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Serotonergic Control of Androgen-Induced Dominance

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BONSON, K. R., R. G. JOHNSON, D. FIORELLA, R. A. RABIN AND J. C. WINTER. *Serotonergic control of androgen-induced dominance.* PHARMACOL BIOCHEM BEHAV 49(2) 313–322, 1994.—The present study investigates the role of serotonergic systems in anabolic steroid-induced aggression. An animal model of aggressive dominance was used to assess the chronic effects of testosterone propionate. When rats that had become dominant following administration of testosterone propionate received serotonergic agonists with selectivity for the 5-HT_{1A} receptor (8-OH-DPAT, buspirone, gepirone), the 5-H_{1B} receptor (eltoprazine, TFMPP), or the 5-$HT_{2A/2C}$ receptor (DOM), a dose-dependent decrease in dominance was demonstrated. Pretreatment with three serotonergic antagonists (pizotyline, pirenpirone, and pindolol) blocked agonist-induced reductions in dominance in varying degrees. Nonserotonergic agonists with CNS depressant effects were also tested in dominant animals. The benzodiazepine, chlordiazepoxide, did not reduce dominance except at doses that interfered with motor behavior. The opioid agonist, morphine, dose dependently decreased dominance, but this effect was reversible with administration of the serotonergic antagonist, pirenpirone, suggesting the antidominant effect of morphine had a serotonergic component. Biochemical experiments demonstrated that following chronic testosterone propionate, there was a decrease in levels of 5-HT and 5-HIAA in the hippocampus but not in the striatum or the frontal cortex. Chronic testosterone propionate also caused an increase in the affinity of [^{3}H]8-OH-DPAT for the 5-HT_{1A} receptor but no corresponding change in the density of 5-HT_{1A} binding sites in the hippocampus. There was also no change in the properties of the 5-HT_2 receptor in the frontal cortex following chronic testosterone propionate. These data suggest that serotonergic systems may play an important role in the control of anabolic steroid-induced aggressive dominance.

Aggression Anabolic steroids Testosterone propionate Serotonergic drugs

THROUGHOUT the past decade, there has been an increase in the nonmedical use of anabolic steroids by athletes and body-builders who wish to rapidly increase muscle mass and strength (12,15) and to improve athletic performance (56). In addition, it has been estimated that one-quarter million adolescents in the United States have either tried or are currently using anabolic steroids (5). Chronic self-administration of anabolic steroids by humans has led to the recognition that these drugs can induce alterations in behavior. The most frequently reported behavioral change associated with high-dose, long-term androgen use by humans is that of heightened aggression and irritability (21,38,44,55). Aggression and social dominance in human males have also been correlated with high levels of endogenous testosterone (11,21). Similarly, when animals are chronically exposed to the anabolic steroid testosterone propionate, there are distinct increases in aggressive behavior (1,2,10,47).

At present, the mechanism of action of anabolic steroids in inducing aggression is unclear. However, a decrease in the activity of systems of the central neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) has been experimentally shown to be related to naturally occurring aggression (23,25,48,49). Conversely, when animals were given drugs that increase activity in 5-HT systems, there was a lessening of naturally occurring aggression (6,8,17,39).

The hypothesis that central serotonergic systems are involved in anabolic steroid-induced aggressive behavior is supported by experimental evidence that suggests that an inverse relationship exists between levels of testosterone and serotonin. Chronic administration of testosterone decreases seroto-

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nin content in the diencephalon (28) and can increase the density of 5-HT_{1A} receptors in the hypothalamus (29) as well as decrease the density of 5-HT_3 receptors in the amygdala (30). Conversely, reduction in endogenous testosterone levels through orchiectomy results in an increase in serotonin content of the brain (45), which is reversible with testosterone replacement (46). In addition, castration has paradoxically been shown to both decrease the number of central 5-HT_1 receptors (13) and to cause a supersensitivity to agents that induce behavioral changes characteristic of 5-HT_{1A} agonists (14,27).

With respect to the behavioral relationship between androgens and serotonin, a previous report by our group showed that testosterone-induced aggressive dominance is reduced by the acute administration of the serotonin agonist quipazine (4). This antidominant effect was reversible with serotonergic antagonists. The present study extends this investigation using behavioral and biochemical experiments that systematically address which subtypes of serotonergic receptors may mediate androgen-induced aggression.

ABBREVIATIONS

CDP, chlordiazepoxide; DOM, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC, high performance liquid chromatography; 5-HT,5-hydroxytryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-(di-n-propylamino)tetralin; TFMPP, trifluromethylphenylpiperazine.

METHOD

Animals

Male Fischer 344 rats (120–150 g) purchased from Charles River Breeding Laboratories (Wilmington, MA) were used in all experiments. Animals were housed individually in a controlled environment with free access to water. During behavioral testing, rats were food deprived to maintain 80% of normal body weight. Rats used for biochemical studies were not used in the behavioral studies.

BEHAVIORAL STUDIES

Training to Competition Task

The model of aggression used in this study has been previously described (4) and is a modification of another behavioral method (19). Briefly, rats were individually trained to consume 20 mg sucrose pellets that were automatically dispensed every 20 s into a receiving cup in a test cage (12 × 12 × 10″). After each rat had learned to obtain 30 consecutive pellets from the receiving cup, rats were assigned to permanent pairings on the basis of similar body weight. Each rat pair was then allowed to compete during a 10 min trial for sugar pellets that were delivered into the receiving cup every 20 s. Because the receiving cup is only wide enough to accommodate one rat snout, rats must actively compete to gain access to each sugar pellet. The number of pellets consumed by each rat during each trial was recorded by an observer.

