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Reversal of Testosterone-Induced Dominance by the Serotonergic Agonist Quipazine

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BONSON, K. R. AND J. C. WINTER. *Reversal of testosterone-induced dominance by the serotonergic agonist quipazine.* PHARMACOL BIOCHEM BEHAV 42(4) 809–813, 1992. – Anabolic steroids and other androgens, such as testosterone propionate (TP), have a faciliatory role in the expression of aggressive behavior. Based upon literature indicating an inverse relationship between aggression and the central neurotransmitter serotonin (5-HT), the present study was undertaken to investigate the role of 5-HT in androgen-induced aggression. In this study, an animal model of aggression involving competition between male rat pairs for sugar pellets was used to investigate the effects of TP. When TP was administered daily (30 mg/kg) to nondominant rats, these animals became dominant. Dominant behavior was found to be stable throughout the study with continued daily administration of TP. To test the serotonergic component of TP-induced aggression, the serotonergic agonist 2-(1-piperazinyl) quinolone dimaleate (quipazine) was administered acutely to TP-dominant rats. Quipazine dose dependently reduced aggressive dominance in TP-dominant rats, as well as in naturally dominant rats. When the serotonergic antagonists pirenpirone or pizotyline were coadministered with quipazine to either group of dominant rats, they blocked the effect of quipazine in reducing dominance. However, when 1-[1*H*-Indol-4-yloxy]-3-[isopropylamino]-2-propanol (pindolol), a drug that acts at both β-adrenergic receptors and at 5-HT_{1A} and 5-HT_{1B} receptors, was coadministered with quipazine there was a reversal of the quipazine effect on aggression only in TP-dominant rats. These results indicate that androgen-induced aggression may involve a complex alteration in serotonergic neurotransmission.

Aggression Anabolic steroids Testosterone propionate Quipazine Pirenpirone Pizotyline Pindolol

THE primary androgen produced by the testis is testosterone, a hormone responsible for male secondary sexual characteristics. Over the past 10 years, there has been a dramatic rise in the nonmedical use of synthetic testosterone and its derivatives, known as anabolic steroids (21). Athletes and bodybuilders are the principal users of anabolic steroids in an attempt to quickly increase muscle mass and strength (11).

Although there have been extensive investigations into the effects of androgens on peripheral systems in the body (21), there is a paucity of information regarding the interaction of these hormones with the CNS. The chronic use of anabolic steroids by humans has led to the recognition that these drugs do affect the CNS, as manifested by changes in behavior. This has become especially apparent with the awareness that exogenous androgens can heighten human aggression (14).

Testosterone is an androgen that has been shown to cause aggression in many animal models (1,3,19). Studies with human males have also demonstrated that high levels of plasma testosterone are correlated with aggression and social dominance (5,7). Investigations into naturally occurring aggression have suggested an inverse relationship between aggression and the central neurotransmitter serotonin [5-hydroxytryptamine (5-HT)]. In these studies, an increase in serotonergic neurotransmission lessens aggressive behavior (10,16) while a decrease in serotonin at receptor sites increases aggression (9,17,20). It is therefore plausible that aggression induced by androgens could be mediated by the serotonergic system.

A quantifiable test of animal aggression based upon competition between rats for reinforcers has been previously described (6,10). In this behavioral test, aggressive dominance is measured through the emergence of intragroup rank ordering of animals based upon the ability of rats to compete successfully for the reward. The advantage of this model is objective measurement of aggression, which contrasts with other animal models of aggression based upon a phenomenology of fighting behavior between animals. After a stable dominance hierarchy develops, drugs may be used to determine the pharmacological mechanisms of aggression. Previous experiments using this model have shown that administration of 2-(1-piperazinyl) quinolone dimaleate (quipazine), a serotonergic agonist, to naturally dominant rats causes a dose-dependent decrease in dominant behavior (6,10).

