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BRIEF COMMUNICATION

Effects of Clonidine and Yohimbine on the Social Play of Juvenile Rats

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NORMANSELL, L. AND J. PANKSEPP. *Effects of clonidine and yohimbine on the social play of juvenile rats.* PHARMACOL BIOCHEM BEHAV 22(5) 881–883, 1985.—The social play of juvenile rats was observed following administration of either the α-2-adrenergic agonist clonidine (0.1, 0.5, 1, 5, 10, and 50 μg/kg) or antagonist yohimbine (0.5, 1, 2, and 5 mg/kg). Using pins (one animal on its back with the other on top) as the dependent measure, we found that clonidine reliably reduced the amount of play at all but the lowest dose tested, while yohimbine had no effect at any but the highest dose. In addition, we tested a clonidine-yohimbine interaction to assess neurospecificity of the results. While yohimbine alone (0.5 mg/kg) had no effect on play, it partially reversed the clonidine-induced suppression, indicating that the effect may be mediated to some degree through an α-2-adrenergic mechanism.

Rats Social play Clonidine Yohimbine Norepinephrine

WITH the introduction of an easily quantifiable, objective indicator of play behavior in juvenile rats [9], factors influencing this dynamic social behavior can now be systematically and conveniently investigated. Several reports have now described the effects of various psychopharmacological agents on play including catecholamine agonists [4] and antagonists [3,8]. But so far, only manipulations of the endogenous opioid system (via administration of an agonist and an antagonist) have been found to produce opposite effects on play behavior. Morphine increases play [8] while naloxone, an opiate antagonist, decreases play [2,8].

Clonidine, an α-2-adrenergic agonist, shares behavioral and neuropharmacological properties with morphine. It reduces distress vocalizations in chicks [10,11] and in morphine-dependent rats, inhibits naloxone precipitated wet dog shakes [5]. Clonidine also reverses the increased norepinephrine (NE) turnover in the dorsal NE bundle which accompanies morphine withdrawal [7] and has therapeutic value in treatment of the acute symptomatology of opiate withdrawal in humans [6]. Both clonidine and morphine, when micro-injected into the locus coeruleus, inhibit neuronal activity in the dorsal NE system [1].

In the present experiment we investigated the role of the α-2-adrenergic system in the mediation of play in juvenile rats using clonidine as agonist and yohimbine as antagonist. Dose-response relationships for each drug were established and a yohimbine-clonidine interaction was evaluated to determine neurochemical specificity of the effects obtained.

METHOD

Subjects

Thirty-two male and female Long-Evans hooded rats from four litters bred and born at BGSU were used. Animals were housed in family groups in suspended, stainless steel cages (24×40×19 cm) until they were weaned at 21 days of age. Subjects were then assigned a like-sex partner and individually housed in 23×10×13 cm cages. Throughout testing all animals had free access to food and water and the colony room was maintained on a 12:12 hr light-dark cycle, with lights on at 0800.

Apparatus

The play observation chamber has been described elsewhere [9]. Briefly, it was a Lucite test arena (31×31×32 cm) situated within a soundproof outer chamber with a 10×10 cm observation window. An observer scored the play behaviors of each animal during 5 minute test sessions. The primary dependent measure, a pin, was recorded whenever one animal ended up on its back during the course of the apparent rough and tumble play activity. Inter-observer correlations for such observations are routinely above 0.9 in our laboratory. In the present study, the frequency and duration of pins were recorded with a manually activated counter and running time meter, respectively.

RESULTS

Clonidine Dose-Response

Beginning on the day following weaning, pairs of animals were allowed 2 days (5 min periods) of habituation in the test chamber. The 16 pairs of like-sex subjects were randomly assigned to at least 2 of the 6 drug conditions. For each dose, drug and saline control were administered in a counterbal-

TABLE 1

EFFECT OF CLONIDINE ON PINNING BEHAVIOR (MEAN ± SEM) AT EACH OF THE DESIGNATED DOSES

Dose (μg/kg)[1]	Pins/Pair/5 min Clonidine	Saline	% of Control
0.1 (7)	62.9 ± 2.3	65.9 ± 5.4	95%
0.5 (8)	46.5 ± 5.8*	53.8 ± 5.3	86%
1 (4)	18.8 ± 10.4*	59.0 ± 7.9	32%
5 (4)	4.0 ± 2.0*	59.5 ± 3.1	7%
10 (4)	0.5 ± 0.3†	56.0 ± 2.7	1%
50 (4)	0†	61.0 ± 5.3	0%

[1]Number in parentheses indicates number of pairs.
t-Tests for matched samples; *$p<0.05$, †$p<0.01$.

