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Evaluation of Adrenergic, Cholinergic and Dopaminergic Involvement in the Inhibition of Hyperreactivity and Interanimal Aggression by the Medial Hypothalamus in the Rat[1]

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ALBERT, D. J., R. C. K. WONG, K. N. BRAYLEY AND H. C. FIBIGER. *Evaluation of adrenergic, cholinergic and dopaminergic involvement in the inhibition of hyperreactivity and interanimal aggression by the medial hypothalamus in the rat.* PHARMAC. BIOCHEM. BEHAV. 11(1) 1–10, 1979.—Intracranial infusions were made bilaterally through permanently implanted cannulas ending in the medial hypothalamus. The rats were first screened for changes in reactivity, muricide and intermale aggression using infusions of the local anesthetic, lidocaine. Those animals which showed an increase in reactivity and/or muricide were then infused with a transmitter antagonist. The results showed the the α-adrenergic antagonists tolazoline and phentolamine reproduced the induction of increased reactivity muricide and intermale aggression. The dopaminergic antagonist haloperidol produced only a slight increase in reactivity to the experimenter. Cholinergic (atropine) or β-adrenergic (propranolol, hydralazine) antagonists were without effect. In a subsequent experiment the induction of hyperreactivity and muricide by infusion of the local anesthetic lidocaine or the α-adrenergic antagonist tolazoline and phentolamine reproduced the induction of increased reactivity, muricide and intermale aggression. The dopaminergic antagonist haloperidol produced only a slight increase in reactivity to the depleted by 76%. Also, prior injection of 6-OHDA directly into the hypothalamic infusion site did not attenuate the induction of hyperreactivity or muricide by lidocaine or tolazoline. In neither of these experiments did the depletion of noradrenaline itself produce an increase in reactivity or muricide relative to vehicle-injected control animals. In a final experiment the induction of hyperreactivity and muricide by tolazoline was not counteracted by the noradrenergic agonist clonidine. It is inferred that the medial hypothalamic system controlling reactivity and muricide is not noradrenergic and that at the doses used in the present experiment, the blocking action of tolazoline does not appear to be specific to α-adrenergic synapses.

Atropine Haloperidol Hydralazine 6-Hydroxydopamine Intermale aggression Medial hypothalamus Mouse killing Phentolamine Propranolol Reactivity Tolazoline Ventral noradrenergic bundle

THE EXISTENCE of an α-adrenergic system modulating reactivity and aggression in the region ventral to the anterior septum has recently been suggested [4]. Infusion of two α-adrenergic antagonists, tolazoline or phentolamine, induces increased reactivity while β-adrenergic (propranolol, hydralazine), cholinergic (atropine, scopolamine) and dopaminergic (haloperidol) antagonists are without effect. This evidence that a noradrenergic transmitter substance is involved in the modulation of reactivity coincides with a variety of previous findings pointing to an involvement of noradrenergic systems in the modulation of aggression (see [4, 8, 40] for recent reviews).

The question immediately arises as to whether the medial hypothalamus which seems to be involved in the inhibitory modulation of reactivity and aggression might also be sensitive to α-adrenergic antagonists. Electrolytic lesions there increase reactivity to an experimenter and induce muricide in some animals [15,32]. More recently it has been shown that temporary lesions produced by microinfusion of the general blocking agent lidocaine also induce reactivity and muricide, and in addition, intermale aggression [5]. In a previous attempt to assess the pharmacology of this system [23], crystalline implants of noradrenergic, cholinergic or dopaminergic transmitter antagonists were without effect. However, no

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independent evidence was presented at that time to show that the implants were in close proximity to the medial hypothalamic neurons inhibiting reactivity and aggression.