The present experiments examined the effects of testosterone on the CNS. This was done by investigating the ability

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of testosterone propionate to cause an increase in dominant behavior in male rats. Subsequently, the ability of the serotonergic agonist quipazine to reverse testosterone-induced dominance was examined. Finally, serotonergic antagonists were administered in combination with quipazine to determine their ability to block the effects of quipazine on dominance.

METHOD

Male Fischer 344 rats (120–150 g) obtained 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 were then assigned to permanent pairings based upon initial body weight. 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.

Two weeks after arrival, rats were trained to recognize and consume sucrose pellets (20 mg; Noyes Co., Lancaster, PA) through daily exposure to pellets in an easily accessible dish. After rats readily ate the sucrose pellets, they were placed individually into the testing apparatus (Modular Small Animal Cage, Coulbourn Instruments, Inc., Lehigh Valley, PA) each day for training. The test cage (12 × 12 × 10″) is equipped with an automatic pellet-dispensing system that delivers pellets every 20 s into a receiving cup inside the cage. Individual training of rats in the test cage continued until each rat learned to obtain 30 consecutive pellets from the receiving cup.

Following individual training, rat pairs were placed in the test cage for a 10-min competition trial. Sucrose pellets were delivered automatically into the receiving cup every 20 s during the trial. Because the receiving cup is only large enough to accommodate one rat snout, rats must actively compete to gain access to the sugar pellet. An observer recorded the number of pellets consumed by each rat during each trial. Rat pairs received one trial per day until a stable hierarchy of competitive success developed. A rat was considered "dominant" in the pair if it consumed an average of 20 or more pellets 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."

After a stable dominance hierarchy was established, nondominant rats in each pair began to receive 30 mg/kg testosterone propionate (1 ml/kg, SC) dissolved in sunflower oil following daily competition trials. Dominant rats received daily SC injections of oil after behavioral testing. After 14 days, the performance of nondominant rats was assessed to determine whether daily administration of testosterone propionate caused a significant increase in dominant behavior.

Nondominant rats that had a significant increase in dominance following chronic administration of testosterone were reclassified as "testosterone dominant" and used to assess the ability of serotonergic agonists to decrease dominance. Before agonist trials began, each rat in a pair received a saline pretreatment trial to establish a baseline in response to pretrial injection.

For each drug trial, only one rat in each pair received quipazine IP 30 min before behavioral testing; the other rat received saline. At the conclusion of each quipazine trial, the testosterone-dominant rat was left in the test cage without its paired mate for a second 10-min period, during which time sugar pellets continued to be dispensed. This was done to assess whether any decrease in competitive behavior from quipazine was due to a decrease in motor or feeding behavior.

Following each test day when one rat in the pair received a dose of quipazine, there were 2 control days when neither rat received an injection. Throughout the duration of testing with quipazine, testosterone-dominant rats continued to receive 30 mg/kg testosterone propionate following daily competition trials while the other rats in each pair received oil injections.

As a positive control, quipazine was also tested in rat pairs in which neither animal was receiving testosterone propionate. The procedure was identical to that for testosterone-receiving rats except the dominance hierarchy in these rat pairs had developed and was maintained without administration of an androgen.

Several serotonergic antagonists were administered separately to determine whether the effects of quipazine on dominant behavior could be reversed. The serotonin antagonists pizotyline (BC-105) and pirenperone were given IP with a 60-min pretreatment time. The β-adrenoceptor antagonist 1-[1*H*-Indol-4-yloxy]-3-[isopropylamino]-2-propanol (pindolol) was also tested because of its antagonistic activity at both 5-HT_{1A} and 5-HT_{1B} sites (8,13,18). Other researchers have found that the combination of quipazine with another β-adrenergic/serotonergic antagonist, propranolol, was able to reduce shock-elicited fighting in rats (15). Pindolol was administered IP 30 min before behavioral testing. The antagonists were given both alone and in conjunction with 3 mg/kg quipazine (IP, 30 min pretreatment time).

Testosterone propionate and pindolol were purchased from Sigma Chemical Co. (St. Louis, MO). Quipazine was purchased from Research Biochemicals Incorporated (Wayland, MA). Pirenpirone was donated by Janssen Pharmaceuticals (Beerse, Belgium). Pizotyline (BC-105) was donated by Sandoz (Hanover, NJ).