Daily competition trials continued until each rat pair had developed a stable hierarchy of competitive success. A rat was considered dominant in the pair if it consumed an average of 20 or more pellets out of 30 possible pellets per trial over 15 consecutive trials. By default, the other rat in the pair consumed less than 10 pellets over the same period and was designated nondominant. Out of 11 rat pairs trained to perform in the competition task, 1 pair did not meet the initial criterion for a natural dominance hierarchy and was eliminated from the experimental pool of subjects.

Influence of Androgen in Induction of Dominance Behavior

Following the establishment of a stable dominance hierarchy, nondominant rats began to receive 30 mg/kg testosterone propionate (SC) after daily competition trials. Dominant rats in each pair received daily vehicle injections of sunflower oil following behavioral testing. In the present study, as previously demonstrated (4), androgen administration to nondominant rats for 14 days induced a significant increase in dominant behavior in all ten rats which received the steroid. Those nondominant rats that had received testosterone propionate and consistantly obtained the majority of sugar pellets in competition against their paired mates were reclassified as testosterone dominant. Testosterone-dominant rats were used to assess the ability of centrally acting drugs to decrease testosterone-induced aggressive dominance. By default, those rats that had formerly been classified as dominant were reclassified as nondominant in relation to the new androgen-induced dominance hierarchy.

Influence of Centrally Acting Drugs on Testosterone-Induced Dominance Behavior

For each drug trial, only one rat in each pair received a centrally acting drug via intraperitoneal injection; the other rat received vehicle. When all behavioral trials in this phase of the study had been completed, both the testosterone-dominant and the nondominant rats had received each of the drugs in separate trials. Following each test day when one rat in each pair received a dose of a drug, there were two control days when neither rat received an injection. On control days, rats were still run in competition trials to assure the stability of the dominance hierarchy in between drug trials. Throughout the duration of drug testing, testosterone-dominant rats continued to receive 30 mg/kg testosterone propionate daily following competition trials while the nondominant rat in each pair received vehicle. In all cases, testosterone-dominant rats obtained more than 25 of 30 possible sugar pellets on control days.

Measurement of Impairment of Ability to Obtain Pellets

To test whether centrally acting drugs impaired the ability of rats to obtain sugar pellets, rats that had received a drug were immediately given a second 10 min trial in the test cage following the conclusion of the drug trial, but without the presence of their paired mates. An observer recorded the number of pellets each rat consumed in this second trial while also noting other behaviors occuring during that period.

BIOCHEMICAL STUDIES

The hippocampus, the striatum, and the frontal cortex were dissected according to a previously described method (20). One hemisphere of each dissected brain region was used in radioligand binding studies; the other hemisphere was used to measure levels of 5-HT and 5-HIAA. Rats were sacrificed 24 h following the final administration of either testosterone propionate or oil vehicle.

Radioligand Binding Studies

Tissue was homogenized in 50 mM Tris-HCl (pH 7.4) using a Dounce tissue grinder and was centrifuged at 39,000 × *g*

for 15 min at 4°C. The resulting pellets were resuspended in 10 ml of Tris-HCl buffer and were stored at −70°C. At the time of assay, samples were thawed and were incubated at 37°C for 10 min to remove endogenous 5-HT (34). Each sample was then centrifuged at 39,000 × *g* for 15 min. Final pellets were resuspended in Tris-HCl buffer containing 4 mM $MgCl_2$, 10 μM pargyline, and 0.1% ascorbic acid.

Changes in the properties of the 5-HT_{1A} receptor subtype were determined by measuring the binding of [^{3}H]8-OH-DPAT to hippocampal tissue (54). The assays were carried out in a final volume of 0.5 ml consisting of radioligand (0.05 nM to 10 nM), 50 mM Tris-HCl buffer (pH 7.4), or 10 μM 5-HT for determination of nonspecific binding, and 0.4 ml of the tissue suspension. Following a 25 min incubation at 37°C, assays were terminated by vacuum filtration using a Brandel cell harvester, and the filters were washed twice with 5 ml of cold 50 mM Tris-HCl buffer (pH 7.4). Radioactivity was measured by liquid scintillation spectroscopy after incubating the filters in scintillation cocktail overnight. Specific binding was defined as the difference in amount of radioligand binding in the presence and absence of 10 μM 5-HT.

The 5-HT_2 receptors were assessed using [^{3}H]ketanserin (53). The procedure for binding with this receptor subtype was as for the 5-HT_{1A} receptor with the following changes: the brain tissue was frontal cortex, the radioligand was [^{3}H]ketanserin at a concentration range of 0.1 nM to 5.0 nM, and the nonspecific binding was determined as the difference between the presence and absence of 10 μM cinanserin.

Measurement of 5-HT and 5-HIAA Levels

Levels of 5-HT and 5-HIAA were assessed using a modification of a previously described method (24). Hippocampal, striatal, or frontal cortical tissue was homogenized using a polytron tissue grinder in a solution containing 50 mM Tris-HCl buffer (pH 7.4), 0.0336 mM $HClO_4$, and 30 ng/ml *N*-omega-methyl-5-hydroxytryptamine oxalate (NMET) as an internal standard. Homogenates were centrifuged for 15 min at 39,000 × *g* with the resulting supernatents stored at −70°C.

