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Cholinergic Agonists Suppress Play Fighting in Juvenile Rats

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WILSON, L. I., R. A. BIERLEY AND W. W. BEATTY. *Cholinergic agonists suppress play fighting in juvenile rats.* PHARMACOL BIOCHEM BEHAV **24**(5) 1157–1159, 1986.—Previous research has suggested that acetylcholine might activate play fighting in juvenile rats through its actions on central muscarinic receptors. To test this hypothesis we evaluated the effects on play fighting by the muscarinic agonists pilocarpine and arecoline given alone or in combination with the muscarinic antagonists scopolamine or methylscopolamine. Scopolamine, but not methylscopolamine which penetrates the brain poorly, suppressed play as indexed by frequency of pinning. Pilocarpine and arecoline also suppressed pinning at higher doses. Concurrent treatment with various agonist-antagonist dose combinations produced additive rather than counteractive effects. These data do not support the supposition that central muscarinic circuits are involved in the activation of play fighting.

Play fighting Social play Social development Cholinergic influences on behavior Scopolamine Pilocarpine Arecoline Rats

SCOPOLAMINE and its quaternary analogue methylscopolamine are cholinergic antagonists that exert their effects on muscarinic receptors by blocking the receptor sites. Acute administration of scopolamine, which has both central and peripheral effects, depresses the play fighting of juvenile rats at doses which are not sedating [1,11]. Methylscopolamine, which does not readily cross the blood-brain barrier, does not have this effect [1,11], suggesting that the scopolamine effect is exerted centrally.

Recently, Thor and Holloway [12] gave rats seven daily treatments of scopolamine and demonstrated an increase in play that persisted for at least a week following termination of the injections. They attributed this effect to a drug induced proliferation of muscarinic receptors, a well documented effect of chronic exposure to antimuscarinic drugs [6,14].

The above findings [1, 11, 12] suggest that acetylcholine modulates play through its action on central muscarinic cholinergic receptors. However, there are a number of studies showing a general suppression of play to a wide variety of substances [2, 10, 13] so this suggestion should not be accepted uncritically.

Panksepp, Siviy and Normansell [10] have shown that the nicotinic cholinergic antagonist, mecamylamine, stimulates play and blocks the suppressive effects of nicotine. If cholinergic receptors, in general, mediate play, it should be possible to manipulate muscarinic receptors in a similar manner. Since scopolamine suppresses play, the administration of muscarinic agonists should facilitate play. Likewise, muscarinic agonists should counteract the effects of scopolamine administered concurrently. In the present experiments we tested these two predictions by examining the effects of the muscarinic agonists pilocarpine and arecoline on play fighting. These drugs were administered in varying doses either alone or in combination with the muscarinic antagonists scopolamine or methylscopolamine. The methylscopolamine condition was included to protect against the possibility that the central effects of the agonists (hypothetically stimulation of play fighting) might be masked by their powerful peripheral effects on the parasympathetic system. Previous research on the effects of cholinergic agonists on muricide [15] suggested such masking effects can occur.

METHOD

Animals

Juvenile male rats of a Sprague-Dawley strain obtained from the Holtzman Co., Madison, WI at 21 days of age were the subjects. Separate groups (N=9 pairs each) were used in the two experiments. The rats were caged singly in an air conditioned animal room maintained at 22±3°C with free access to food and water in the home cage. The animal room was illuminated by overhead fluorescent fixtures from 0800 to 2100. Testing occurred during the daylight phase of the L:D cycle.

