number of attacks for 50 shocks prior to drug injections for the above respective groups was 19.5 ± 1.4, 20.3 ± 1.5, 10.5 ± 1.5 and 14.6 ± 4.2. Rats were fought 6–8 hours after the last injection.
†$p<0.01$ Dunnett's t-test, 2-tailed comparing change in attacks with control.

TABLE 6

EFFECT OF CHRONIC β-ADRENERGIC ANTAGONISTS ON β-ADRENERGIC RECEPTORS IN RAT CEREBRAL CORTEX*

Group	$K_d \times 10^{10}$ (M)	B_{max} (fmoles/mg prot)
Control	2.08 ± 0.24	42.6 ± 5.0
Metoprolol	2.22 ± 0.24	43.1 ± 2.7
d-Propranolol	2.01 ± 0.16	42.1 ± 3.4
l-Propranolol	2.31 ± 0.16	57.6 ± 5.3†

*Rats were injected for 14 days bid with saline, metoprolol (10 mg/kg), d-propranolol (5 mg/kg) or l-propranolol (5 mg/kg). Eighteen hours after the last drug injection rats were decapitated. Values were determined by Scatchard analysis and are expressed as the mean ± S.E.M. for 7–8 animals. Details of the IHYP binding assay are described in the Method Section.
†$p<0.05$, Dunnett's t-test (2-tailed).

Cerebral cortical membranes were prepared and β-adrenergic receptors were measured using IHYP as described in the Method section of Experiment 1. In the first part of Experiment 3, the B_{max} for the control and pindolol groups were compared by t-test. In the second part, the B_{max} data were analyzed with an ANOVA and the groups statistically compared to the control with Dunnett's test.

RESULTS

There was a significant increase in shock-induced fighting with pindolol treatment. The change in fighting was approximately three times the control value ($p<0.02$; see Table 4A). Pindolol also increased the B_{max} for β-adrenergic receptors in these aggressive rats ($p<0.05$; see Table 4B). Metoprolol and the stereoisomer d-propranolol did not increase shock-induced fighting, while l-propranolol the active stereoisomer did (see Table 5, $p<0.01$). Only l-propranolol induced an increase in receptor number (Table 6, $p<0.05$). Thus, the increased fighting was induced only by the stereoisomer of propranolol which also increased the number of β-adrenergic receptors. Increased fighting could also be induced by another β-adrenergic antagonist, pindolol, and in this case it, too, was associated with increased receptor number. Metoprolol failed to bring about a behavioral change with the dose

given and failed, as well, in altering the number of cortical β-adrenergic receptors.

EXPERIMENT 4

Finally, we wished to determine how long the increase in fighting would persist following cessation of chronic propranolol treatment and whether enhanced fighting occurred only during periods when receptor density was increased and receptors were open. In unpublished studies we have noted that the specific IHYP binding for rats injected one hour prior to sacrifice was 10–15% as compared with the 65–70% specific binding for rats injected six to eight hours prior to sacrifice. This is consistent with β-adrenergic receptors being well-occupied one hour after treatment with propranolol, but open at six to eight hours. The findings from Experiments 1 and 2 suggest that we were no longer able to detect an increase in receptor density after 48 hours, but we could at eight hours. These previous experiments suggested that we would see facilitated fighting six hours after drug injection, but not at one hour nor at 48 hours following injection.

METHOD

Animals

These were 32 adult male Sprague-Dawley derived rats of 350–400 g.

Apparatus

The same as in Experiment 1.

Behavioral Procedure

Again, after one week of housing in the laboratory, the rats were ear punched, numbered and randomly paired. They received three days of baseline testing for shock-induced fighting. There was a saline control group (8 pair) which received one ml of isosaline bid, IP, and an experimental group (8 pair) which received 10 mg/kg of propranolol, bid, IP. Injections continued for 14 days. The rats were then fought at 1, 6, 12, 24, 36, and 48 hours following the final injection of drug. Change from baseline levels of fighting were compared by a repeated measures ANOVA. Comparisons during withdrawal were made comparing the drug treated group with the control group by Dunnett's *t*-test.

RESULTS

There was a significant difference between groups over time in the amount of shock-induced fighting ($p<0.01$). Figure 2 illustrates that fighting was markedly increased over baseline levels from 6 to at least 24 hours for the propranolol group. When compared with the control group, this increase was statistically significant at 6 and 12 hours. We attribute the increase in fighting seen at 24 hours within the control group to random variance. As a consequence of this isolated increase in control fighting, we cannot claim that the propranolol fighting effect lasts a full 24 hours.