5-HT and 5-HIAA levels in thawed samples were determined using a reverse phase high performance liquid chromatography (HPLC) system with a micro-Bondapak C-18 3.9 × 300 mm column (Waters Associates, Inc.) and a LC-4 amphoteric electrochemical detector (Bioanalytical Systems, Inc.). A mobile phase containing 0.1 M trichloracetic acid, 0.01 M sodium acetate, 0.1 mM EDTA, and 20% methanol (pH 3.85) was used to elute samples from the HPLC column. The flow rate for all tissue samples was 1.2 ml/min. Following column separation, 5-HT and 5-HIAA were oxidized at 0.6 V.

Levels of 5-HT and its metabolite 5-HIAA were determined by quantifying peak height and retention times in comparison to the internal standard. These data were then compared against known standards for 5-HT and 5-HIAA to establish actual concentration of the biogenic amines in each brain region. Levels of 5-HT and 5-HIAA are expressed as ng/mg protein in supernatent solution.

Drugs and Chemicals

Testosterone propionate was obtained from Sigma Chemical Co. (St. Louis, MO) and was dissolved in sunflower oil for injection in a volume of 1.0 ml/kg. The following drugs were used in the acute phase of behavioral experiments: 8-OH-DPAT HBr (Research Biochemicals, Inc., Wayland, MA); buspirone HCl (Bristol-Myers Co., Evansville, IN), gepirone HCl (Bristol-Myers); eltoprazine HCl (Duphar B.V., Weesp, the Netherlands); TFMPP HCl (Aldrich Chemical Co., USA); DOM (National Institue on Drug Abuse, Baltimore, MD); pizotyline (Sandoz Pharmaceuticals, East Hanover, NJ); pirenpirone (Janssen Pharmaceuticals, Beerse, Belgium); (±)pindolol (Sigma); chlordiazepoxide HCl (Hoffman-LaRoche, Inc., Nutley, NJ); morphine (NIDA). Pirenpirone was prepared at a concentration of 0.08 mg/ml and was administered in a volume of 2.0 ml/kg for a final dose of 0.16 mg/kg. Pindolol was dissolved in a minimal quantity of 8.5% lactic acid and diluted with water. All other drugs were dissolved in 0.9% saline and injected at a volume of 1.0 ml/kg. [^{3}H]8-OH-DPAT and [^{3}H]ketanserin were purchased from New England Nuclear, Inc. (Wilmington, DE).

Statistical Analysis

All comparisons of behavioral data were accomplished by means of individual applications of Wilcoxons's signed ranks test. Data from the competition plots were analyzed by nonlinear regression using the IBM-PC version of EBDA-LIGAND (32). For between group comparisons of the radioligand binding measurements and the HPLC measurements, a two-tailed Student's *t*-test was used. Differences were considered significant if they would be expected to arise by random sampling alone with a $p < 0.05$.

RESULTS

Effect of Agonists on Dominant Behavior

5-HT_{1A} agonists. The number of sucrose pellets consumed by testosterone-dominant rats during a competition trial was dose dependently decreased following acute pretrial administration of the 5-HT_{1A} agonists 8-OH-DPAT, buspirone, or gepirone (Fig. 1). In addition to the antidominant effect produced by these three drugs, these compounds also induced a flattening of body posture characteristic of 5-HT_{1A} agonists at the highest doses tested. This effect occurred without interfering with the motor ability of rats to obtain sugar pellets. Indeed, when dominant rats that had received one of the 5-HT_{1A} agonists were run in the second 10 min trial without their paired mates, all rats consumed each of 30 possible sugar pellets. When a flattened body posture was present during competition trials, this effect persisted in the second trial, demonstrating that the effects of the drug had not dissipated. When nondominant rats were tested with 8-OH-DPAT, buspirone or gepirone, there was no significant change in nondominant status.

5-HT_{1B} agonists. Acute pretrial administration of the 5-HT_{1B}/5-HT_{1A} agonist eltoprazine to testosterone-dominant rats resulted in a dose-dependent reduction in aggressive dominance (Fig. 2). When the same rats were tested in the second 10-min trial, each rat obtained every sugar pellet that was dispensed. Nondominant rats that had been given eltoprazine did not show an alteration in performance.

Similarly, TFMPP, a 5-HT_{1B}/5-HT_{2C} agonist, also induced a dose-dependent decrease in the number of sugar pellets consumed by testosterone-dominant rats when administered acutely prior to competition trials (Fig. 2). However, at the highest dose of TFMPP that was tested (8 mg/kg), rats displayed distinct behavioral disturbances that interfered with their ability to obtain sugar pellets. This disruption of behavior occurred during the competition trials as well as during the second 10-min trial. Rats tested with TFMPP were unable to move their rear legs normally, in a manner that has been called hindleg abduction (9). In addition, TFMPP induced a bizarre