Procedure

All behavioral tests were conducted in a 51×32×47 cm high box made of plywood and clear plastic. The chamber was housed in a dark room and illuminated by red incandescent bulbs (see [1] for details). The animals were assigned at random to test pairs which remained intact for the duration of the study. Starting at 23 days of age each pair was placed

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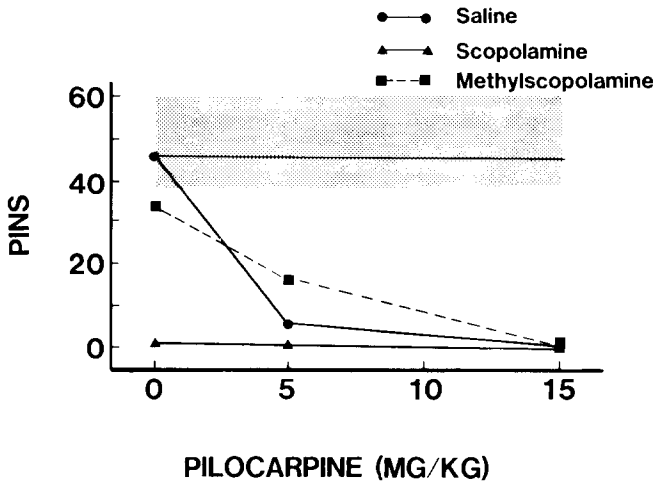

FIG. 1. Mean number of pins at varying doses of pilocarpine given in combination with saline, scopolamine HBr (1 mg/kg) or scopolamine methylbromide (1 mg/kg). Horizontal lines denote overall mean performance under no treatment conditions; shaded area is the range of means on no treatment test days.

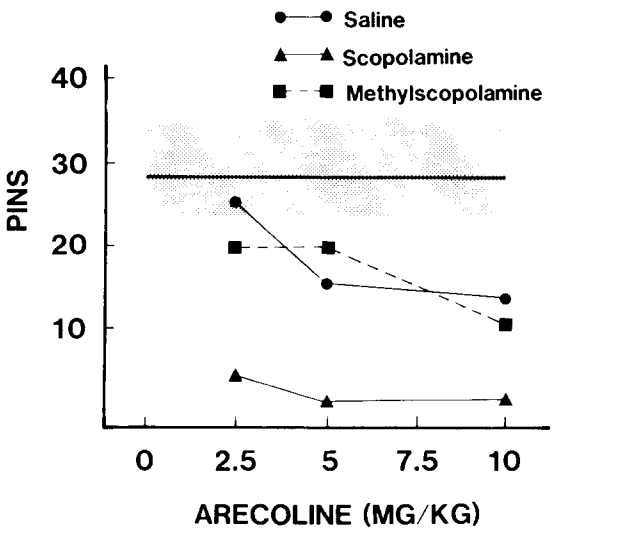

FIG. 2. Mean number of pins at varying doses of arecoline given in combination with saline, scopolamine HBr (1 mg/kg) or scopolamine methylbromide (1 mg/kg). Horizontal lines denote overall mean performance under no treatment conditions; shaded area is the range of means on no treatment test days.

into the chamber for 10 min once each day. The first 2 days were considered adaptation and behavior was not scored. Beginning on the third day when the rats were 25 days old and continuing for the next 18 days, a single observer recorded the number of pins (one rat on its dorsal surface with the other rat standing over it) made by the pair for each session. Previous research in our laboratory has shown that interrater reliabilities are high for this measure ($rs>0.90$) when it is scored in this way and that the number of pins is highly correlated with other indices of play fighting [8].

Drug treatments began on the 4th day and were administered on alternate days until 9 drug test days had been given. On the intervening days behavior was scored without drug treatment. All drugs were purchased from Sigma Chemical Co., St. Louis, MO, and injected IP 20 min before testing in a volume of 1 ml/kg. All drugs were dissolved in saline. Both members of a test pair received the same combination of drug treatments on drug test days.

In the first study rats received pilocarpine HCl (0, 5 or 15 mg/kg) in combination with saline (1 ml/kg), scopolamine HBr (1 mg/kg) or scopolamine methylbromide (1 mg/kg) resulting in 9 treatment condition. Each combination of treatments was administered once to each test pair.

In the second study the design was the same except that pairs received arecoline HBr (2.5, 5.0, or 10.0 mg/kg) in combination with saline (1 ml/kg), scopolamine HBr (1 mg/kg) or scopolamine methylbromide (1 mg/kg). The pH of the arecoline solution varied from 4.75 to 4.40 with increasing dose. As in the first experiment drug treatments were given on alternate days in a counterbalanced order. The mean performance on the 9 no-treatment days served as the behavioral baseline for the statistical analysis. Other procedures were identical to the first experiment.