The results were analyzed by the Wilcoxon's signed-ranks test for paired observations and are expressed as the mean with variation given in terms of the SE. Differences between groups were considered significant if they would be expected to arise by random sampling alone with $p < 0.05$.

RESULTS

Figure 1 shows that daily administration of testosterone propionate (30 mg/kg) to nondominant rats for 2 weeks significantly increased the acquisition scores of these rats in the competitive task. By default, the competitive score of the other rat in the pair that did not receive testosterone propionate decreased (data not shown).

Specific behavior of those rats receiving testosterone propionate while in the competition cage varied from trial to trial. On some occasions, a testosterone-dominant rat would attempt to obtain each sugar pellet before its paired mate could through rapid head-weaving with the other rat while animals were in front of the receiving cup. On other occasions, a testosterone-dominant rat would engage in more classic aggressive phenomenon such as initiating upright fighting or by maneuvering the other rat onto its back in a defeat position.

Since the weight of rats in each pair were always within 10 g of each other, the increase in dominance in testosterone-receiving rats was not due to an increase in body mass. With continued administration of the drug throughout the remainder of the experiment, testosterone-receiving rats further increased the percentage of sugar pellets obtained in an average trial. Thus, the reversal of the natural dominance hierarchy by testosterone propionate was found to be stable over time with chronic administration of the drug.

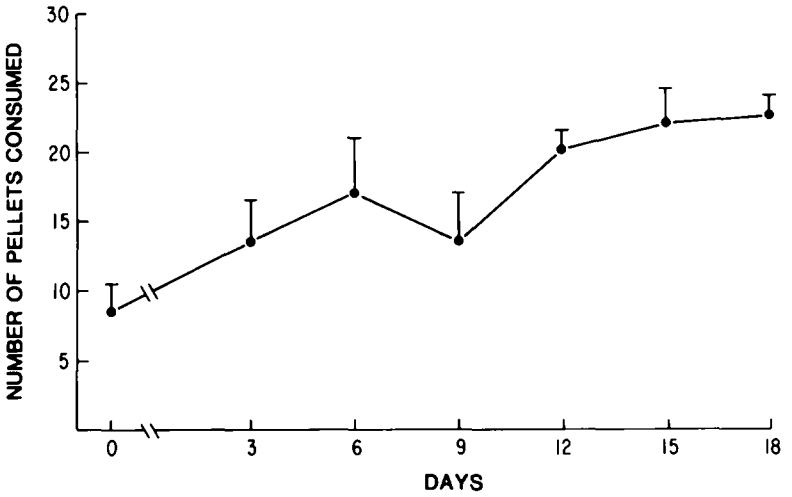

FIG. 1. Reversal of natural dominance hierarchy by daily administration of testosterone propionate (30 mg/kg) to nondominant rats. Each point represents the mean number of sugar pellets consumed in each trial ± SE (n = 5).

Acute administration of quipazine to testosterone-dominant rats caused statistically significant decreases in competitive scores at doses of 1.0 and 3.0 mg/kg (Fig. 2). When quipazine was administered to the other rat in each pair that was not receiving testosterone propionate (and was not behaving dominantly), there was no change in performance in the competition trials (data not shown). Similar to the results with testosterone-dominant rats, pretrial injection of quipazine to naturally dominant rats caused a dose-dependent decrease in competitive success (Fig. 2). Significant decreases in dominant behavior were found at doses of 0.3, 1.0, and 3.0 mg/kg quipazine. When naturally nondominant rats were given quipazine, they continued to behave unaggressively (data not shown). Although rats that had been administered quipazine did not compete well, they did readily consume sugar pellets once the other rat in each pair had been removed from the test cage.

