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Suppression of Mouse Killing by Lateral Hypothalamic Infusion of Atropine Sulfate in the Rat: A General Behavioral Suppression[1]

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ALBERT, D. J. *Suppression of mouse killing by lateral hypothalamic infusion of atropine sulfate in the rat: A general behavioral suppression.* PHARMAC. BIOCHEM. BEHAV. 12(5) 681–684, 1980.—Male hooded rats which had been induced to kill mice by food deprivation had intracranial cannulas implanted into the lateral hypothalamus. Injections of 3 μl to each side of the brain blocked mouse killing in 12 out of 15 animals when the injected substance was the general blocking agent lidocaine. Atropine sulfate produced a significant suppression of mouse killing at a dose of 15 μg to each hemisphere but not with doses of 1.8 or 6.0 μg. The 15 μg but not the 1.8 μg dose also suppressed food intake. It is argued that the cholinergic antagonist atropine sulfate only suppresses mouse killing at high doses which produce a general suppression of behavior.

Aggression Atropine sulfate Cholinergic Lateral hypothalamus Mouse killing

CHOLINERGIC neurons have been implicated in the lateral hypothalamic control of mouse killing in the rat. The cholinergic agonist carbachol facilitates [4,5] or induces [8, 14, 19] mouse killing when injected into the lateral hypothalamus but not in the medial hypothalamus. Adrenergic agonists injected at the same sites are without effect [4]. Injections of a cholinergic antagonist have been shown to block mouse killing in one study [19] but not in another [4]. The agonist and antagonist counteract one another when injected together [19].

While this evidence for a cholinergic system modulating mouse killing appears substantial, important deficiencies become apparent when the experiments are considered in detail. The minimum dose of carabachol required to induce attack is 20 μg, considerably higher than that used in other instances where this cholinergic agonist affects thirst, avoidance learning, temperature regulation, EEG activity (see [15] for numerous examples). Also, there is a delay of 1 to 3 hr following the injection before the attack behavior appears [8,19]. This is much longer than the latency with which other chemically induced behaviors appear. The dose of cholinergic antagonist used to suppress attack is also high (methyl atropine, 20 μg) when compared to that required to alter thirst and avoidance learning (1–5 μg, [6, 11, 12]). However, no one has examined whether the high dose of antagonist is necessary.

EXPERIMENT 1: SUPPRESSION OF MOUSE KILLING BY ATROPINE SULFATE

The object of this first experiment was to obtain a dose-response curve for the suppression of attack by a cholinergic antagonist. If suppression of attack occurs only with unusually high doses of antagonist it will question the direct involvement of cholinergic neurons in the induction of attack. The antagonist injected was atropine sulfate because this antagonist has been widely used.

METHOD

Subjects

Fifteen naive male hooded rats weighing 350 to 400 g (Canadian Breeding Farms and Laboratories) were used. All were preselected for mouse killing from a larger group of rats. This was done by housing the rats individually and maintaining them on a food deprivation schedule (20 g/day). They were tested for mouse killing once a day for 7 to 10 days. Those that killed within 1 min on two successive days were used.

Surgery

All animals were first anesthetized with sodium pentobarbital anesthesia (60 mg/kg). Bilateral stainless steel cannulas (23 g) aimed at the lateral hypothalamus were then put in place (see [2,3] for details of the cannulas). The cannula placements are shown in Fig. 1.

Behavioral Testing

Four days following surgery the animals were again placed on a food deprivation schedule. After they had killed

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TABLE 1

MOUSE KILLING FOLLOWING THE INFUSION OF VARIOUS SUBSTANCES INTO THE LATERAL HYPOTHALAMUS. FIGURES IN PARENTHESES ARE SEM

Substance infused	N	Latency to kill Pretest	Postinjection	Proportion not killing (postinfusion)
Lidocaine	15	18 (±1.5)	227 (±8.2)‡	9/15*
1.8 μg atropine	8	23 (±4.3)	16 (±2.8)	0/8
6.0 μg atropine	13	16 (±1.6)	60 (±9.0)	3/13
15 μg atropine	13	26 (±2.6)	158 (±11.6)§	5/13†
0.9 NaCl	15	16 (±1.1)	16 (±2.0)	0.15

*$p<0.01$ from saline.
†$p<0.05$ from saline.
‡significantly different from pretest $p<0.001$.
§significantly different from pretest $p<0.01$.

a mouse within 30 sec of its presentation in their home cage on two successive days, behavioral testing with drug injections began. Four to five days were required to reach this criterion.

Examination of the effect of drugs on mouse killing began by placing a mouse in the rat's home cage. If the rat killed the mouse within 45 sec testing continued. If the kill took longer, no further testing was done on that day. Animals which were to continue had the plug removed from their cannula and a 30 ga stainless-steel injection needle inserted. The needle had a 45° bevel at the tip and when in place extended 0.5 mm beyond the tip of the cannula. The needles were connected to a 50 μl syringe (Hamilton) by 1 m of polyethylene tubing (Intramedic, PE 50). The tubing was sheathed with a wire spring to prevent the rat from biting through it.