RESULTS AND DISCUSSION

The effects on pinning of pilocarpine, alone or in combination with methylscopolamine or scopolamine, are shown in Fig. 1. A repeated measures analysis of variance, which did not include the no-treatment days, revealed significant treatment effects, $F(8,64)=43.81$, $p<0.001$. Subsequent tests showed that all treatment combinations differed reliably from saline alone, $ts(8)\geq 3.43$, $ps\leq 0.02$. In combination with saline, pilocarpine produced a dose-dependent suppression of pinning (5 vs. 15 mg/kg, $t(8)=4.06$, $p<0.01$). The high pilocarpine dose virtually eliminated pinning. The effect of the low dose of the agonist was partially reversed by concurrent treatment with methylscopolamine $t(8)=2.75$, $p<0.05$, but not by scopolamine, $t(8)=1.14$, $p>0.20$. As expected from previous work [1,11] scopolamine caused a greater suppression of play than methylscopolamine, $t(8)=8.81$, $p<0.001$.

As shown in Fig. 2 the effects of arecoline on pinning were generally similar to those of pilocarpine. Analysis of variance including the no-treatment baseline revealed significant drug treatment effects, $F(9,72)=11.40$, $p<0.001$. Relative to the no-treatment baseline both the 5 and 10 mg/kg doses of arecoline reduced pinning, $ts(8)\geq 3.43$, $ps\leq 0.02$, but the lowest dose (2.5 mg/kg) was ineffective ($t<1$). Concurrent treatment with methylscopolamine did not alter the effect of arecoline, $ts(8)\leq 1.26$, $ps\geq 0.20$. Treatment with scopolamine virtually abolished pinning regardless of the dose of arecoline given at the same time, $ts(8)\geq 7.00$, $ps\leq 0.001$.

Since the 1 mg dose of scopolamine virtually eliminated pinning it might be argued that the failure of either agonist to alleviate this suppression effect was due to an insufficient dose of the agonist drug relative to the dose of antagonist drug administered. In unpublished work we evaluated this possibility by examining the influence of moderate doses of pilocarpine (1 or 5 mg/kg) in combination with lower doses of scopolamine (0.125 or 0.500 mg/kg). Neither dose of pilocarpine counteracted the suppression of play by either dose of scopolamine.

We also tested yet a third cholinergic agonist, oxotremorine (unpublished observations), and found that it depressed play at doses as low as 15 μg/kg. Due to rapid development of tolerance to oxotremorine as has been observed by others [3,4], we were not able to systematically study oxotremorine's effects on play, but any dose that produced an effect was clearly depressant.

Noting that a great many drugs can reduce the frequency of play, Panksepp *et al.* [10] suggested that investigators conduct a careful analysis of agonist-antagonist interactions before inferring a specific role for a neurotransmitter system in the regulation of play. In the present experiments none of the muscarinic agonists tested increased play fighting (as indexed by pinning) when given alone, and none antagonized the suppression of play produced by scopolamine. In fact, the muscarinic agonists suppressed play at the higher doses tested and these effects were additive with those of the antagonist scopolamine. Thus, while centrally active muscarinic antagonists potently suppress play, the present findings do not support the view that central muscarinic circuits have a specific role in the activation of play fighting.

On pharmacological grounds a stronger case can be made for the specific influence of nicotinic receptor systems in the control of play fighting. Panksepp *et al.* [10] have shown that nicotine suppresses play while mecamylamine, a nicotinic antagonist, stimulates play and blocks the suppressive effect of nicotine. While these data suggest a specific influence of nicotinic cholinergic systems in the control of play it is not at present clear whether the effects of nicotine and mecamylamine on play arise from the peripheral or central actions of these drugs. Thus a specific influence of central cholinergic systems in the regulation of play is not yet clearly established.

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