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Effects of Cholinergic Agonists on the Dorsal Immobility Response

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POTTER, T. J., G. A. COTTRELL AND C. VAN HARTESVELDT. *Effects of cholinergic agonists on the dorsal immobility response.* PHARMACOL BIOCHEM BEHAV **36**(1) 77–80, 1990.—The effects of pilocarpine, arecoline, and physostigmine on the dorsal immobility response in ovariectomized female rats were tested. The effect of pretreatment with the cholinergic antagonist scopolamine was also tested. Pilocarpine, arecoline, and physostigmine all significantly decreased the duration of the dorsal immobility response in a dose-dependent way. Scopolamine significantly blocked the effect of pilocarpine. Thus, cholinergic agonists attenuate the dorsal immobility response via their effect on cholinergic systems in the central nervous system.

Pilocarpine Arecoline Physostigmine Scopolamine Dorsal immobility response Rat

PREVIOUS research has shown that two kinds of immobility responses in several species may be modulated by cholinergic manipulations. Tonic immobility is elicited in some species by inversion and restraint; the animal responds by remaining immobile for a period of time (3). The duration of this response varies across species and may be manipulated pharmacologically. For example, the cholinergic agonists physostigmine and pilocarpine increase tonic immobility in ducks; physostigmine shortens it in guinea pigs (11). Scopolamine, a cholinergic antagonist, shortens tonic immobility in chickens (5) and ducks but increases it in guinea pigs (11). Catalepsy, a second kind of immobility, is induced by drugs or lesions. This behavior is characterized by a waxy flexibility in which the animal tends to remain in an imposed position. Pilocarpine, a cholinergic agonist, has been shown to induce catalepsy in the rat (1, 6, 12), as have dopamine antagonists (7). Both tonic immobility and catalepsy are kinds of immobility that can be experimentally induced, but are not naturally occurring behaviors in adult rats.

The dorsal immobility response (DIR) refers to the immobility displayed by an animal while being carried either by its parent or by a predator. The DIR is experimentally induced by grasping the dorsal skin at the base of the skull, above the shoulder blades, and lifting until no part of the animal is touching the ground. The animal responds by immediately becoming immobile and remains in this position for a period of time until it displays escape-like behaviors. The DIR has been demonstrated in a variety of rodents (9). It can also be manipulated pharmacologically; haloperidol, a dopamine antagonist, potentiates it (7). The effects of cholinergic agonists and antagonists have been tested on tonic immobility and catalepsy, but not on the DIR. Therefore, in the present experiments we investigated the effects of three cholinergic agonists, pilocarpine, physostigmine, and arecoline, on the DIR.

In the first experiment the effect of the muscarinic agonist pilocarpine was tested. In the second experiment the effects of a peripheral cholinergic blocker, methylscopolamine, and a central blocker, scopolamine, were compared. In order to establish that the effect of pilocarpine on the DIR was due to its characteristics as a cholinergic agonist arecoline, another muscarinic cholinergic agonist, was used in the third experiment. Finally, physostigmine, a cholinesterase inhibitor and indirect cholinergic agonist, was used in the fourth experiment.

GENERAL METHOD

Animals

Female Long-Evans hooded rats were obtained from Charles River. All animals were housed individually in a colony room on a 14:10 light-dark cycle. The rats were maintained on food and water ad lib throughout the experiments.

Surgical Procedure

Rats were 150–175 g upon arrival and were ovariectomized three days after arrival using ether (Fisher Scientific) as an anesthetic. The rats were ovariectomized because ovarian steroid hormones have been shown to affect the DIR (8). Animals were allowed a 2-week recovery period prior to experimentation. They were approximately 2 months old at the time of testing.

Behavioral Testing

Animals were removed from the colony room in their home cages and placed in the experimental room. The DIR was the sole behavioral measure in all four experiments. The DIR was induced by grasping the dorsal skin at the base of the skull, above the shoulder blades, and lifting until no part of the animal touched the

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ground. The animals responded by immediately becoming immobile. Duration of the response was measured from onset until animals displayed escape-like behavior including physical contact with the hand or arm of the experimenter or until 300 sec had elapsed. Criteria for termination of the trial were thus more stringent than in previous research, in which only escape-like behavior was required (8,9). Three consecutive trials were administered separated by a 30-sec intertrial interval. The averaged scores for the three trails were used for statistical evaluation.

Animals used in Experiment 1 were randomly selected and regrouped for use in Experiment 2. Separate groups of new animals were used in Experiments 3 and 4. Methods for each experiment are presented below.