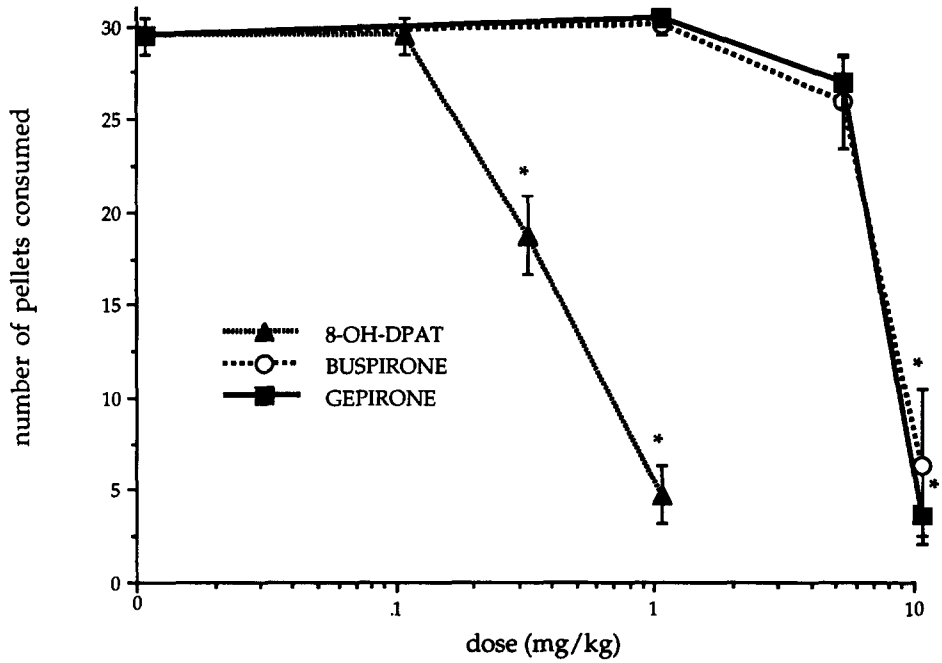

FIG. 1. Effect of 8-OH-DPAT (pretreatment time, 20 min), buspirone (−20 min) and gepirone (−20 min) on dominant behavior in testosterone-dominant rats. Each point represents the mean number of sugar pellets consumed in each trial ± SE (n = 4–6). *$p < 0.05$ when compared to control condition.

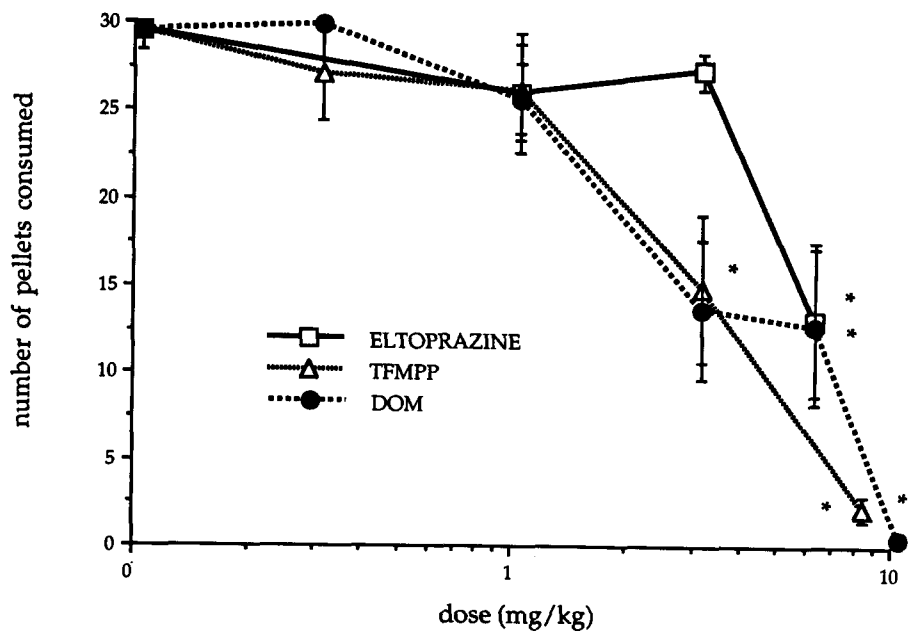

FIG. 2. Effect of eltoprazine (pretreatment time, 30 min), TFMPP (−30 min) and DOM (−20 min) on dominant behavior in testosterone-dominant rats. Each point represents the mean number of sugar pellets consumed in each trial ± SE (n = 4–6). *$p < 0.05$ when compared to control condition.

behavior whereby a rat would make mouth movements as if it were eating something out of its paws when there was not actually anything in its grasp. This behavior has been characterized as purposeless chewing (9). Rats that had been tested with TFMPP also periodically would stop other behaviors and scan the horizon as if they were visually tracking nonexistant objects in the air. The term hallucinogenic pause has been used to describe this behavior (52). Administration of TFMPP to nondominant rats did not cause a change in the number of sugar pellets obtained during a competition trial.

5-HT$_2$ agonist. The 5-HT$_{2A}$/5-HT$_{2C}$ agonist DOM caused a dose-dependent reduction in competition scores in testosterone-dominant rats when administered acutely before behavioral testing (Fig. 2). At the highest dose of DOM that was given (6.0 mg/kg), dominant rats not only did not perform well in the competition task, they also exhibited the purposeless chewing and the hallucinogenic pause that was seen following TFMPP administration. In addition, DOM caused head shakes and contractions of the back muscles in rats, behaviors that are associated with stimulation of the 5-HT$_2$ receptor (9). These behaviors occurred both during the competition trial as well as the second 10-min trial. When DOM was tested in nondominant rats, there was no change in nondominant behavior.

