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Cholinergic, Dopaminergic, Noradrenergic, or Glutaminergic Stimulation Ventral to the Anterior Septum Does Not Specifically Suppress Defensive Behavior[1]

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ALBERT, D. J. *Cholinergic, dopaminergic, noradrenergic, or glutaminergic stimulation ventral to the anterior septum does not specifically suppress defensive behavior.* PHARMAC. BIOCHEM. BEHAV. 14(1) 35–39, 1981.—Three experiments investigated neurotransmitters which might function in the neural system ventral to the anterior septum modulating defensiveness in the rat. In the first experiment, a dose dependent suppression of defensive behavior to the experimenter was produced by intracranial infusion of carbachol or physostigmine but not dopamine, norepinephrine, or glutamate. The suppression of defensiveness did not occur when a carbachol-atropine sulfate mixture was infused. In a second experiment the cholinergic antagonists, atropine methyl nitrate or atropine sulfate, did not increase reactivity when infused ventral to the anterior septum although the nonspecific blocking agent lidocaine was effective. In a final experiment, the infusion of carbachol ventral to the anterior septum which had suppressed defensiveness was found to suppress eating and general activity as well, thus suggesting that the effect of carbachol on defensiveness was the result of a nonspecific suppression of behavior. It is concluded that the specific modulation of defensiveness by the neural system ventral to the anterior septum is not mediated by acetylcholine, dopamine, noradrenaline, or glutamate.

Aggression Cholinergic Defensiveness Dopaminergic Glutaminergic Hyperreactivity Noradrenergic Septum

THE region ventral to the anterior septum is thought to play a central role in the inhibitory modulation of defensive behavior in the rat. Electrolytic or temporary chemical lesions in this region increase defensiveness to the experimenter [3, 4, 6, 7, 8] while electrical stimulation produces the reverse effect. These changes in defensiveness are manifested in changes in reactivity to stimuli presented by the experimenter.

An attempt has been made to identify the neurotransmitter that may be functioning in the neural system ventral to the anterior septum which modulates defensiveness. The infusion of a nonspecific transmitter antagonist has been shown to heighten defensiveness to the experimenter but this result has not been duplicated when specific cholinergic, dopaminergic, or noradrenergic antagonists are infused at the low doses which do not produce nonspecific effects [5,7]. In view of the evidence that cholinergic [3, 22, 29, 30], dopaminergic [13, 23, 34] and noradrenergic synapses [13, 23, 34] exist in the region ventral to the anterior septum, a further investigation of their involvement in the modulation of defensive behavior seemed desirable.

EXPERIMENT 1: INFUSION OF CHOLINERGIC, DOPAMINERGIC, NORADRENERGIC, OR GLUTAMINERGIC AGONISTS VENTRAL TO THE ANTERIOR SEPTUM

The object of the first experiment was to determine whether chemical stimulation ventral to the anterior septum by various transmitter agonists would decrease the rat's reactivity to stimuli presented by the experimenter (defensiveness). In order to ensure a high level of reactivity, this behavior was heightened by a prior lesion of the medial hypothalamus. It has been previously demonstrated that electrical stimulation of the region ventral to the anterior septum will suppress hyperreactivity induced by the medial hypothalamic lesion [1, 3, 11].

METHOD

Animals

Seventeen naive male hooded rats from the Canadian Breeding Farms and Laboratories were used. They were housed in individual cages following surgery.

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Procedure

Surgery. Under sodium pentobarbital anesthesia, electrolytic lesions were made bilaterally in the ventromedial hypothalamus. A stainless steel electrode (anode) was used with a 2.0 mA current for 25 sec. The coordinates were: 0.0 mm anterior to bregma, 0.7 mm lateral to the midline, and 9.0 mm ventral to the surface of the cortex (mouth bar 5.0 mm above the interaural line). Immediately afterward, cannulas (23-ga stainless steel) were stereotaxically implanted bilaterally into the region ventral to the anterior septum, just lateral to the vertical arm of the diagonal band of Broca and medial to the rostral limb of the anterior commissure. The coordinates were: 3.0 mm anterior, 1.0 mm lateral, and 6.8 mm ventral. Plugs were kept in the cannulas when they were not in use. Injection needles were made from 30 ga stainless steel tubing and extended 0.5 mm beyond the end of the cannula [4, 5, 6].