EXPERIMENT 1

Hyperreactivity and Interanimal Aggression Induced by Intrahypothalamic Infusions of α-Adrenergic but not β-adrenergic, Cholinergic or Dopaminergic Antagonists in the Rat

The object of the first experiment was to use infusions of various agents into the medial hypothalamus to examine the pharmacology of the system there controlling reactivity and aggression. The procedure was to implant bilateral cannulas and then infuse the general blocking agent lidocaine. Animals that showed an increase in reactivity with lidocaine infusion were considered to have their cannulas in close proximity to the neural system inhibiting these behaviors. These animals were subsequently infused with an α-adrenergic, β-adrenergic, cholinergic, or dopaminergic transmitter antagonist at doses which have been effective in previous experiments [9, 22, 30, 33, 38, 45] using intracranial administration. The effect of the antagonists on reactivity, muricide and intermale aggression was then observed.

METHOD

Animals

The animals were 80 male hooded rats weighing 300–400 g (Canadian Breeding Farms and Laboratories). They were housed in group cages prior to surgery and in individual cages thereafter.

Surgical Procedure

Injection cannulas made from 23 g tubing were implanted bilaterally into the medial hypothalamus using sodium pentobarbital anesthesia and standard surgical procedures. The coordinates used were: posterior to bregma 0.0 mm, lateral to the midline 1.2 mm, ventral to the cortical surface 8.5 mm. The mouth bar was set 5.0 mm above the interaural line. Immediately following surgery, cannula plugs were put in place. Injection needles for the cannulas were made from 30 g tubing [5]. When in place the injection needle extended 0.5 mm beyond the end of the cannula.

Behavioral Testing

Behavioral testing was done between 7 and 14 days postoperatively. The cannula plugs were removed and the injection needles inserted without the use of anesthesia. The animal was then placed on the floor of the test chamber, an open box measuring 60 × 60 × 60 cm (for details see [5]). Each injection needle was continuously connected to a control syringe (50 μl, Hamilton) by one metre of polyethylene tubing (P.E. 50, Intramedic). The needle was secured to the cannula by a polyethylene collar covering the joint between the injection needle and the top of the cannula.

The rat was allowed to explore the test box for 5 to 10 minutes before testing began. Behavioral testing began by ascertaining the animal's behavioral baseline prior to any infusion. The animal was examined for reactivity to the experimenter using a 3-point scale to score the response to each of 6 stimuli (presentation of a pencil, tap on the back, presentation of a glove, prod on the side, grasping of tail, and grasping around abdomen) and on the amount of vocalization and amount of biting in response to the test stimuli (see Albert and Richmond [2,3] for details of this procedure). The maximum score possible was 24. The rat was then examined for muricide by placing an adult albino mouse next to it for 15–20 sec and then lastly for intermale aggression by placing an adult hooded rat next to it for 15–20 sec. Intermale aggression consisted of a biting attack on the target animal. The animals were always tested in the sequence described with the following exception. Once muricide had occurred during the infusion period, muricide and intermale aggression were not tested again until the final postinfusion reactivity test. The number of mouse kills was limited in this way because our experience has been that with repeated mouse killing, the behavior sometimes becomes habitual and independent of the hypothalamic infusion.

After this behavioral baseline was established, the rat was infused with 1 μl of lidocaine (Xylocaine, 2%, Astra) every 5 min up to a total of 4 μl (bilaterally). The infusion rate (1 μl/3 min) was controlled automatically by a Sage Instruments pump. Behavioral observations of reactivity and aggressiveness to the experimenter, muricide and intermale aggression began approximately 15 sec following the infusion of each μl. Following the infusion, behavioral testing continued every 5 min to confirm that behavior returned to its pre-injection baseline.

Two to 3 days following the lidocaine infusion, all animals showing an increase in reactivity (5 points or more) or muricide were tested with the identical procedure except that the infusate was one of the following transmitter antagonists: atropine (0.4 μg/μl, cholinergic, Glaxo-Allenbury), tolazoline (25 μg/μl, α-adrenergic, Ciba), phentolamine (5 μg/μl, α-adrenergic, Rogitine, Ciba), hydralazine (20 μg/μl, β-adrenergic and monoamine oxidase inhibitor, Apresoline, Ciba), propranolol (1 μg/μl, β-adrenergic, Inderal, Ayerst) and haloperidol (5 μg/μl, dopaminergic, Haldol, McNeil). All solutions infused were the commercial preparations. Only one of the substances (tolazoline) in a solution with additives was substantially effective in altering reactivity and aggression. Tolazoline has been observed to produce the same effects on reactivity and aggression when dissolved in 0.9% NaCl as reported here (unpublished results). A control group was infused with 0.9% NaCl.