Nonserotonergic agonists. The benzodiazepine, chlordiazepoxide, had no significant effect on competitive behavior when it was administered acutely before behavioral testing to testosterone-dominant rats at doses of 3.0 and 10.0 mg/kg (Fig. 3). However, at 20.0 mg/kg, dominant rats showed a significant reduction in their ability to compete for sugar pellets. At this dose of chlordiazepoxide, rats appeared limp and could not easily move around the test cage. It was this lack of motor ability that interfered with consumption of sugar pellets even in the absence of competition in the second 10-min trial. When chlordiazepoxide was administered acutely in nondominant rats, there was no change in the dominance status of these animals at any of the doses tested.

When the opioid agonist, morphine, was administered acutely to testosterone-dominant rats prior to competition trials, there was a significant reduction in the number of sugar pellets obtained by the animals only at the highest dose tested (6.0 mg/kg). However, during the second 10-min trial, all rats consumed all available sugar pellets at each dose of morphine that was tested. Nondominant rats that had been given morphine did not show a change in competitive performance.

Effect of Antagonists on Agonist-Induced Behavior

Pretreatment with pizotyline significantly reversed the antidominant effect of gepirone (10.0 mg/kg) but not that of 8-OH-DPAT (1.0 mg/kg) or buspirone (10.0 mg/kg) when these agonists were given to testosterone-dominant rats prior to competition trials (Fig. 4). Similarly, pirenpirone antagonized the antidominant effects of gepirone but not 8-OH-DPAT or buspirone. However, when dominant rats were pretreated with pindolol, there was a significant reversal of the antidominant effects of both 8-OH-DPAT and gepirone.

Although pirenpirone significantly reversed the antidominant effect of morphine (6.0 mg/kg), it did not have a similar effect of behavior induced by eltoprazine (6.0 mg/kg) (Fig. 5). Pretreatment with either pizotyline or pindolol caused no significant reversals in the competitive behavior induced by morphine or eltoprazine.

Acute administration of the serotonergic antagonists, pizotyline, pirenpirone, or pindolol, alone to testosterone-dominant rats did not cause a significant reduction in dominance behavior in comparison to control conditions (data not shown).

Biochemical Experiments

Radioligand binding. Following chronic administration of testosterone propionate, there was no significant change in the

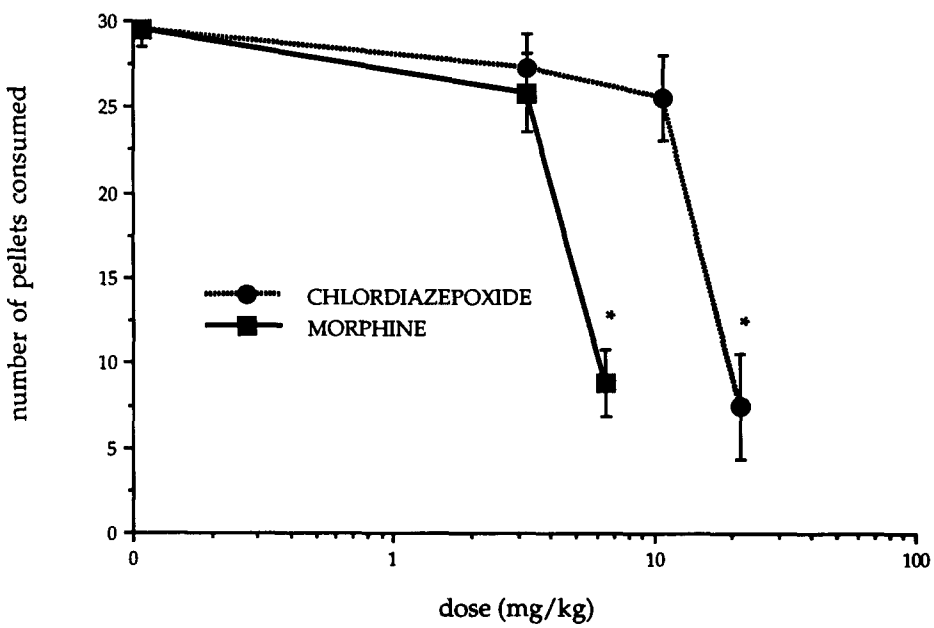

FIG. 3. Effect of chlordiazepoxide (pretreatment time, 20 min) and morphine (−20 min) on dominant behavior in testosterone-dominant rats. Each point represents the mean number of sugar pellets consumed in each trial ± SE (n = 4–6). *$p < 0.05$ when compared to control condition.