Drugs

All drugs were dissolved in 0.9% saline. The following drugs were used: pilocarpine hydrochloride, Sigma (P-6503); scopolamine hydrochloride, Sigma (S-1013); scopolamine methyl bromide, Sigma (S-8502); arecoline hydrobromide, Sigma (A-6134); and physostigmine sulfate, Sigma (E-8625). All drug doses were calculated on the basis of the weight of the salt. Drugs were administered subcutaneously to the animal's right flank.

EXPERIMENT 1

Method

Baseline scores were obtained for all animals as follows. Animals were injected twice with 0.25 ml saline separated by a 15-min interval. Fifteen minutes after the second injection animals were tested in the above manner. Rats were then grouped according to their baseline scores to produce 5 groups of 7 or 8 animals per group in which the group average baseline scores were approximately equal. Following a two-week rest period animals were injected with 1 mg/kg scopolamine methyl bromide to block the peripheral effects of pilocarpine. Fifteen minutes later the animals were injected SC with one of 5 doses of pilocarpine [0.05 (n=7), 0.5 (n=7), 1.25 (n=8), 2.5 (n=7), and 5.0 (n=8) mg/kg]. Animals were tested for the DIR 15 min after the second injection. The DIR scores for the 3 trials were then averaged and compared to the saline baseline scores and to each other.

Results

Pilocarpine reduced DIR durations in a dose-dependent manner (see Fig. 1). A two-way ANOVA indicated that there were significant differences between the scores of the pilocarpine dose groups, $F(4,32)=7.09$, $p<0.001$, within pilocarpine dose/saline baseline groups, $F(1,32)=115.19$, $p<0.001$, as well as a significant interaction, $F(4,32)=12.62$, $p<0.001$. Subsequent analyses with one-way ANOVAs indicated that while there were no significant differences between the scores of any of the groups for the saline baseline measure, there were significant differences between groups for the different doses of pilocarpine. The DIR scores were inversely related to dose with the highest dose, 5.0 mg/kg, producing the greatest reduction. Further post hoc analyses were conducted using Duncan's New Multiple Range test to discover which doses were significantly different from one another. This test indicated that the 5.0 mg/kg group's scores were significantly different from those of all dose groups ($p=0.01$) except for the 2.5 mg/kg group. The 2.5, 1.25, and 0.5 mg/kg groups were each significantly different from one another ($p=0.01$); the 0.05 mg/kg group was not significantly different from the 0.5 mg/kg group. In order to discover which pilocarpine dose groups were significantly different from their saline baseline scores, further post hoc analysis was carried out using a *t*-test for related measures. The 5.0, 2.5, and 1.25 mg/kg groups all differed significantly from their saline baseline scores ($p=0.001$) but the other two dose groups did not.

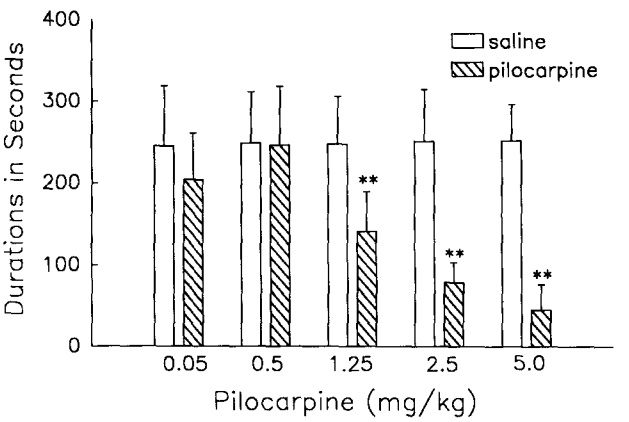

FIG. 1. The duration of the dorsal immobility response significantly decreased as the dose of pilocarpine increased. Bars represent mean durations ±1 standard deviation. Double asterisks indicate that the pilocarpine group was significantly different from the saline baseline ($p=0.001$).

EXPERIMENT 2

Method

Following a 2-week rest period 14 animals from Experiment 1 were randomly selected and separated into 2 groups, n=7 per group. Their saline baseline scores were determined as indicated in Experiment 1. Rats in group 1 were injected with 1.0 mg/kg scopolamine methyl bromide followed 15 min later by 5.0 mg/kg pilocarpine. Rats in group 2 were injected with 1.0 mg/kg scopolamine followed 15 min later by 5.0 mg/kg pilocarpine. Animals were tested for the DIR 15 min after the last injection. The groups pretreated with the different agents were compared with each other and with the baseline saline scores.