GENERAL DISCUSSION

The first experiment replicates the earlier reports [25] of dopamine antagonists decreasing shock-induced fighting. The enhanced fighting seen with chronic propranolol administration contrasted with the findings of both Sheard [20] and Vassout and Delini-Stula [26] who used an acute injection

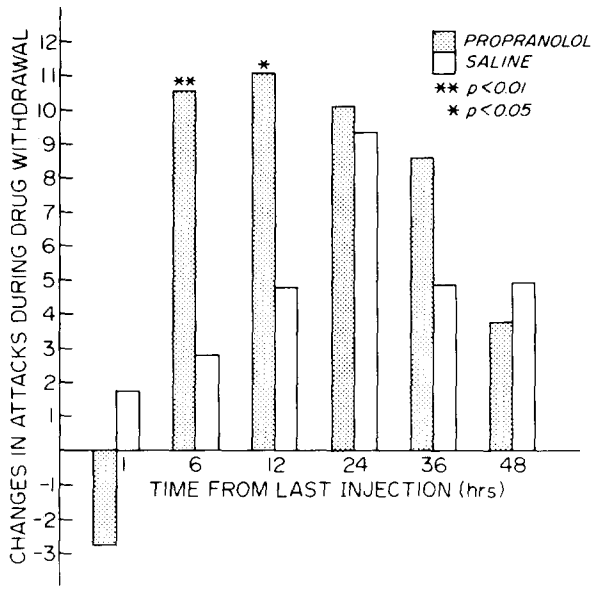

FIG. 2. Effect of withdrawal from chronic propranolol injections on shock-induced fighting in rats. Rats were subjected to footshock as described in the Method section at the various time periods following 14 days of injections bid of 10 mg/kg propranolol or saline. Drug versus control, Dunnett's *t*-test: $*p<0.05$; $**p<0.01$.

paradigm. However, Experiment 2 confirmed their findings that an acute injection of propranolol produced a decrease in shock-induced fighting. This contrasted with the increase in fighting observed after two weeks of treatment seen when rats were tested six to eight hours after injection. The timing of the increased fighting occurs when there is an increase in the number of β-adrenergic receptors which should be "open" due to the relatively brief halflife of propranolol in rats.

The behavioral enhancement of fighting was only seen in experiments where there was a concommitant increase in the number of cortical β-adrenergic receptors. This occurred with pindolol and with l-propranolol. It did not occur with metoprolol in the dose used or with the stereoisomer d-propranolol. The difference in behavioral and biochemical effects between the two propranolol stereoisomers suggests that the effects are not due to a general membrane alteration which is a known effect of both isomers.

The duration of the increased fighting followed the period when β-adrenergic receptors were increased in number, but unoccupied by drug. Both the behavioral effect and the increased receptor number have disappeared by 48 hours after the last injection. The experiments underscore the importance of chronic pharmacological studies to contrast with acute studies. They also affirm the need to understand the brain as a plastic organ with changing characteristics.

The observations of the experiments reported are consistent with the hypothesis that shock-induced fighting can be increased by increasing the number of β-adrenergic receptors in brain, assuming there is little or no concurrent change in adrenergic neurons presynaptically. The absolute number of β-adrenergic receptors, however, is an insufficient predictor of aggressive behavior. Desipramine treatment facilitates shock-induced fighting [7] but also reduces the number of

β-adrenergic receptors [28]. In this case, the tricyclic antidepressant also possesses the uptake blocking properties which may overshadow the changes in receptor number. A hypothesis of facilitated aggressive behavior occurring in the rat secondary to the development of a supersensitivity to endogenously released NE may also explain why this aggressive behavior is not immediately facilitated following neurotoxin treatment (6-hydroxydopa) when amine levels are rapidly lowered, but rather develops slowly beginning on day three and increasing for at least six days following neurotoxin treatment [24].

The findings in this study do not rule out the possibility that the changes in fighting are, in part, elicited by changes in the pain threshold of the rats. It should be noted, however, that rats can show either increased or decreased shock-induced fighting behavior when pain thresholds are lowered [4].

These experiments lend further support to a noradrenergic theory of affective aggressive behavior. Less specific arguments include the observation that subacute administration of tricyclic antidepressants [7], monoamine oxidase inhibitors [7], rubidium [9,21], REM deprivation [8,15], or chronic immobilization stress [13] all facilitate shock-induced fighting and all have been reported to augment central noradrenergic functioning [6]. The disappearance of NE centrally during aggressive behavior in the cat [17] or rat [22] also support this hypothesis. In the human, Brown *et al.* [2] have also reported a positive correlation between aggression scores in military personnel and the CSF levels of the NE metabolite 3-methoxy-4-hydroxyphenolglycol (MHPG).

Lastly, this study does not set out to suggest that the noradrenergic system is the only, or even predominant, system influencing shock-induced fighting in the rat. The serotonergic [12] and cholinergic [18] systems, for example, also appear to be involved with this behavior. Perturbation of β-adrenergic receptors may also initiate feedback effects on other neural networks. Moreover, propranolol and other β-adrenergic antagonists may have pharmacological actions on other systems. For example, Weinstock and Weiss [27] have reported that chronic propranolol treatment is also correlated with serotonergic receptor blockade in isolation-induced aggression in mice. Future studies will be necessary to determine whether β-adrenergic receptor number plays a significant role in other types of aggressive behavior.

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