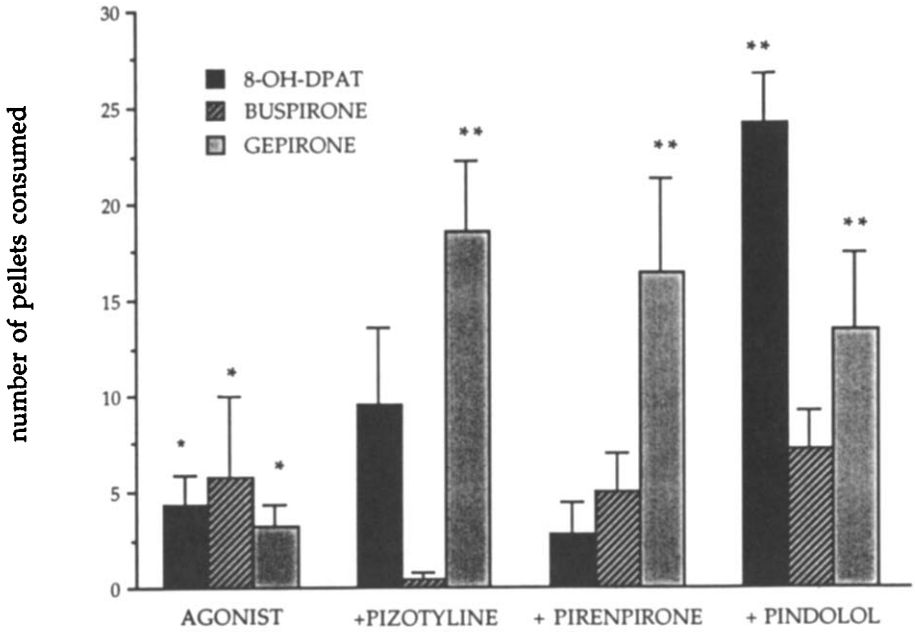

FIG. 4. Effect of the serotonergic antagonists pizotyline (10.0 mg/kg, pretreatment time, 60 min), pirenpirone (0.16 mg/kg, −60 min), and pindolol (5.0 mg/kg, −30 min) on dominant behavior when administered prior to administration of the serotonergic agonists 8-OH-DPAT (1.0 mg/kg, −20 min), buspirone (10.0 mg/kg, −20 min), and gepirone (10.0 mg/kg, −20 min) to testosterone-dominant rats. Bars represent the mean number of sugar pellets consumed in each trial ± SE (n = 4–6). *$p < 0.05$ when compared to control condition, **$p < 0.05$ when compared to agonist alone condition.

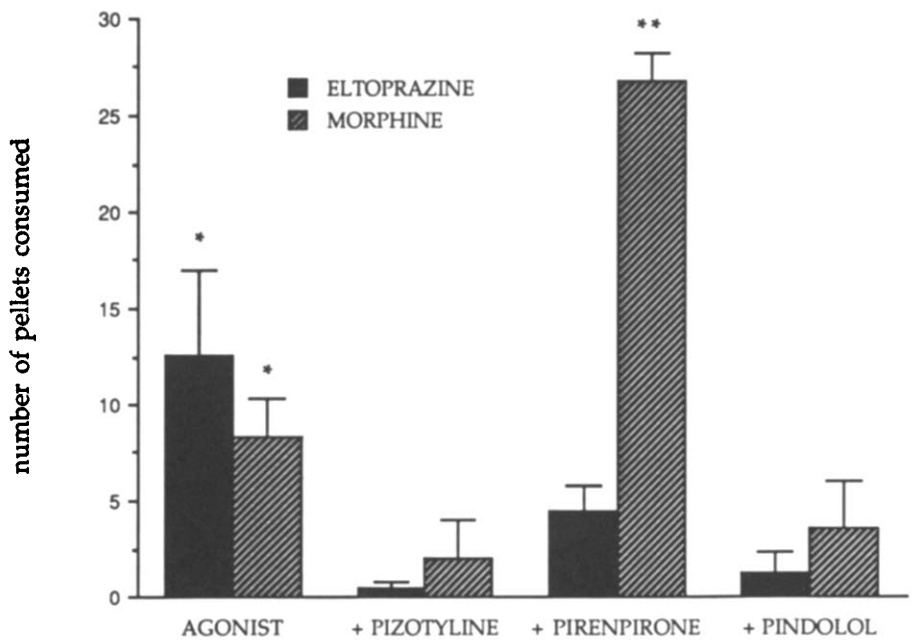

FIG. 5. Effect of the serotonergic antagonists pizotyline (10.0 mg/kg, pretreatment time, 60 min), pirenpirone (0.16 mg/kg, −60 min), and pindolol (5.0 mg/kg, −30 min) on dominant behavior when administered prior to administration of the serotonergic agonist eltoprazine (6.0 mg/kg, −30 min) or the opioid agonist morphine (6.0 mg/kg, −20 min) to testosterone-dominant rats. Bars represent the mean number of sugar pellets consumed in each trial ± SE (n = 4–6). *$p < 0.05$ when compared to control condition, **$p < 0.05$ when compared to agonist alone condition.

B_{max} (density) of 5-HT_{1A} sites in hippocampal tissue (Table 1). However, there was a significant decrease in the K_d of 5-HT_{1A} receptors in the hippocampi of rats that had been treated chronically with the androgen, indicating an increase in affinity. In contrast, chronic exposure to testosterone propionate did not cause a significant change in either the density (B_{max}) or affinity (K_d) of 5-HT_2 sites in frontal cortical tissue.

Measurement of 5-HT and 5-HIAA levels. In the hippocampus, chronic administration of testosterone propionate led to a significant decrease in levels of 5-HT and 5-HIAA (Table 2). However, there was no change in levels of 5-HT or 5-HIAA in the striatum or in the frontal cortex of those animals that had been treated chronically with testosterone propionate.

DISCUSSION

The present data support the contention that there is a serotonergic component to anabolic steroid-induced aggression. When agonists selective for various 5-HT receptor subtypes were given to testosterone-dominant rats, they dose dependently decreased aggression. Biochemical evidence showing a reduction in levels of 5-HT and its metabolite 5-HIAA following chronic administration of testosterone propionate also support a serotonergic component underlying the induction of aggressive behavior. Overall, these results extend initial observations that the serotonergic agonist quipazine can decrease testosterone-induced aggression, and that this decrease is reversible with serotonergic antagonists (4).

