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Effects of Yohimbine on Isolation-Induced Aggression, Social Attraction, and Conspecific Odor Preference in Mice

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KEMBLE, E. D., M. BEHRENS, J. M. RAWLEIGH AND B. M. GIBSON. *Effects of yohimbine on isolation-induced aggression, social attraction, and conspecific odor preference in mice.* PHARMACOL BIOCHEM BEHAV **40**(4) 781–785, 1991.— Yohimbine treatment inhibited isolation-induced attack in mice but had no effect on defense. The drug also increased social distances and produced a transient decrease in preference for conspecific male odors. The antiaggressive actions of yohimbine parallel those reported for the anxiogenic β-carbolines and for phenylpiperazine "serenic" agents. The results emphasize the importance of supplementing conspecific agonistic encounters with additional behavioral measures such as nonagonistic social attraction in evaluating antiaggressive drugs. The decreased responsiveness to conspecific odors seen in Experiment 3 also suggests that increased conspecific avoidance may be mediated, in part at least, by altered olfactory processes.

Yohimbine Attack Defense Social attraction Odor preference

VARIOUS forms of resident-intruder aggression are used extensively in the evaluation of putative antiaggressive drugs such as the recently synthesized family of phenylpiperazine compounds (DU 27716, 27225, 28412, 28853). These serotonergic agonists uniformly inhibit resident-intruder, isolation-induced and maternal aggression in rodents while leaving defensive behavior unchanged or increased [e.g., (4, 15, 25–27)]. Further, the drugs may also actually increase social investigatory behaviors such as conspecific sniffing. The selective inhibition of attack, paired with normal or enhanced social behavior, has led to their characterization as "serenics." Although this pattern of results is promising, such agonistic encounters provide little information about the sensory and emotional/motivational mechanisms mediating their effects. In previous research with one of these compounds (DU 27716), for example, we found that the drug produced rather robust anxiogenic effects (20, 22, 24, 31). If the antiaggressive actions of DU 27716 are mediated in part by increased fearfulness, then other known anxiogenic compounds might be expected to have similar effects. The adrenoceptor antagonist yohimbine displays a consistent anxiogenic profile in a wide range of animal testing paradigms [e.g., (6, 9, 11, 16, 17)] and in humans (5) and thus seemed a promising candidate for further study. The present experiments examine the effects of this drug on conspecific aggression and some possible mechanisms of action.

EXPERIMENT 1

Experiment 1 assessed the effects of yohimbine on intermale attack induced by social isolation. If, as our earlier results suggest, drugs which potentiate fearfulness also inhibit attack but not defense, then one might expect yohimbine to produce antiaggressive effects similar to those of DU 27716.

METHOD

Subjects and Apparatus

The subjects were 72 male CD-1 albino mice weighing 29.9–42.2 g at the time of the experiment. After weaning the mice were housed in groups of 4–8 in glass aquaria with a sawdust substrate and ad lib access to Purina Lab Chow and water. Five days prior to testing 36 mice (residents) were individually housed in $38 \times 20 \times 25$ cm glass aquaria equipped as described above. The remaining 36 mice served as intruders and were group housed until the time of testing. Intruders weighed 3.9–5.6 g less than the residents with whom they were tested. Testing was conducted in the residents' aquaria under incandescent illumination (48 ft.c). All behaviors were videorecorded for later analyses.

Procedures

The 36 resident mice were randomly assigned to weight-balanced groups ($N = 9$) designated to receive Low (0.5 mg/kg), Medium (1.0 mg/kg), or High (2.0 mg/kg) doses of yohimbine or an equivalent volume of isotonic saline (Saline Group) by intraperitoneal injection. The yohimbine dosages selected have been shown by others [e.g., (9, 16, 30)] to produce clear anxiogenic effects. Immediately after injection, the mice were re-

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turned to their home aquaria. Thirty min later an intruder was introduced into the aquarium and the behavior of resident and intruder recorded for 15 min.

Behavioral Measures

Lateral attack and chasing behaviors were summed to yield overall measures of both the frequency and duration of offensive attack behavior. Fleeing, defensive upright and boxing were combined to form frequency and duration indices of defensiveness. The frequency and duration of two social investigatory behaviors (nose-to-nose sniffing, anogenital sniffing) were also analyzed. Because 10 of 27 drug-treated mice showed little or no agonistic behavior, overall group comparisons were carried out by means of Kruskal-Wallis analyses of variance and individual group comparisons by U-tests.