Results

Scopolamine significantly blocked the effects of pilocarpine (see Fig. 2). A two-way ANOVA indicated significant differences between, $F(1,12)=8.51$, $p=0.012$, and within, $F(1,12)=98.18$, $p<0.001$, groups as well as a significant interaction, $F(1,12)=15.79$, $p=0.002$. Subsequent one-way ANOVAs indicated that while the saline baseline scores of the 2 groups did not differ from one another, the scores of the group pretreated with scopolamine before receiving pilocarpine were significantly higher than those of the group pretreated with methylscopolamine, $F(1,12)=18.63$, $p=0.001$. Further post hoc analyses using *t*-tests for related measures indicated that the scores of both the group pretreated with methylscopolamine ($p<0.001$) and the group pretreated with scopolamine ($p=0.011$) were significantly lower than those of the saline baseline.

EXPERIMENT 3

Method

Twenty-five rats were obtained, ovariectomized as described, and randomly placed into one of 4 groups of 6 or 7 rats. All groups

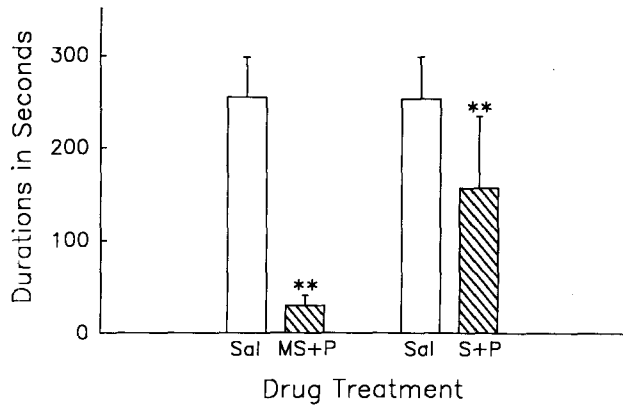

FIG. 2. Pretreatment with scopolamine (S) significantly blocks the pilocarpine (P)-induced decrease in the dorsal immobility response. Relative to the saline (Sal) baseline scores, pilocarpine produced a significant decrease in the dorsal immobility response whether the animals were pretreated with methylscopolamine (MS, $p<0.001$) or scopolamine (S, $p=0.011$). These differences are indicated by double asterisks. However, the pilocarpine-induced decrease in the dorsal immobility response was significantly less in the scopolamine-pretreated group (S+P) than the methylscopolamine-pretreated group (MS+P), $p=0.001$. Bars represent mean durations ± 1 standard deviation.

first received 1 mg/kg methylscopolamine. Fifteen min later animals were given either saline (n = 6) or one of the three doses of arecoline (5.0 mg/kg, n = 7; 7.5 mg/kg, n = 6; or 10.0 mg/kg, n = 6). Animals were tested 15 min later for the DIR.

Results

Arecoline reduced DIR duration in a dose-dependent way (see Fig. 3). A one-way ANOVA indicated that this reduction was significant, $F(3,21)=12.08$, $p<0.001$. Duncan's New Multiple Range test showed that the scores of the 7.5 and 10.0 mg/kg dose groups were not significantly different from one another, but both were significantly lower than the 5.0 mg/kg group ($p<0.01$) and the saline group ($p<0.01$). The scores of the 5.0 mg/kg group were not significantly different from those of the saline group.

The scores of animals in the saline group in the present

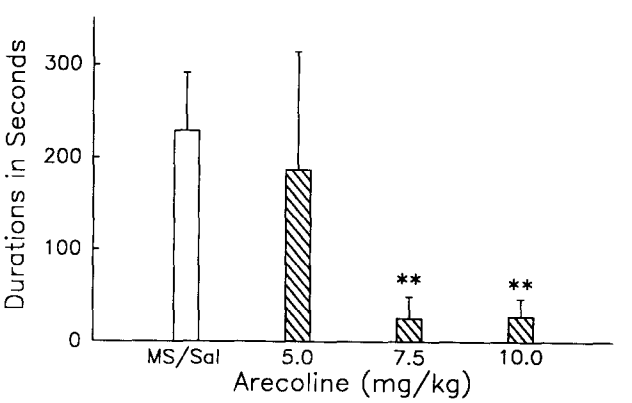

FIG. 3. The duration of the dorsal immobility response significantly decreased as the dose of arecoline increased. Bars represent mean durations ± 1 standard deviation. Double asterisks indicate that the scores for pilocarpine were significantly different from those of the methylscopolamine-pretreated saline (MS/Sal) group ($p<0.01$).