Behavioral testing. Following a minimum of 7 days postsurgical recovery, testing began in a gray box (60×60×60 cm) with an open top and wood shavings on the floor. The animal's baseline level of reactivity was assessed using a standard series of stimuli, which included the response to a pencil, a gloved hand, and grasping of the body (see [4, 5, 6, 7], or [8] for details). If the baseline rating was 5 or greater (out of a maximum of 24), the animal was retained in the experiment. Animals with lower scores were discarded only because their lower scores limited the quantitative suppression that could be obtained with the chemical stimulation.

Rats to be tested further were *very lightly* etherized with ethyl ether to permit insertion of the chemical infusion needles. The needles were connected to a syringe (50 μl, Hamilton) by 1 m of polyethylene tubing (PE 50, Intramedic). A piece of polyethylene tubing covered the joint between the top of the needle collar and the cannula, insuring a leak proof connection.

The animal's level of reactivity to the experimenter was again assessed at 5 and 10 min following insertion of the infusion needles in order to obtain a stable baseline.

Chemical infusion began 5 min after the third assessment of reactivity to the experimenter. A rate of infusion of 1 μl/3 min was maintained by a Sage Instruments pump. However, in order to allow time for diffusion in the vicinity of the injection needle tip, the infusion pump was on only every other minute. Therefore, the infusion of 1 μl was spread over a 5 min period. Behavioral testing was done in a 2 min period following infusion of each μl. If 2 μl infused in this way induced no change in reactivity, a final microliter was infused over a 3 min period. Following the infusion the animal was tested every 5 min until its behavior returned to the preinfusion baseline.

The drugs injected were: norepinephrine bitartrate or hydrochloride, dopamine hydrochloride, monosodium glutamate, and physostigmine methyl bromide. Ascorbic acid (0.5%) was used with the catecholamines to retard oxidation. The vehicle was distilled water or sterile saline. Order of drug infusion was randomized. Each animal was infused on a maximum of 4 occasions with a minimum of 2 days (and more usually 4 days) between each test.

Following behavioral testing, the animals were killed. Their brains were removed and placed in formal saline and later sectioned and stained.

Data analysis was done using an analysis of variance followed by *t*-tests comparing pre- and postinfusion measures of reactivity.

TABLE 1

THE MEAN CHANGE (± SD) IN REACTIVITY TO THE EXPERIMENTER PRODUCED BY THE INFUSION OF VARIOUS SUBSTANCES VENTRAL TO THE ANTERIOR SEPTUM

Substance	N	Baseline	Mean change from baseline
Glutamate (60 μg)	7	11.4 (±2.5)	+1.3 (±4.0)
Dopamine (12 μg)	5	9.0 (±1.7)	+0.7 (±0.8)
Norepinephrine (12 μg)	5	10.3 (±2.0)	+3.6 (±1.6)
Carbachol (0.5 μg)	5	7.6 (±2.9)	+0.3 (±1.2)
Carbachol (2.0 μg)	11	10.1 (±2.7)	−5.8 (±3.7)†
Carbachol (4.0 μg)	4	11.5 (±2.6)	−8.1 (±1.9)†
Carbachol (2.0 μg) plus atropine (4.0 μg)	6	9.3 (±3.5)	+1.5 (±1.8)
Physostigmine (10 μg)	3	8.3 (±2.4)	−2.0 (±3.5)
Physostigmine (20 μg)	6	8.8 (±2.9)	−3.1 (±2.7)†
NaCl (0.9%)	6	9.2 (±3.1)	+2.0 (±1.7)

Significant decrease from baseline, *$p<0.05$, †$p<0.001$.

RESULTS AND DISCUSSION

A significant suppression of reactivity to the experimenter was produced by the cholinergic agonists carbachol and physostigmine relative to the preinjection baseline, $F(9,48)=40$; $p<0.001$; Table 1. The change in reactivity was calculated as the difference between the reactivity at the third baseline test and the reactivity 5 min following the end of drug infusion. The decrease in reactivity produced by the cholinergic agonists was apparent after the first microliter (i.e. 1 μg carbachol), reached a maximum following the second microliter or 5 min later and returned to baseline an average of 30 min following the end of the infusion. The suppression of reactivity to the experimenter induced by carbachol was completely abolished by the cholinergic antagonists atropine sulfate (Table 1).