Following behavioral testing, the animals were killed with CO_2. Their brains were placed in formol-saline and later sectioned on a cryostat and then stained with thionin.

Statistical analysis of the results utilized Dunnett's test for comparing several means with a single control and *t*-tests [52]. Nonparametric comparisons of muricide and intermale aggression frequency were done with Fisher's exact probability test. Unless otherwise indicated *p* values are two-tailed.

RESULTS

The α-adrenergic antagonists, tolazoline and phentolamine, were most effective in reproducing the hyperreactivity induced by the local anesthetic (Table 1, Fig. 1). With both of these groups, the increases in reactivity are significantly higher than those produced in saline-infused control animals (T_d=5.4, $p<0.01$; T_d=4.4, $p<0.01$). The hyperreactivity in each case was slightly but significantly lower than that produced by the general blocking agent lidocaine (t=2.5,

TABLE 1

THE EFFECT OF INJECTING LIDOCAINE OR VARIOUS TRANSMITTER ANTAGONISTS INTO THE MEDIAL HYPOTHALAMUS ON REACTIVITY TO THE EXPERIMENTER. TABULATED ARE THE MEAN INCREASES ABOVE THE PREINJECTION BASELINE AND THE STANDARD DEVIATIONS

Blocking Agent	N	Lidocaine	Drug
α-Adrenergic			
Tolazoline	8	9.6 (±3.6)	6.8 (±2.7)†
Phentolamine	7	10.9 (±4.0)	6.1 (±3.0)†
β-Adrenergic			
Hydralazine-Propranolol	8	8.9 (±3.3)	0.9 (±1.3)
Cholinergic			
Atropine	9	8.1 (±2.6)	0.1 (±0.5)
Dopaminergic			
Haloperidol	8	8.2 (±2.0)	3.9 (±2.9)*
Control			
0.9% NaCl	8	8.6 (±2.4)	0.2 (±0.8)

*Significantly different from control $p<0.05$.
†Significantly different from control $p<0.01$.

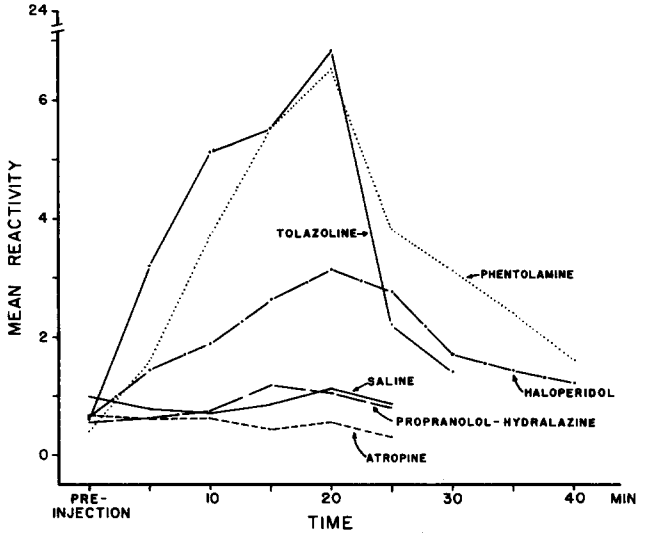

FIG. 1. The time course of the behavior change produced by the infusion of various substances in the medial hypothalamus. Infusions of 1 μl occurred prior to the 5, 10, 15 and 20 min tests. The data are the actual reactivity scores obtained, not increases above the preinjection baseline.