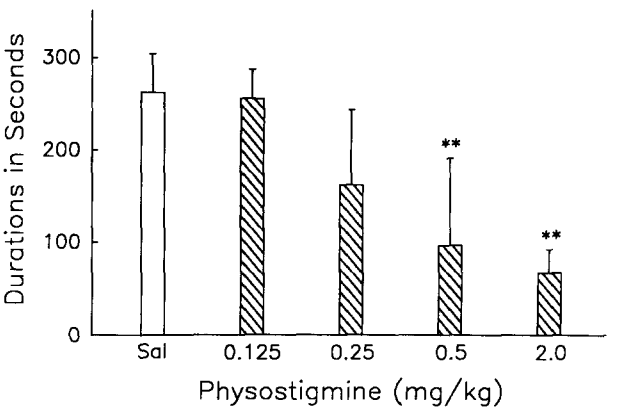

FIG. 4. The duration of the dorsal immobility response significantly decreased as the dose of physostigmine increased. Bars represent mean durations ± 1 standard deviation. Double asterisks indicate that the scores for physostigmine were significantly different from those of the saline (Sal) group ($p<0.01$).

experiment, which had methylscopolamine pretreatment, were compared with those of the saline baseline groups in Experiments 1 and 2, which did not have this pretreatment. They were very comparable, and did not significantly differ.

EXPERIMENT 4

Method

Thirty-five animals were obtained, ovariectomized as described above, and placed into five groups, n = 7 per group. Animals in these groups were then injected either with saline or with one of four doses of physostigmine (0.12 mg/kg, 0.25 mg/kg, 0.5 mg/kg, or 2.0 mg/kg). Because pilot tests indicated that physostigmine at these doses did not elicit the parasympathetic effects of the direct cholinergic agonists previously tested, and because it was established in Experiment 2 that the effects of cholinergic agonists on the DIR are at least in part centrally mediated, methylscopolamine was not given.

Results

Physostigmine reduced the DIR in a dose-dependent manner (Fig. 4). A one-way ANOVA indicated that this reduction was significant, $F(4,30)=10.53$, $p<0.001$. Post hoc analyses conducted using Duncan's Multiple Range test showed that while the highest dose (2.0 mg/kg) produced the greatest reduction in the duration of the DIR, the three highest doses were not significantly different from one another. The scores of the 2.0 and 0.50 mg/kg doses were significantly different from those of the saline group, while the scores of neither the 0.25 nor the 0.125 mg/kg dose were.

DISCUSSION

The results of the experiments described here indicate that cholinergic mechanisms in the central nervous system decrease the duration of the DIR: the DIR is decreased by several different cholinergic agonists, and this agonist-induced decrease is significantly blocked by a cholinergic antagonist. This decrease in the duration of an immobility response is the opposite of the effect of cholinergic agonists on catalepsy (1, 6, 12), indicating that these responses are mediated in different ways. The discrepancy between the size of the dose of pilocarpine required to decrease the

DIR in this experiment and to induce catalepsy in previous research is one indication that different mechanisms may be involved. In the present experiment as little as 1.25 mg/kg pilocarpine decreased the DIR, while in previous studies as much as 25–100 mg/kg pilocarpine was needed to induce catalepsy (1,6). The facts that systemically administered cholinergic agonists can suppress locomotion and induce catalepsy, and anticholinergic agents can elicit increased locomotor activity, have suggested that central cholinergic systems are involved in behavioral inhibition. However, at some levels in the central nervous system such as the spinal cord and medulla, cholinergic agonists activate simple locomotor responses (4). The site of cholinergic agonist-induced DIR reduction is not yet known.

An additional unknown is the possible relationship between cholinergic and dopaminergic systems with reference to the DIR. These neurotransmitter systems are thought to be antagonistic to one another with respect to locomotor activity and catalepsy [e.g., (6)], that is, cholinergic agonists and dopamine antagonists affect these behaviors in the same way. However, cholinergic agonists and dopamine antagonists have opposite effects on the DIR. Thus, there is no simple way to characterize dopaminergic-cholinergic interactions with respect to behavior.

Recently, evidence of cholinergic mediation of the transport response has been reported (10). The transport response is an immobility response displayed by preweanling rat pups when lifted by the dorsal skin of the neck. Although this behavior is most commonly observed when the dam grasps the pup in her mouth prior to transport, it can be induced experimentally in the same manner as the DIR (2). Wilson and Cromey (10) found that pilocarpine decreased the intensity of the transport response at the same low doses used in the present experiment; atropine (a cholinergic blocker) increased it, and scopolamine blocked the pilocarpine-induced decrease in this response. While the transport response involves immobility, it also includes strong adduction of both the fore- and hindlimbs (2), which was the response measured in this developmental study. The fact that cholinergic agonists both decrease limb adduction in rat pups and decrease the DIR in adult rats at the same dose levels suggests that these two behavioral inhibitory responses are closely related.

ACKNOWLEDGEMENT

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