RESULTS

The results of yohimbine on offensive and defensive behaviors are summarized in Fig. 1. It can be seen (upper panel) that drug treatment strongly suppressed the frequency of offensive behaviors ($H = 14.20$, $p < 0.005$). Individual comparisons revealed that both Low, $U(9,9) = 11$, $p < 0.05$, and High, $U(9,9) = 3$, $p < 0.002$, but not Medium ($p > 0.10$) groups differed significantly from the Saline Group. Similarly, yohimbine treatment produced a highly significant overall decrease in the duration of offensive behaviors ($H = 14.49$, $p < 0.005$) with Medium, $U(9,9) = 17$, $p = 0.05$, and High, $U(9,9) = 3$, $p < 0.002$, but not Low ($p > 0.10$) doses differing significantly from Saline Control levels. Although there appeared to be some decrease in defensive behaviors at the highest dosage (lower panel, Fig. 1), there were no statistically reliable drug effects on either the frequency or duration of these behaviors ($ps > 0.10$). Additional analyses also examined possible drug effects on defensive behaviors when they were expressed as a percentage of total social interactions (offense + defense + sniffing). These comparisons also failed to reveal statistically significant drug effects on either frequency or duration measures ($ps > 0.10$).

The frequency and duration of nose-to-nose and anogenital sniffing are summarized in Fig. 2. It can be seen (upper panel) that yohimbine treatment produced a marked overall increase in the frequency of nose-to-nose sniffing ($H = 14.63$, $p < 0.002$). Both the Low, $U(9,9) = 15$, $p < 0.05$, and High, $U(9,9) = 5$, $p < 0.002$, doses significantly increased sniffing over control levels while the Medium dose produced a marginally significant increase, $U(9,9) = 18$, $p < 0.10$. There was also a highly significant increase in the duration of nose-to-nose sniffing following yohimbine treatment ($H = 13.88$, $p < 0.003$). Individual comparisons revealed that the Low, $U(9,9) = 13$, $p < 0.02$, and High, $U(9,9) = 7$, $p < 0.002$, doses differed significantly from Saline levels while the Medium dose produced a marginally significant evaluation, $U(9,9) = 18$, $p < 0.10$. Although the frequency and duration of anogenital sniffing (lower panel, Fig. 2) seemed to be elevated by the highest yohimbine dose, the data from this group was quite variable and there were no statistically significant drug effects ($ps > 0.10$).

EXPERIMENT 2

The selective suppression of offensive attack behaviors by yohimbine noted in Experiment 1 were presumably mediated by its well-documented anxiogenic effects. This interpretation is quite consistent with the strong suppression of attack noted following other fear-inducing manipulations such as footshock (14) or mere exposure to a potential predator (3). If this analysis is correct, then yohimbine might also be expected to increase mutual avoidance among nonaggressive conspecifics as well. Utilizing a simple index of social gregariousness [e.g., (23)], we have found that the antiaggressive actions of DU 27716 (22,24) and DU 28853 (19) are also accompanied by increased social distances. Moreover, several anxiogenic compounds (including yohimbine) have been shown to decrease, while anxiolytics increase, interactions in a somewhat different test of social reactivity [e.g., (10, 12, 16)]. This experiment therefore examined the effects of yohimbine on the average interanimal distances maintained by pairs of drug- or saline-treated mice. Nonlactating female mice were selected for this experiment to reduce levels of agonistic interaction between saline-treated mice.