$p<0.05; t=2.6, p<0.05$). Animals that showed high increases in reactivity with lidocaine were usually the animals that showed the highest increases with the α-adrenergic antagonists.

The dopaminergic antagonist haloperidol produced an increase in reactivity (mean=3.9) that was substantially less than that produced by the α-adrenergic antagonists or the general blocking agent lidocaine but which was, nevertheless, significantly greater than that produced by isotonic saline in the control group ($T_d=2.8$, $p<0.05$). The mean score for this group is inflated by the score of one animal. The median increase in reactivity for the group is 2.7. The significance of the difference between this and the control group is primarily due to the consistency with which haloperidol induced rather small increases in reactivity.

The increases in reactivity induced by the other drugs were not significantly different from those induced by isotonic saline.

The differences between groups in the reactivity increase caused by the lidocaine infusions are not reliable [$F(5/42)=0.9$, NS]. The pretest baseline scores of all animals were between 0 and 3.

The differential effectiveness of the various transmitter antagonists is again visible in the results of the interanimal aggression tests (Table 2). With one exception, the only animals to show muricide were those infused with α-adrenergic antagonists. In the intermale aggression tests tolazoline but not phentolamine induced a significantly greater frequency of attack than did saline. Animals which displayed muricide or intermale aggression with the α-adrenergic antagonists were those that exhibited this behavior during infusion of the general blocking agent.

Detailed descriptions of the reactivity, muricide and intermale aggression induced by lidocaine can be found elsewhere as can a detailed examination of the brain sites yielding these effects [5,6].

The infusion sites are shown in Fig. 2.

In the process of screening the entire group of operated animals with lidocaine, 32 animals were discarded for not showing a reactivity increase of 5 points or more.

Additional Observation

Since tolazoline was the most effective of the specific

TABLE 2

THE EFFECT OF INJECTING LIDOCAINE OR VARIOUS TRANSMITTER BLOCKING AGENTS INTO THE MEDIAL HYPOTHALAMUS ON THE PROPORTION OF ANIMALS SHOWING MURICIDE OR INTERMALE AGGRESSION.

Drug	Muricide		Intermale Aggression	
α-Adrenergic	Lidocaine	Drug	Lidocaine	Drug
Tolazoline	5/8	6/8*	3/8	4/8†
Phentolamine	6/7	3/7‡	4/7	1/7
β-Adrenergic				
Hydralazine-Propranolol	4/8	1/8	4/8	0/8
Cholinergic				
Atropine	6/9	0/9	5/9	0/9
Dopaminergic				
Haloperidol	2/8	0/8	3/8	0/8
Control				
0.9% NaCl	4/8	0/8	2/8	0/8

*Significantly different from the effect of 0.9% NaCl; $p<0.01$.
†Significantly different from th effect of 0.9% NaCl; $p<0.05$, one-tailed test.
‡Significantly different from the effect of 0.9% NaCl; $p=0.08$, one-tailed test.

TABLE 3

A COMPARISON OF THE MEAN INCREASE IN REACTIVITY AND AGGRESSION PRODUCED BY THREE DIFFERENT DOSES OF TOLAZOLINE. STANDARD DEVIATIONS ARE SHOWN IN PARENTHESES

		Lidocaine		Tolazoline	
Dose	N	Increase in Reactivity	Frequency of Muricide	Increase in Reactivity	Frequency of Muricide
25.0 μg/μl	8	9.6 (±3.6)	5/8	7.4 (±3.3)	6/6
12.5 μg/μl	5	11.5 (±3.4)	5/5	8.4 (±2.8)	5/5
6.0 μg/μl	3	9.2 (±4.0)	3/3	1.8 (±1.3)	0/3