Glutamate (60 μg), dopamine (12 μg), and norepinephrine (12 μg) were each ineffective in suppressing the hyperreactivity induced by the medial hypothalamic lesions. With each of these agonists there was actually a slight increase in reactivity as there was with 0.9% NaCl.

Examination of brain sections indicated that all cannulas were in the intended area ventral to the anterior septum (see [5, 6, 7] or [8] for illustrations of this area).

The suppression of reactivity by carbachol and physostigmine infused ventral to the anterior septum and the blocking of this effect by the simultaneous infusion of atropine sulfate suggests that a cholinergic mechanism in this region may modulate reactivity. The quantities of agonists required to induce these behavioral effects are similar to those which induce drinking [15, 17, 20, 21, 25] and alter avoidance behavior [17,18]. The failure of dopamine, noradrenaline, and glutamate to alter reactivity is congruent with earlier observations [7] and does not appear due to the injection of inadequate quantities since behavioral effects have been obtained previously with smaller doses of these agents [12, 16, 19, 26, 27, 28, 31].

EXPERIMENT 2: INJECTION OF CHOLINERGIC ANTAGONISTS VENTRAL TO THE ANTERIOR SEPTUM

The effectiveness of carbachol and physostigmine in sup-

TABLE 2

THE MEAN CHANGE IN REACTIVITY TO THE EXPERIMENTER (±SD) CAUSED BY THE INFUSION OF VARIOUS SUBSTANCES VENTRAL TO THE ANTERIOR SEPTUM

Substance	N	Baseline	Change
Atropine sulfate (20 μg)	5	0.9 (±0.9)	+1.0 (±0.9)
Atropine sulfate (10 μg)	7	1.3 (±0.9)	+1.5 (±1.2)*
Atropine sulfate (4 μg)	5	0.7 (±1.0)	+0.2 (±1.2)
Atropine methyl nitrate (10 μg)	5	1.4 (±1.1)	+1.7 (±1.4)
Lidocaine (80 μg)	12	1.3 (±1.3)	+6.0 (±2.1)†
NaCl (0.9%)	13	1.3 (±1.0)	+0.1 (±0.5)

Significant difference from baseline, *$p<0.05$, †$p<0.001$.

pressing the hyperreactivity to the experimenter induced by medial hypothalamic lesions is unexpected since infusion of atropine sulfate into this region has been reported previously to be without effect [7].

The present experiment reexamined the effect of atropine sulfate on reactivity using a variety of doses.

METHOD

Thirteen male rats of the strain described above were used. Infusion cannulas were implanted as described in the first experiment. Behavioral testing was similar to that of the previous experiment. The infusion needles were inserted without the use of ether. After two preinjection baseline reactivity tests (see previous experiment for procedure), the infusion of various drugs began. A maximum of 4 μl was injected to each hemisphere over a 20 min period. The injection rate was 1 μl/3 min. A 2 min period for assessment of reactivity was allowed after each μl infused. On completion of the infusion the rat continued to be tested every 5 min until behavior returned to the preinfusion baseline.

Each animal was infused on a maximum of 4 occasions with a minimum of 48 hrs between infusions. The drugs infused were atropine sulfate, atropine methyl nitrate, and lidocaine. Each was dissolved in distilled water except for lidocaine which was an injectable commercial preparation without epinephrine.

Following the experiment the rats were killed and their brains were sectioned and stained. The cannulas of all animals were in the area intended.

RESULTS AND DISCUSSION

As has been reported previously, lidocaine infusion produced an increase in reactivity to the experimenter from the preinfusion baseline, $F(5,41)=16$; $p<0.001$; $t=9.6$, $p<0.001$ (Table 2). Infusions of the cholinergic antagonist atropine sulfate produced a small but significant increase in reactivity at a dose of 10 μg. This effect did not occur at the 20 μg dose. Atropine methyl nitrate also did not significantly increase reactivity. The change in reactivity was calculated by subtracting the second preinjection baseline reactivity rating from the highest reactivity rating obtained during or 5 min following the drug infusion.

In contrast to the failure to produce a consistent and substantial suppression of reactivity with the atropine derivatives, substantial alterations of drinking and avoidance behavior have been observed at doses of 1 to 5 μg [17,20].