That anabolic steroid-induced aggression can be mediated by serotonergic systems is consistent with previous work indicating that other forms of aggression are also functionally related to serotonin. For example, aggressive behavior is induced in both animals and humans when there are decreases in the activity in serotonergic systems (23,48,49). Conversely, increases in the activity of serotonergic systems have been shown to decrease aggressive behavior in animals and humans (6,8,18,39).

Although information is limited regarding which specific subtypes of the serotonin receptor might be involved in aggressive behavior, the 5-HT_{1A} site appears to have a role in the mediation of aggression. Previous investigations with the 5-HT_{1A} agonists 8-OH-DPAT, buspirone, or gepirone have demonstrated that these drugs have the ability to decrease naturally occuring dominance (18,28,51). Similarly, the present study shows that when these drugs were given to testosterone-dominant rats, each of these agents reduced the rats' aggressive dominance in a dose-dependent manner. Additional evidence for a 5-HT_{1A} component is seen in the ability of pindolol, a drug with 5-HT_{1A} antagonistic properties, to reverse the antidominant effects of 8-OH-DPAT and gepirone.

The antagonist data is not completely consistent for all the 5-HT_{1A} drugs tested, however. Although pindolol was not able to reverse the antiaggressive effects of buspirone, as it had for gepirone and 8-OH-DPAT, this may be explained by work showing that pindolol actually acts as a partial agonist at 5-HT_{1A} sites (22,35). This suggests that pindolol may, instead, contribute to a reduction in dominance caused by a 5-HT_{1A} agonist, dependent on the differential binding profile of a particular drug. Similarly, although the 5-HT_2 antagonists pizotyline and pirenpirone did not affect the antidominant effects of 8-OH-DPAT or buspirone, these antagonists were able to reverse the reduction in aggression resulting from administration of gepirone. Although this inconsistency is difficult to explain, other workers have shown that 5-HT_2 antagonists can enhance 5-HT_{1A} agonist-induced behavior (3), suggesting a functional interaction between these two receptors.

A dose-dependent decrease in aggression in testosterone-treated animals was also seen in the present study following administration of eltoprazine, a drug with affinity at 5-HT_{1B}/5-HT_{1A} receptors (41). Eltoprazine has previously been shown to reduce naturally occurring aggression in rats (41). This drug is one of a series of new compounds called serenics which

TABLE 1

EFFECT OF CHRONIC TESTOSTERONE PROPIONATE ADMINISTRATION ON BINDING OF SEROTONERGIC RADIOLIGANDS

	B_{max} (fmol/mg protein)	K_d (nM)
[^{3}H] 8-OH-DPAT		
Control	273 ± 41	2.1 ± 0.2
TP treated	265 ± 48	*1.4 ± 0.1
[^{3}H] ketanserin		
Control	300 ± 44	1.8 ± 0.3
TP treated	242 ± 8	1.5 ± 0.2

Binding assays were performed as described in the Method section. [^{3}H]8-OH-DPAT was incubated at six concentrations ranging from 0.5 to 10.0 nM in hippocampal tissue. [^{3}H]ketanserin was incubated at six concentrations ranging to 0.5 to 10 nM in frontal cortical tissue. Analyses of the saturated data were performed with the program LIGAND (Munson and Rodbard, 1980). Values are the means ± SE (n = 6–7).

*$p < 0.05$.

TABLE 2

CHANGES IN LEVELS OF 5-HT AND 5-HIAA FOLLOWING CHRONIC ADMINISTRATION OF TESTOSTERONE PROPIONATE

	(ng/mg Protein)	
	5-HT	5-HIAA
Hippocampus		
Control	285 ± 13	89 ± 7
TP treated	*211 ± 22	*69 ± 5
Striatum		
Control	230 ± 35	90 ± 6
TP treated	168 ± 23	75 ± 9
Frontal Cortex		
Control	122 ± 11	25 ± 2
TP treated	89 ± 14	20 ± 2

Measurement of 5-HT and 5-HIAA levels was performed as described in the Method section. Brain tissue was homogenized with Tris-HCl buffer, $HClO_4$, and NMET as internal standard. Levels of 5-HT and 5-HIAA were determined using reverse phase high performance liquid chromatography with a micro-Bondapak column and an electrochemical detector. Mobile phase consisted of trichloroacetic acid, sodium acetate, EDTA, and methanol to elute samples from the HPLC column. Values are the means ± SE (n = 7–8).

*$p < 0.05$.

reduce aggression without causing sedation or muscle relaxation (36). Eltoprazine is currently in development for use in patients who exhibit hyperaggressive behavior (41).

In the present study, administration of pizotyline or pirenpirone, antagonists at 5-HT_2 sites, were unable to reverse the effects of eltoprazine, as would be expected. Yet there was also a lack of reversal with pindolol, a drug with 5-HT_{1A}/5-HT_{1B} antagonist effects. Instead, pindolol appeared to cause a trend toward potentiating the reduction in dominance seen following administration of eltoprazine. Based on the partial agonist profile of pindolol at 5-HT_{1A} sites (22,35), this suggests that the antiaggressive effect of eltoprazine may indeed be mediated through the 5-HT_{1A} receptor.