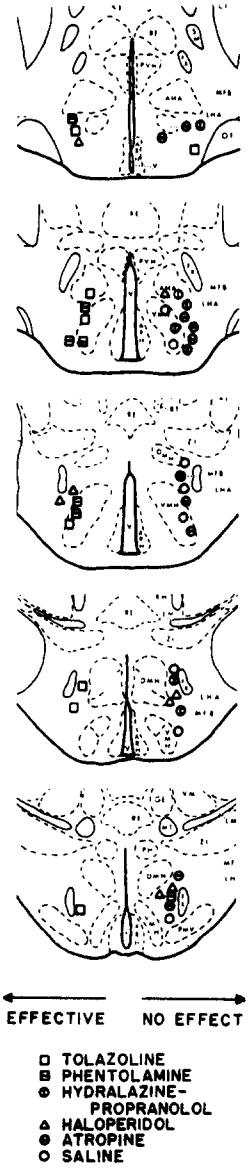

FIG. 2. The placement of the injection cannulas in animals receiving various transmitter blocking agents. Injections of lidocaine produced increases in reactivity of at least 5 points and/or interanimal aggression at each site, but only the sites on the left were effective when the indicated transmitter blocking agent was used.

transmitter antagonists in reproducing the effect of lidocaine, a dose response curve was obtained for this substance. The animals were tested in the manner already described except that at the two lower dose levels, 3 instead of 4 μl were injected into each hemisphere.

The results are shown in Table 3. The data from the animals infused with 25 μg/μl (Table 1) are also included in this table. The results for the group injected with 12.5 μg/μl are entirely similar to those of the group injected with 25 μg/μl. The effect of the drug appears to drop off sharply when the concentration is lowered to 6.3μg/μl.

DISCUSSION

The overall pattern of results suggests that a medial hypothalamic α-adrenergic inhibitory system modulates reactivity and aggression. The α-adrenergic antagonists induced each of the behaviors that were induced by the general blocking agent. For the most part they induced these behaviors to a similar degree as the lidocaine infusions. An aspect of these results that deserves special emphasis is that the behavior changes were induced by both α-adrenergic antagonists. In contrast, the β-adrenergic, dopaminergic, or cholinergic antagonists were ineffective in reproducing the effect of lidocaine.

There is considerable support for the conclusion that noradrenergic systems are involved in the inhibition of reactivity and aggression. Three recent reviews each conclude that increasing noradrenaline levels in the central nervous system decreases muricide [7, 8, 40]. This is consistent with the present observation that noradrenergic antagonists induce mouse killing. However, these conclusions are based upon observations following systemic administration of amphetamine, L-DOPA, or tricyclic antidepressants [28, 29, 42]. The indirectness of these manipulations greatly limits confidence in conclusions drawn from them. There does not seem to be any biochemical or pharmacological evidence available that can be closely tied to the increase in reactivity and muricide that is produced by medial hypothalamic lesions.

EXPERIMENT 2

No Effect of Noradrenergic Depletion of the Dorsal and Ventral Noradrenergic Bundles on Reactivity and Muricide or on the Induction of these Behaviors by Medial Hypothalamic Infusion of Lidocaine or Tolazoline

The availability of an agent for chemically lesioning the central catecholamine systems makes it possible to determine

TABLE 4

BEHAVIORAL EFFECTS OF INFUSING LIDOCAINE OR TOLAZOLINE INTO THE MEDIAL HYPOTHALAMUS IN RATS OR IN RATS WITH DORSAL AND VENTRAL BUNDLES DEPLETED OF NA. STANDARD DEVIATIONS ARE IN PARENTHESES

Group	N	Lidocaine			Tolazoline		
		Preinjection Baseline	Increase in Reactivity	Frequency of Muricide	Preinjection Baseline	Increase in Reactivity	Frequency of Muricide
NA Depleted	8	1.6 (±0.7)	8.6 (±3.1)	6/8	1.2 (±0.9)	6.9 (± 2.8)	5/8
Control	7	1.7 (±0.9)	8.2 (±1.9)	2/7	0.8 (±0.9)	6.2 (± 1.8)	2/7

whether the induction of reactivity and aggression by the α-adrenergic antagonists phentolamine and tolazoline occurs by virtue of a specific interference with noradrenergic transmission. This agent is the neurotoxin, 6-hydroxydopamine (6-OHDA). The 6-OHDA is selectively taken up by noradrenergic and dopaminergic neurons and is toxic to them, thus creating a chemical lesion [27, 50, 51].