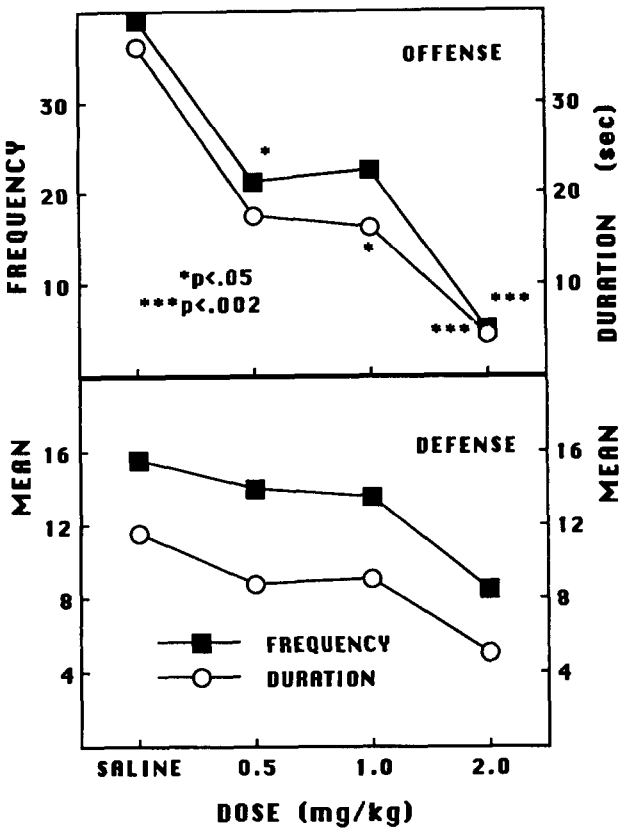

FIG. 1. Mean total frequency and duration of offensive (upper panel) and defensive (lower panel) behaviors by saline- and yohimbine-treated residents.

METHOD

Subjects

The subjects were 30 pairs of female CD-1 albino mice weighing 21.1–40.0 g. The subjects were housed in groups of 8–10 with ad lib access to Purina Lab Chow and water. Pairs of mice differed by no more than 6.0 g in body weight and were randomly designated to receive Low (0.5 mg/kg, $N = 8$) or High (2.0 mg/kg, $N = 8$) doses of yohimbine or an equivalent volume of saline ($N = 14$).

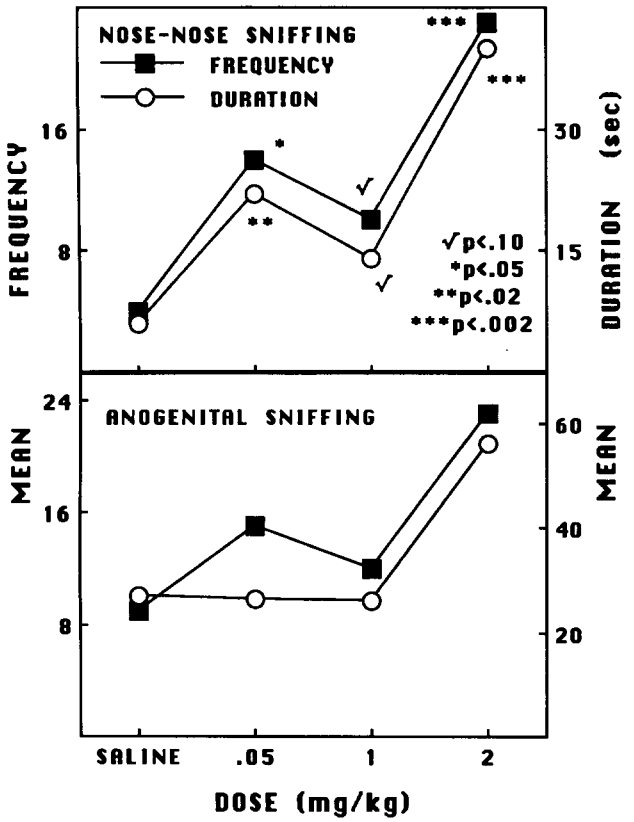

FIG. 2. Mean total frequency and duration of nose-to-nose (upper panel) and anogenital (lower panel) sniffing by saline- and yohimbine-treated residents.

Apparatus and Procedures

Testing was conducted in a 64.0 × 10.0 × 10.0 cm linear runway which contained a 28.0 × 10.0 × 10.0 cm startbox at either end separated from the runway by a sliding door. The floor and walls of the runway and startboxes were of flat black plywood, and the ceiling was of clear Plexiglas. The floor of the startboxes and runway were divided into numbered 10.0 cm intervals by thin white lines. Interobservation intervals were timed by stopwatch.

The mice were initially adapted to the apparatus for 10 min/day for two days. Prior to testing, both members of a pair received identical dosages of yohimbine or saline by intraperitoneal injection and were individually housed for thirty min. The members of the pair were then placed in opposite startboxes for 20 s and both sliding doors opened simultaneously. The distance separating the two mice was then recorded at 15-s intervals during the 10-min test.