Biochemical data also support a role for the 5-HT_{1A} receptor in the mediation of androgen-induced aggressive dominance. Following chronic administration of testosterone propionate, there were statistically significant decreases in the levels of 5-HT and 5-HIAA in the hippocampus, an area of the brain with a high density of 5-HT_{1A} receptors. These results are consistent with the hypothesis that increased levels of androgens at a dose that induce animal aggression will reduce levels of serotonin. However, chronic administration of testosterone propionate did not cause a concomitant upregulation of the 5-HT_{1A} receptor in the hippocampus in response to reduced levels of 5-HT. This result contrasts with a previous report (29) that showed an increase in the number of 5-HT_{1A} receptors in hypothalamic tissue after animals had received testosterone propionate chronically.

It is possible that the lack of change in the density of 5-HT_{1A} receptors following chronic androgen administration may be due to the resistance of this receptor to regulation. Other workers have found that chronic administration of 5-HT_{1A} agonists fails to cause either up- or downregulation of 5-HT_{1A} receptors (33, 40). Similarly, chronic administration of the female sex hormone estrogen does not alter the number of 5-HT_{1A} receptors in hippocampal tissue (7). These reports raise the possibility that a change in receptor density may not be an important factor in the functional response of the 5-HT_{1A} receptor following long-term exposure to the male sex hormone testosterone.

Two serotonergic drugs that do not have significant affinity at 5-HT_{1A} sites, TFMPP and DOM, were also tested behaviorally for their ability to affect androgen-induced aggression. Both of these drugs were able to significantly reduce testosterone dominance, but only when administered at doses that interfere with general behavior. TFMPP has previously been demonstrated to decrease naturally occurring aggression but only at doses that disrupt motor behavior (28).

The various disturbances in normal behavior that occurred following administration of TFMPP, a 5-HT_{1B}/5-HT_{2C} agonist, or DOM, a 5-HT_{2A}/5-HT_{2C} agonist, consisted of purposeless chewing, the apparent hallucinogenic pause, and backwards walking. It is clear that these unique behaviors were the cause of the decrease in dominance, rather than a specific antiaggressive effect resulting from stimulation of selective receptor subtypes by these drugs. However, it is possible that the behaviors themselves may have arisen from a common biochemical mechanism because both TFMPP and DOM have action at either the 5-HT_{2C} or 5-HT_{2A} receptor subtype and homology has been shown to exist between the genes that encode for these receptors (16). Thus, it seems reasonable to conclude that agonists that act at either 5-HT_{2A} or 5-HT_{2C} receptors would not be useful as drugs that could control anabolic steroid-induced aggression because of the nonselective behavioral side effects.

The biochemical data from the present study also do not support a role for the 5-HT_2 receptor in the mediation of androgen-induced dominance. Chronic administration of testosterone propionate did not cause a statistically significant change in the levels of 5-HT or 5-HIAA in the frontal cortex, a region of the brain with a high density of 5-HT_2 receptors. This treatment also did not cause a change in the K_d or the B_{max} of the 5-HT_2 receptor. This lends additional evidence to the conclusion that the 5-HT_2 receptor does not play a direct role in the control of aggressive dominance induced by anabolic steroids.

Although it seems clear that activation of certain serotonergic systems can decrease anabolic steroid-induced aggression, this antiaggressive effect may not be limited to those drugs that specifically affect serotonergic receptors. In the present study, this possibility was investigated by testing the ability of two central nervous system depressants that lack primary effects on serotonergic systems to decrease aggressive behavior in testosterone-dominant rats.

The benzodiazepine, chlordiazepoxide (CDP), did not cause a reduction in competition scores in testosterone-dominant rats until the highest dose was tested. At that dose, CDP induced a significant decrease in dominant behavior resulting from the inability of rats to move about the test cage. A similar reduction in aggression accompanied by a reduction in motor behavior has been previously described (50). These data suggest that CDP does not cause a specific antiaggressive effect but rather induces generalized neurobehavioral deficits.

The opioid agonist, morphine, induced a dose-dependent decrease in aggression in testosterone-dominant rats without interfering with the ability of rats to move or consume sugar pellets. Although this might suggest the antidominant effect resulted from opioid and not serotonergic activation, several investigators have shown that a functional interaction exists between opioid systems and serotonergic systems in relation to antinociception (31,42). Morphine has also been shown to increase serotonergic metabolism in the cortex (43). Data from the present study with serotonergic antagonists indicate that the antiaggressive effect of morphine could be mediated through a central serotonergic mechanism. Although pindolol and pizotyline were unable to block the antidominant effect of morphine, pirenpirone did significantly reverse the reduction in aggression resulting from morphine administration. This result is consistent with a previous report demonstrating that the antinociceptive effects of morphine could also be attenuated by pirenpirone (37).

In summary, the data in the present study support the hypothesis that anabolic steroid-induced aggressive dominance is mediated by changes in serotonergic systems in the CNS. Specifically, the testosterone-induced alterations in aggressive behavior may be related in part to changes in activity at the 5-HT_{1A} receptor subtype which is found in greatest density in the hippocampus. Although this premise is supported by both behavioral and biochemical data, it does not preclude the involvement of other subtypes of serotonin receptors or mediation by other neurochemical systems in the CNS. These results, however, suggest that 5-HT_{1A} agonists may be useful as drugs that could specifically control aggressive outbursts in humans who have been chronically self-administering anabolic steroids.

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