In this experiment we examined whether the induction of reactivity or muricide by either the general blocking agent lidocaine or the α-adrenergic antagonist tolazoline would be impaired by severe damage to two of the main noradrenergic fiber systems, the dorsal and ventral noradrenergic bundles [51]. If the medial hypothalamic systems mediating reactivity and interanimal aggression are noradrenergic, the damage to these systems caused by the 6-OHDA could have several effects. First, it could cause an increase in reactivity and aggression just as do nonspecific lesions in this area [5, 15, 32]. Second, because the medial hypothalamic noradrenergic system is damaged, the intracranial infusion of pharmacological agents which would further decrease its level of activity could have a different effect than in intact control animals.

METHOD

Animals

The animals were 15 male hooded rats.

Surgical Procedure

Injections of 6-OHDA (Regis Chemical Co.) were made using a 10 μl Hamilton syringe with a 37 ga tip. The volume injected was controlled with a micrometer drive. The 2 μg/μl of 6-OHDA was dissolved in a solution of 9.0 mg/ml of NaCl and 0.3 mg/ml ascorbic acid. Two μl were injected into each hemisphere over a 10 min period into the region just rostral to the locus coeruleus where the dorsal and ventral noradrenergic bundles begin to separate [51]. The injection coordinates were 1.0 mm anterior to the interaural line, 1.5 mm lateral to the midline, and 3.0 mm dorsal to the interaural line. The mouth bar was located 4.2 mm below the level of the interaural line. Control animals were injected with the vehicle alone.

Infusion cannulas were implanted bilaterally in the medial hypothalamus immediately following the 6-OHDA injections. The placement coordinates were the same as in the previous experiment.

Behavioral Testing

Behavioral testing, which was done blind, began 14 days following surgery. The procedure was the same as that described in Experiment 1. All animals were infused with lidocaine and then three days later with tolazoline (25 μg/μl).

Biochemical Analysis

Three days following the second behavioral testing, the animals were killed by cervical dislocation. The brain was immediately removed and analyzed for noradrenaline using the procedures described by McGeer and McGeer [34].

RESULTS

There was no difference between the noradrenaline (NA) depleted and control groups on the initial baseline reactivity level (1.6 and 1.7 respectively; $t=0.2$, $p>0.20$; Table 4). Similar baseline scores were obtained on the second test day.

The group depleted of NA by 6-OHDA also did not differ from the control group in the effectiveness with which lidocaine elicited hyperreactivity (8.6 and 8.2, respectively; $t=0.3$, $p>0.20$: Table 4) or muricide (6/8 and 2/7, respectively: $p>0.10$).

Infusion of the α-adrenergic antagonist tolazoline induced changes in reactivity and muricide very similar to those produced by lidocaine.

As in Experiment 1, tolazoline induced hyperreactivity and muricide in the same animals that showed such effects with lidocaine.

Biochemical Analysis

Biochemical analysis revealed a NA content of 0.309 (± 0.090) μg/g brain in the four control animals examined. The mean NA content of the animals injected with 6-OHDA was 0.079 (±0.030) μg/g brain, 74% below the control level. The recovery of the standard was 72%. The data in this and subsequent experiments are not corrected for this.

DISCUSSION

The depletion of NA indicates that the neurons in the dorsal and ventral noradrenergic bundles are extensively damaged. Accordingly, if these noradrenergic systems include the hypothalamic neurons inhibiting reactivity and muricide, the behavioral result should have been similar to that of an electrolytic lesion. The similar behavioral baseline ratings of NA depleted and control groups clearly indicates that no substantial lesions has been produced in the medial hypothalamic systems controlling reactivity and muricide. Similarly, the absence of an effect of NA depletion on the

induction of increased reactivity and muricide by medial hypothalamic infusion of lidocaine or tolazoline argues that the infusion is not producing its effect on behavior by altering the activity of neurons from the dorsal and ventral noradrenergic systems which were depleted of NA by 6-OHDA.