RESULTS

One (saline-treated) pair of mice displayed brief episodes of tail rattling and lateral attack posture without biting. No other aggressive behavior was observed. When compared to the Saline Group (mean = 34.5 cm), both Low (mean = 40.8 cm) and High (mean = 40.5 cm) doses of yohimbine increased mean interanimal distances. At the highest dosage, however, autogrooming and persistent floor-sniffing were noted in four of eight pairs and considerably increased variability. Analysis of variance, therefore, yielded only a marginally significant overall drug effect, $F(2,27) = 2.82$, $p < 0.10$. When attention was restricted to the lower dose, however, significant group differences emerged, $t(20) = 2.22$, $p < 0.05$.

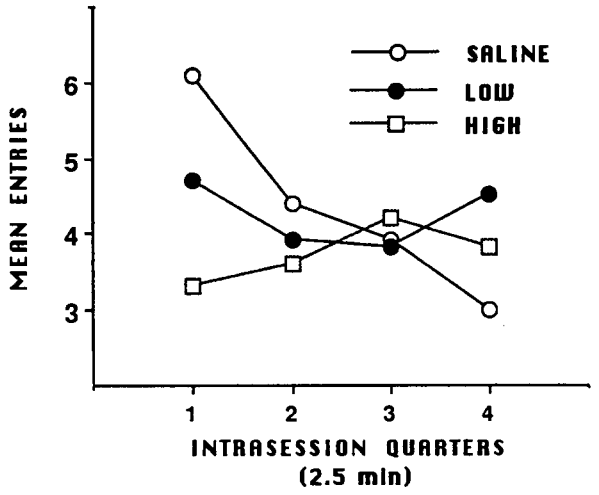

FIG. 3. Mean entries into conspecific odor compartment by saline- and yohimbine-treated mice.

EXPERIMENT 3

The drug-induced increase in nose-to-nose sniffing noted in Experiment 1 suggests an olfactory component in the drug's effects on aggression and social attraction. Indeed, the antiaggressive effects of DU 27716 are also accompanied by increased conspecific sniffing [e.g., (4)] and altered olfactory preference and discrimination (21,35). Experiment 3, therefore, examined the effects of yohimbine on preference for conspecific odors.

METHOD

Subjects

The subjects were 24 male albino CD-1 mice weighing 36.8–46.4 g. The mice were individually housed in 25.0 × 19.0 × 13.0 cm stainless steel cages containing a sawdust substrate and ad lib Purina Lab Chow and water. Prior to testing the mice were divided into three weight-balanced groups of 8 mice designated to receive Low (0.5 mg/kg) or High (2.0 mg/kg) doses of yohimbine or an equivalent volume of isotonic saline (Saline).

Apparatus

Testing was conducted in a 50.4 × 22.0 × 31.0 cm chamber divided into two equal compartments by a partition containing a 7.0 × 10.0 cm opening at floor level. The walls and ceiling of the apparatus were constructed of clear Plexiglas and the floor of 1.3 cm hardware cloth. The chamber floor was elevated 5.0 cm from the base of the apparatus and odorous materials were placed in a 47.0 × 21.0 × 1.0 cm aluminum tray placed beneath the floor. Conspecific odors were provided by sawdust bedding which had been soiled for 15 days by a group of 10–12 male

mice housed in a 45 × 24.0 × 21.0 cm polycarbonate cage containing a 4.0 cm sawdust substrate. Unsoiled sawdust bedding was utilized as the second odor. The compartment containing the soiled bedding was alternated after each behavioral test and the apparatus and aluminum tray thoroughly cleaned between trials. The apparatus was housed in a semiacoustic chamber and observations carried out from an adjacent room. Number of entries into the odorous compartment was recorded for four consecutive 2.5-min intervals by 28-V DC programming equipment and durations timed by stopwatch.

Procedure

The subjects were adapted to the apparatus for 15 min/day for three days. On the fourth day mice received the appropriate drug or saline injection and were immediately returned to their home cages for 30 min. Five min prior to the beginning of the test 250 ml of soiled and unsoiled bedding material were spread evenly beneath the apparatus floor over the most distal 18.0 cm of the aluminum tray at opposite ends. The mouse was then placed in the center of the compartment containing unsoiled bedding and number of entries into the compartment containing soiled bedding and time spent in this compartment recorded during the 10-min test.