TABLE 2

EFFECT OF YOHIMBINE ON PINNING BEHAVIOR (MEAN ± SEM AT EACH OF THE DESIGNATED DOSES

Dose (mg/kg)[1]	Pins/Pair/5 min Yohimbine	Saline	% of Control
0.5 (8)	27.3 ± 3.1	28.0 ± 4.9	98%
1 (8)	33.3 ± 3.2	30.0 ± 5.4	111%
2 (7)	29.9 ± 2.8	28.3 ± 5.5	106%
5 (7)	11.7 ± 4.5*	23.7 ± 3.1	49%

[1]Number in parentheses indicates number of pairs.
t-Tests for matched samples; *$p<0.05$.

anced fashion but no animal was treated on consecutive days.

Clonidine was administered subcutaneously in the nape of the neck in doses of 0.1, 0.5, 1, 5, 10 or 50 μg/kg 20 minutes before testing, which occurred during the last half of the light cycle. Testing consisted of placing the animals in the chamber and recording the frequency and duration of pins for a 5 min period. Dividing total duration by number of pins yielded the average pin duration. Since at each dose, each pair of animals served as its own control, analyses were performed using *t*-tests for matched samples. The stability of the control data in these experiments is noteworthy.

Clonidine reliably reduced the play of juvenile rats in a dose-dependent manner at all but the lowest (0.1 μg/kg) dose (Table 1). No differences were found in average pin duration at any but the 5 μg/kg dose where it was increased by clonidine from 1.4 to 2.2 sec, $t(3)=4.28$, $p<0.05$. It should be noted also that the doses of clonidine and yohimbine used in these experiment produced no outwardly apparent indication that the animals were behaviorally impaired in any way.

Yohimbine Dose-Response

Following the conclusion of clonidine testing, animals were pair housed with their respective partners (to reduce the levels of play so that any reciprocal antagonist-induced increases could be more readily observed) and given one day of rest. From the pool of subjects, pairs were randomly assigned to at least 2 of the 4 drug conditions. At each dose, drug and saline control were administered in a counterbalanced fashion and no animal was treated on consecutive days. Animals were individually housed in 23×10×13 cm cages for 3 hours before testing.

Yohimbine was administered subcutaneously in doses of 0.5, 1, 2, or 5 mg/kg, 20 min before testing. Observation of play and analyses of data were conducted as before.

Yohimbine had no effect on the frequency of pinning behavior except at the highest dose tested (5 mg/kg) where it reduced the behavior (Table 2). Yohimbine-treated animals did not differ from controls in average pin duration at any dose tested.

Clonidine-Yohimbine Interaction

At the conclusion of yohimbine testing, animals were individually housed and given one day of rest. On each of 4 consecutive days, each of the 16 pairs of subjects was tested in one of the following conditions: clonidine (5 μg/kg), yohimbine (0.5 mg/kg), clonidine (5 μg/kg) + yohimbine (0.5 mg/kg) mixture, or saline. Order was counterbalanced and each animal received all treatment conditions.