The effects of serotonergic antagonists on quipazine-induced behavior are seen in Fig. 3. Both pizotyline (BC-105) (10.0 mg/kg) and pirenpirone (0.16 mg/kg) significantly reversed the effects of quipazine (3.0 mg/kg) on dominance in both testosterone- and naturally dominant rats. However, when the selective 5-$HT_{1A/1B}$ antagonist pindolol (5.0 mg/kg) was given in combination with quipazine (3.0 mg/kg) there was a statistically significant reversal of the effects of quipazine in testosterone-dominant rats. This contrasts with the combined effect of quipazine and pindolol in naturally dominant rats, where the reversal was only partial and nonsignificant.

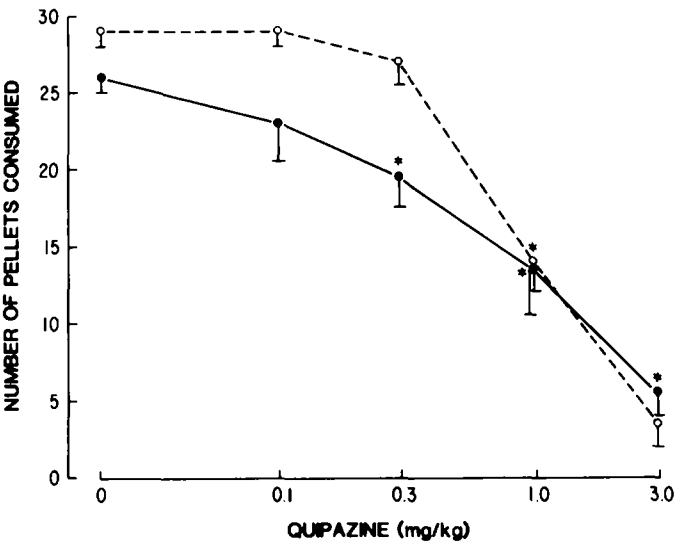

FIG. 2. Effect of quipazine on dominant behavior in testosterone-dominant (○) and naturally dominant (●) rats. Each point represents the mean number of sugar pellets consumed in each trial ± SE (n = 10). *$p < 0.05$ when compared to control group.

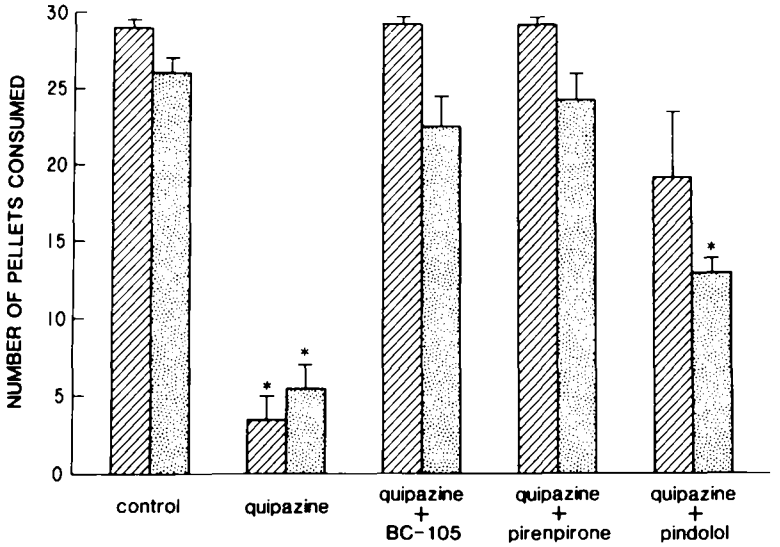

FIG. 3. Effect of the serotonergic antagonists pizotyline, pirenpirone, and pindolol, when coadministered with quipazine on aggressive behavior in testosterone-dominant (hashed bars) and naturally dominant (speckled bars) rats. Bars represent the mean number of sugar pellets consumed in each trial ± SE (n = 5). *$p < 0.05$ when compared to control group.

Administration of the serotonergic antagonists pizotyline (BC-105) (10.0 mg/kg) or pirenpirone (0.16 mg/kg) alone did not have any effect in reducing competitive success in either testosterone- or naturally dominant rats (data not shown). When pindolol (5 mg/kg) was given alone to testosterone-dominant rats, there was also no change in dominance levels. However, administration of 5 mg/kg pindolol to naturally dominant rats produced a small but significant decrease in competitive success when compared to control values (20.5 ± 3.5 vs. 26.0 ± 1.0).