The rat was returned to its home cage, and after a 3 min interval, infusion of the test substance began. A Sage Instruments syringe pump was set to infuse at a rate of 1 μl/3 min to each side of the brain. A total of 3 μl was injected bilaterally. Slow infusion of the drug was used because this procedure has proven effective in previous experiments [2,3].

Following infusion of the test substance, a 1 min interval was allowed to elapse during which the rat was left undisturbed. With the injection needles still in place, a mouse was then placed in the rat's cage and left for a maximum of 5 min. The latency to a killing attack was then recorded. If the rat killed the mouse, the dead mouse was removed shortly afterward. The test session was terminated at the end of the 5 min period. The needles were removed, and the plugs reinserted into the cannulas.

Most animals were tested in this way on 6 occasions. There was a minimum of 48 hr between tests.

The first drug injected in most animals was the general blocking agent lidocaine (Xylocaine, 2%, Astra). This allowed us to ascertain that the cannulas were in an area for suppressing mouse killing. Subsequent injections were of physiological saline (0.9% NaCl), 0.6 μg/μl atropine sulfate (Allen-Hanburys, in distilled water); 2.0 μg/μl atropine sulfate; or 5.0 μg/μl atropine sulfate. The order of injection of these latter solutions was randomized.

At the termination of the experiment the animals were killed and their brains removed. These were subsequently sectioned and stained with cresyl violet.

RESULTS

Lidocaine infusions resulted in a suppression of mouse killing for the full 5 min in 9 out of the 15 animals tested. Three other animals had a latency to kill of 2 min or more. The mean postinfusion latency to kill in the entire group was 227 sec, significantly longer than the preinfusion latency of 18 sec ($t=7$, $p<0.001$) (see Table 1).

Saline injections did not block killing in any of the 15 animals. The mean latency to kill following the infusion (16 sec) also did not differ from the preinjection latency (16 sec, $t=0.1$, $p>0.20$).

Mouse killing was not substantially suppressed in any of the 8 animals infused with the lowest dose of atropine sulfate (1.8 μg). The latency to kill following the infusion (16 sec) was not significantly different from the pretest latency (23 sec, $t=0.1$, $p>0.20$).

With the 6.0 μg dose of atropine sulfate, the mean latency to kill (60 sec) was again not reliably above the preinjection baseline level (16 sec, $t=0.5$, $p>0.20$). Three of 13 animals in this group did not kill in the 5 min test period, but the change in latency of the other animals was negligible.

Following the 15 μg dose of atropine sulfate the mean latency to kill (158 sec) was significantly longer than the pretest latency (26 sec, $t=3.8$, $p<0.01$). Five of 13 animals did not kill, significantly more than with saline ($p<0.01$, Fisher exact probability test). Three other animals had latencies of between 1 and 2 min and the remainder were at the preinjection level.

The distribution of the injection sites is shown in Fig. 1. The injection site from the one animal which had asymmetrically placed cannulas is not shown and the data from this animal has been eliminated. Killing was not suppressed in this animal by any of the drug.

Additional Observations

The sites at which lidocaine was effective in suppressing mouse killing were the same ones at which atropine sulfate was effective. In the animals which killed following the injections, the kill occurred in the species typical manner of a bite on the back behind the head. Lidocaine produced a lowering of activity in some animals. This was noticeable but to a lesser degree with the highest doses of atropine used.

Three animals in which lidocaine had blocked mouse kill-

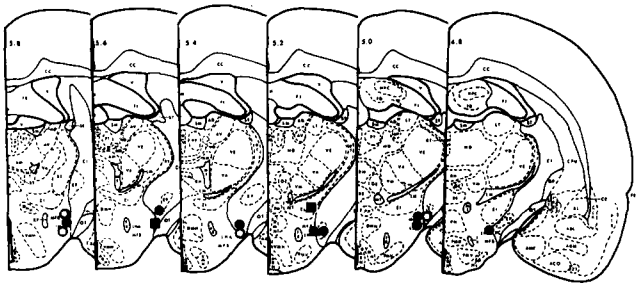

FIG. 1. The location of the injection sites in the hypothalamus. Sites producing suppression only with lidocaine are shown as empty circles. Sites producing suppression with atropine as well as lidocaine are shown as filled circles. Sites where lidocaine was ineffective are shown as squares. Brain sections are from Pellegrino and Cushman [17].

ing for the full 5 min during the first test were retested with lidocaine following the infusion of all other drugs. Each of these animals again showed a complete suppression of mouse killing.