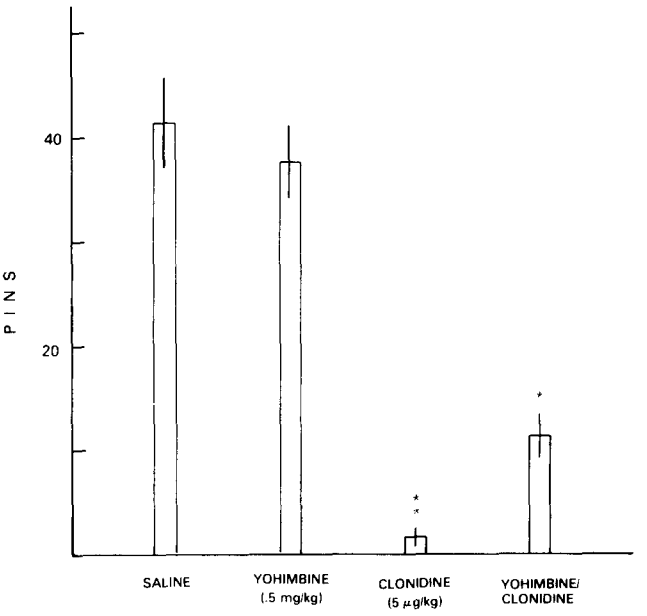

FIG. 1. Effect of clonidine-yohimbine interaction on pinning behavior of juvenile rats. Each bar depicts mean number of pins (±SEM) per pair of animals in a 5 min observation period. Overall analysis, $F(3,45)=48.22$, $p<0.01$. Neuman-Keuls analysis, *$p<0.05$, **$p<0.01$.

Preparation of drugs, injections, and observation procedures were the same as in the previous experiments. Analysis was performed using a one-way analysis of variance with a repeated measure. Individual comparisons were evaluated with a Newman-Keuls test.

Clonidine (5 μg/kg) markedly reduced pinning ($p<0.01$); yohimbine (0.5 mg/kg) had no effect ($p>0.05$) but it did reliably reverse the clonidine-induced suppression of play ($p<0.05$) (Fig. 1). There were no differences in average pin duration among any of the conditions.

DISCUSSION

After the completion of this work, Beatty and colleagues [3] reported that clonidine at doses of 50 μg/kg and above produced marked suppression of play fighting in juvenile rats. The present work extends that effect to the much lower dose of 0.5 μg/kg, with the degree of play suppression being dose related up to 10 μg/kg. Higher doses produce essentially total cessation of play, with animals exhibiting little active social interaction, although the animals do not appear outwardly sedated until doses above 50 μg/kg.

Given the previously reported similar behavioral effects of the α-2-adrenergic agonist, clonidine, and the opiate analgesics [10,11] it was expected that clonidine, as morphine, would increase play. The present finding that clonidine reduces play indicates interesting differences in the action of morphine and clonidine. Elucidation of the exact nature of such differences, however, is outside the scope of the present experiment, for the identification of the psychological processes modulated by clonidine will undoubtedly require the conduct of more subtle behavioral testing.

Additionally, clonidine and yohimbine did not effect play in opposite directions as is the case with morphine and naloxone [8]. For a neurochemical system to be implicated in the control of a specific behavior, increasing or decreasing activity of that system (via administration of an agonist or antagonist) might be expected to modulate that behavior in a similar up/down fashion.

Since yohimbine had no clear play-incremental effect at appropriately low doses, the action of clonidine might best be explained as a non-specific modulatory effect on behavior (e.g., reduced arousal) rather than as a reflection of mechanisms by which activity in play circuits is specifically regulated. Still, it must be emphasized that modest doses of yohimbine which are known to have central pharmacological effects did not impede play, suggesting that α-2-noradrenergic blockage is compatible with the expression of playful activities in the brain. Traditionally, the main pharmacological effect of clonidine is thought to be reduced brain norepinephrine activity mediated via autoreceptor inhibition of norepinephrine cell activity [7,11], but the possibility that the effects on social behavior are mediated by postsynaptic effects remains open. Indeed, clonidine modulation of separation-induced distress vocalizations presently appears to be mediated by postsynaptic NE receptor effects [11]. Of course, the possibility that clonidine produces part of its effects by involvement of other neurochemical systems cannot be ruled out by the present findings.

Considering the existing ambiguity in pinpointing the neural effects of clonidine, it is difficult to assert whether increased or decreased NE activity is compatible with play. Taking previously established lines of evidence, however, we would suggest that heightened NE activity generally reduces play, while reduced NE activity is compatible with the expression of play. This is based largely on the ability of psychomotor stimulants to reduce play behaviors [3,4]. Although the meaning of those results remains highly controversial since the effects cannot be reversed with a variety of catecholamine receptor antagonists [3], the finding that yohimbine is able to at least partially block the ability of clonidine to reduce play does strongly suggest that effect is mediated to some degree through the α-2-adrenergic receptor. Since all brain catecholamine systems probably have some effects on the expression of play, perhaps counteracting ones, the use of wide-spectrum psychomotor stimulants may impede the acquisition of clearly interpretable results. Future evaluations of catecholaminergic processes in the elaboration of play would be well-advised to use more specific pharmacological tools.

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