EXPERIMENT 3: SUPPRESSION OF FOOD INTAKE AND GENERAL ACTIVITY BY CARBACHOL

Since cholinergic antagonists do not consistently increase reactivity to the experimenter, it seems likely that the suppression of reactivity by cholinergic agonists observed in Experiment 1 was one aspect of a general suppression of all behavior rather than a specific suppression of reactivity. The object of this experiment was to determine whether carbachol infused ventral to the anterior septum will suppress two other behaviors, feeding and general activity, as this line of reasoning would predict.

METHOD

Seven male rats of the strain described in the first experiment were used. Medial hypothalamic lesions were made and infusion cannulas implanted as described in the first experiment.

One week after surgery, the rats were put on a food deprivation schedule in which they were fed 25 g/day. On the fourth day of deprivation, each rat was put into a 30×30×45 cm high Plexiglas observation chamber and allowed to eat wet Purina Lab Chow for 10 min.

Measurement of the effect of carbachol infusion on food intake began on the fifth day of deprivation. The infusion procedure was as described in the first experiment. Following chemical infusion, the animals were given a preweighed dish of wet ground Purina Lab Chow. The food dish was removed at the end of 10 min and the amount of unconsumed and spilled food weighed.

Each animal was injected on successive days with 2 μl of carbachol (1 μg/μl) or NaCl (0.9%) in a counterbalanced design. Following each day of eating in the observation box, each rat was given additional Lab Chow in its living cage to bring its daily intake to 25 g.

One week later these same animals were tested in an open field (60×60 cm). The floor was covered with dried corn cob chips and was marked into 15 cm squares by lines of black ink on the corn chips.

Prior to testing each rat was infused following the procedure described in the previous experiment while in its home cage which was placed in the center of the open field. The rat was then dumped gently from its cage into the center of the open field. The number of squares which were entered into by both front feet were counted for each minute over the next 10 min period.

On successive days each rat was injected with 2 μl of

carbachol (1 $\mu g/\mu l$) or saline (0.9% NaCl) in a counterbalanced design.

Following the experiment the rats were killed, their brains were removed and later sectioned and stained with cresyl violet. All cannulas were in the intended area.

RESULTS

The mean amount of food eaten following carbachol infusion was 2.6 g (± 1.9) while 7.2 g (± 1.1) was eaten following saline infusion ($t=4.9$, $p<0.01$).

In the open field, the mean number of squares crossed following carbachol infusion was only 19 (± 20.3), significantly less than the 107 crossed following saline infusion (± 28.5; $t=5.4$; $p<0.01$).

GENERAL DISCUSSION

The cholinergic agonists carbachol or physostigmine each produce a nonspecific suppression of behavior when infused ventral to the anterior septum. This suppression is manifested in a decreased level of reactivity to the experimenter, a decrease in general activity, and a decrease in feeding by a hungry rat. The failure of the infusion of the cholinergic antagonists to produce a large and consistent increase in reactivity to the experimenter also indicates that the suppression of reactivity by the cholinergic agonists is not specific to this dimension of behavior. Together these results indicate that cholinergic fibers in the region ventral to the anterior septum are not involved in the modulation of reactivity to the experimenter.

The failure of these and previous experiments to implicate noradrenergic, dopaminergic, cholinergic, or glutaminergic synapses in the modulation of defensiveness (i.e. reactivity) toward the experimenter raises the question of whether synapses in this region are involved in modulating this dimension of behavior. A tempting alternative is that only fibers of passage through this area are involved. There is at least one reason for supposing that the transmitter(s) mediating defensiveness in the region ventral to the anterior septum has not yet been tried. This is the observation that the neural system mediating defensiveness in the medial hypothalamus also does not appear to function on the basis of a cholinergic, dopaminergic, or noradrenergic transmitter. This consistency between areas would appear to favor the conclusion that the transmitter in the neural system modulating the inhibition of defensiveness has not yet been found.

A parallel should be noted between the failure of intracranial infusion experiments to identify a transmitter substance modulating the inhibition of defensiveness or attack by the medial hypothalamus, the region ventral to the anterior septum, and the lateral septum [3, 5, 7] with a similar state of affairs for the excitatory neural systems in the lateral hypothalamus controlling attack behavior. While some evidence had suggested that intracranial infusion of cholinergic but not noradrenergic substances could modulate attack behavior [9, 10, 20], more recent evidence suggests that the modulation of attack behavior by the cholinergic agents in the lateral hypothalamus was also via nonspecific mechanisms [1, 14, 24, 32].

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