DISCUSSION

The present findings confirm the previous observation that intracranial injection of a cholinergic antagonist into the lateral hypothalamus will suppress mouse killing [12] but they also raise questions about the mechanism by which this occurs. Most significantly, the dose-response data obtained suggests that the suppression of mouse killing occurs at a higher dose than should be necessary to produce a cholinergic blockade. Studies have shown alterations of other behaviors by intracranial injections of considerably smaller doses: appetitive responding (4.0 μg, [20]), avoidance behavior (0.5–5 μg, [10]), drinking (2–3 μg; [13]). Yet, infusion of 6 μg in the present experiment produced only a marginal effect. It did suppress killing in three animals but was without effect in the remaining 10.

EXPERIMENT 2: SUPPRESSION OF FEEDING WITH ATROPINE SULFATE

The possibility that the 15 μg dose of atropine sulfate suppresses killing by a pharmacologically and/or behaviorally nonspecific blocking action must be considered. Nonspecific pharmacological blocking effects have been shown with atropine sulfate [7]. Arguing against a nonspecific pharmacological blocking action is the observation that cholinergic agonists and antagonists apparently interact appropriately in their effects on mouse killing [19]. The possibility of a behaviorally nonspecific effect is not ruled out by previous experiments.

This second experiment examined the behavioral specificity of the suppression of mouse killing by 15 μg of atropine sulfate by observing whether such injections also suppress food intake.

METHOD

Subjects

The 6 animals used were similar to those described in the first experiment. Cannulas were implanted as described above.

Behavioral Testing

Four days following surgery the animals were put on a food deprivation schedule during which they were fed 25 g/day (Purina Lab Chow pellets). On the eighth day, they were allowed to eat wet ground Lab Chow in their home cage instead of the pellets. On the ninth day, each rat was allowed to eat wet ground Lab Chow in the test chamber for a 10 min period. The test chamber was a 25×30×45 cm high Plexiglas chamber.

Measurement of food intake following injections was done on Days 10, 11, and 12. On each of the three test days, each animal was injected with 3 μl of one of the following substances: 0.9% NaCl, 0.6 μg/μl atropine sulfate, or 5 μg/μl atropine sulfate. The order of injection of these substances was randomized. The injection procedure followed was identical to that of the previous experiment (3 μl over 9 min).

Beginning 1 min after each injection was complete, the animal was allowed to eat from a dish of wet ground Lab Chow for 5 min. The food dish was weighed before and after eating. Animals were given Lab Chow pellets in their home cage following testing to bring their total daily food intake up to 25 g/day.

Histology

Following the experiment in the brain of each animal was removed and subsequently sectioned and stained with cresyl violet.

RESULTS

Injection of 15 μg of atropine sulfate into the lateral hypothalamus significantly reduced food intake in the 5 min eating period. Following the 15 μg dose food consumption was 0.9 g (±0.1) as compared to 4.8 g (±0.3) following 0.9% NaCl (t_D 9.0, $p<0.01$). When injected with a low dose of atropine sulfate (1.8 μg), the mean food consumed was 4.3 g (±0.2), not significantly different from that following saline.

The 15 μg dose of atropine sulfate did not obviously incapacitate the animals. Half of the animals began eating as soon as food was presented but stopped within about 1 min. The other animals sniffed the food and nibbled at it but did not really eat (i.e., 0.3 g or less consumed). The animals were active during the feeding period but less so than at the start of the injection. Two animals circled slightly following the 15 μg dose. Animals injected with the lower dose of atropine appeared no different than those injected with saline.

The placements of the cannulas in these animals were similar to those of Experiment 1.

GENERAL DISCUSSION

Atropine sulfate injected into the lateral hypothalamus only suppresses mouse killing when injected at a high dose level. The fact that the high dose also suppresses feeding indicates that the high dose of atropine is producing a nonspecific suppression of behavior rather than a specific suppression of mouse killing. While a high dose of atropine sulfate is required to strongly alter mouse killing and feeding, much lower doses alter drinking [13,18], avoidance responding [10], tremor [9] and learning [10,20].

Anatomical considerations also suggest that atropine sulfate is producing a nonspecific suppression of mouse killing. In the present experiment there is no clear correspondence between the brain regions where atropine suppress mouse killing and those where electrical stimulation elicits this be-

havior [1, 16, 21]. The most effective sites for electrically inducing attack on a mouse appear to be in the lateral hypothalamus at the level of the rostral end of the ventromedial nucleus [1, 16, 31]. In contrast, both the suppression and elicitation of mouse killing by cholinergic agents appear to occur with injections along the entire length of the lateral hypothalamus [8,16].

To summarize, the present experiments confirm that cholinergic antagonists can suppress mouse killing when infused into the lateral hypothalamus. However, the effect occurs only at a high dose which also suppresses food intake. Also, the regions where the drug is effective do not appear to be limited to the sites where electrical stimulation induces killing behavior.

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