DISCUSSION

This study was undertaken to investigate the effect of testosterone propionate on the central serotonergic system. To address this, behavioral changes following daily injections of the hormone were assessed.

The present data show that chronic administration of testosterone propionate to previously nondominant rats causes them to behave dominantly in a competitive model of aggression (Fig. 1). The competitive model was chosen for these experiments because it permits objective measurement of changes in aggressive behavior. Because the primary focus of this study is the effect of androgens on the CNS rather than aggression per se, occurrences of conventional aggressive phenomenon were noted but not scored for statistical significance.

The results from this experiment are consistent with data from other studies using a broad range of behavioral tests indicating that testosterone is positively correlated to increases in aggressive behavior (1-3,5,12). This would seem to validate the use of the competitive model as a nonsubjective method of studying the effects of androgens on aggression. Anecdotal evidence from athletes who chronically self-administer anabolic steroids also suggests that increasing androgen levels leads to an increase in human aggression (14).

At present, the mechanism of action of androgens in inducing aggression is unclear because there is little available data on the effects of these drugs on the CNS. However, an extensive literature exists that is supportive of a theory suggesting a connection between naturally occurring aggression and the central neurotransmitter serotonin. In a variety of animal models, a decrease in serotonin transmission has been correlated with an increase in natural aggression or dominance (10,17,20). Thus, it is plausible that androgen-induced aggression may be caused in part by a prolonged reduction in serotonin transmission. If this were the case, administering a drug that increases serotonin transmission should reduce aggression.

The present experiments show that administration of the serotonergic agonist quipazine to testosterone-dominant rats dose dependently reduces levels of aggression in the competitive model (Fig. 2), presumably through a direct increase in serotonin transmission. This effect occurred without notable changes in motor or feeding behavior. As expected, administration of quipazine to nondominant rats did not alter their competitive behavior since this group of rats normally functioned with low levels of aggression.

When the same experiments were conducted with naturally dominant rats, the response was similar to that with testosterone-dominant rats (Fig. 2). Quipazine dose dependently caused a decrease in the aggression of naturally dominant rats but did not alter the behavior of nondominant rats in the competitive model.

If the effects of quipazine on androgen-induced aggression are specifically related to its action on the serotonergic system, these effects should be reversible through coadministration with serotonergic antagonists. When the serotonin antagonists pizotyline (BC-105) or pirenpirone were given in conjunction with quipazine, the antiaggressive effects of quipazine in both testosterone- and naturally dominant rats were completely blocked (Fig. 3). Neither of the antagonists had any effect

when given alone to either group, suggesting that they are acting as pure antagonists. The similarity of response by the two groups of dominant rats to serotonergic drugs indicates that aggression induced by administration of testosterone propionate is directly related to changes in the serotonergic system.

When pindolol, an antagonist at both 5-HT and β-adrenergic receptors, was administered with quipazine, there was a difference in response between the two groups of dominant rats. In testosterone-dominant rats, coadministration of pindolol and quipazine significantly reversed the antiaggressive effects of quipazine (Fig. 3). Yet, when the same drug combination was given to naturally dominant rats there was only a partial, nonsignificant reversal in the reduction of aggression induced by quipazine. This difference may be explained by examining the effects of pindolol alone. When pindolol was administered by itself to testosterone-dominant rats, there was no effect on aggressive behavior. However, when pindolol was given alone to naturally dominant rats it caused a slight but significant decline in levels of aggression. Because pindolol has been demonstrated to act as a mixed serotonin agonist-antagonist (4,9), it is not unexpected that it would show some of the antiaggressive effects of a serotonin agonist. That these effects were only present in naturally dominant rats suggests that chronic administration of testosterone propionate decreases sensitivity to the effects of pindolol. This may be yet another indication that androgens have a direct effect on the serotonergic system.

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