RESULTS

Mean number of entries into the compartment containing male conspecific odor by drug- and saline-treated groups are summarized in Fig. 3. Yohimbine treatment strongly suppressed entries into the odorous compartment during the first 2.5 min but seemed to have no effect during the remainder of the test. This impression is supported by a highly significant treatment × trials interaction in the analysis of variance, $F(6,84) = 4.69$, $p<0.005$. Individual comparisons revealed that both yohimbine doses decreased entries during the first interval when compared to saline treatment ($ps<0.01$) and that the High dose produced significantly greater suppression than the Low dose ($p<0.05$). Drug treatment had no statistically reliable effect on duration measures whether expressed as totals or corrected for entrance frequency and indexed as mean duration per visit to the odorous compartment ($p>0.10$).

GENERAL DISCUSSION

The present data reveal that the anxiogenic actions of yohimbine are accompanied by a selective reduction in offensive attack during agonistic encounters. It is also interesting to note that the benzodiazepine inverse agonist FG 7142 is also a well-characterized anxiogenic agent (8, 11, 12) and has been reported to decrease aggressive behaviors in rats (2) and to increase timidity in mice (33). As previously mentioned, attack behavior is also strongly suppressed by either painful electric shock (14) or exposure to a potential predator (3). Taken together, the above data suggest that a variety of pharmacological or behavioral manipulations, all having in common the induction of fearfulness, may also mimic the selective antiaggressive effects of the "serenics." Such findings caution against an overreliance on conspecific aggressive encounters in the assessment of putative antiaggressive drugs and underscore the need to examine possible anxiogenic effects. Experiment 2 suggests that measures of social attraction between nonaggressive conspecifics may be useful in addressing this question. Although significant drug effects were noted only at the lowest dose, the data clearly support at least some decrease in social attraction following yohimbine treatment. Similarly, we have found that both DU 27716 and 28853 increase avoidance of both conspecifics and nonconspecifics in the absence of overt aggression (19, 22, 24). Utilizing somewhat different procedures, others also report a rather consistent decrease in social interaction following treatment with various anxiogenics and increases following anxiolytics (9, 12, 16). It must be noted, however, that aggressive acts, though relatively infrequent, are commonly reported in the latter studies and may obscure their interpretation to some extent. We believe that some form of social interaction test which minimizes or eliminates aggressive behavior provides a somewhat more sensitive measure of drug-induced changes in social attraction.

Experiment 3 also indicates that yohimbine decreased preference for conspecific odors. Since the effect was transient, it is possible that olfaction plays a relatively minor role in its antiaggressive effects. It should be noted, however, that the odor donors for this experiment were members of a stable colony in which agonistic behavior was relatively brief and infrequent. It seems possible that odors emitted during an active social engagement might be more salient and strongly affected by the drug. The fact that exposure to the odors of dominant conspecifics produces potent hypoalgesia (29,36) is generally consistent with this suggestion. Further, Dixon (7) suggests that the anxiolytic effects of diazepam are importantly mediated by olfaction. Thus olfactory mediation may be important in both anxiolytic and anxiogenic drug actions. Alternatively, however, the fact that yohimbine decreased odor compartment entries, but not duration, might be argued to simply reflect drug-induced hypoactivity. If so, it is difficult to see why yohimbine increases both nonreinforced and punished lever pressing (18, 30, 32) and number of entries into a novel open field (28). Thus, although some situation-specific hypoactivity remains a possible explanation for the results of Experiment 3, this seems unlikely. It is also possible, of course, that altered reactivity to odors is secondary to some more general drug effect such as increased arousal. Nevertheless, the fact that both yohimbine and DU 27716 inhibit attack, increase conspecific sniffing, and alter performance in olfactory preference tasks must be viewed as suggesting that olfactory processes may be involved in the behavioral effects of both. More importantly, the central role of olfaction in normal murine social and aggressive behavior [e.g., (1, 13, 34)] further suggests that this sensory system may be an important site of action for a wider range of anxiogenic and anxiolytic agents as well. This possibility is now under investigation.

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