There are, of course, lines of reasoning by which the applicability and validity of this argument can be questioned. The most serious objection would seem to be that the level of NA depletion in the hypothalamus may not be sufficient to strongly affect the functioning of noradrenergic systems there. While the depletion overall is 76%, it is known that the level of depletion in the hypothalamus in particular is more likely about 70% [41].

EXPERIMENT 3

No Effect of Hypothalamic NA Depletion at the Infusion Site on Reactivity and Muricide or on the Induction of these Behaviors by Infusion of Tolazoline or Lidocaine

The major limitation of the previous experiment arises from the fact that the depletion of NA was produced by damaging fibers in the dorsal and ventral noradrenergic bundles. While these are major noradrenergic pathways, there is no way to be sure that they contain the medial hypothalamic fibers involved in the control of reactivity and muricide. To more conclusively determine whether NA fibers are involved in the control of reactivity and muricide it is necessary to ensure that NA fibers in the vicinity of the tolazoline injection are depleted of NA.

The present experiment attempted to accomplish this NA depletion at the infusion site by making 6-OHDA injections at that very site immediately prior to the implantation of the cannulas.

METHOD

Animals

The animals were 12 male hooded rats.

Surgical Procedure

Surgery was as described in the first experiment. The injections of 6-OHDA or vehicle were made into the precise medial hypothalamic site at which the infusion needle tips would be located (see Experiment 2). Four μg of 6-OHDA in 2 μl of vehicle were injected into each side of the brain. Immediately after the injections, the cannulas were put in place (see Experiment 2). Because we intended to do histology on the behaviorally tested animals, a separate group of 6 animals injected with 6-OHDA and 3 with the vehicle were operated at the same time as the others and then set aside for biochemical analysis.

Behavioral Testing

Behavioral testing, which was again done blind, began at 48 hr following surgery with the infusion of lidocaine. The use of the short delay prior to testing was done with the intent of having the behavioral testing done as soon as the NA levels were strongly depressed by the 6-OHDA. Infusion of tolazoline was done at 72 hr following surgery. Details of the infusion and testing procedure were the same as in Experiment 1, except that only 3 rather than 4 μl of each drug was infused.

Biochemical Analysis

Biochemical analysis was also carried out at 72 hr following surgery. In this experiment, the hypothalamus was dissected out and it alone was subjected to the biochemical analysis.

RESULTS

The depletion of NA in the medial hypothalamus did not in itself produce an increase in reactivity or muricide (Table 5). At the preinjection test, 48 hr following the 6-OHDA injection, the mean baseline reactivity rating was 1.1 for the 6-OHDA-injected group and 0.7 for the vehicle-injected controls ($t=0.7$, $p>0.20$). At 72 hr, the baseline scores were 1.1 for both the 6-OHDA-and vehicle-injected groups.

Lidocaine infusion into the medial hypothalamus caused a comparable increase in reactivity in the two groups (6.9 and 7.9; $t=0.6$, $\pm$ 0.20; Table 5). Three of 7 animals in the experimental group killed mice compared to three of 5 animals in the control group ($p>0.20$).

Tolazoline injection at 72 hr following surgery produced increases in reactivity and muricide similar to that caused previously by lidocaine.

The temporal pattern of the increase in reactivity during the infusion sequence was the same for both groups and with both drugs. The increase in reactivity started with the first μl injected and increased with each additional μl as is shown in Fig. 1.

The location of the infusion sites was similar to those found to elicit hyperreactivity and muricide in Exeriment 1. There were no systematic placement differences between groups.

TABLE 5

BEHAVIORAL EFFECTS OF INFUSING LIDOCAINE OR TOLAZOLINE INTO THE MEDIAL HYPOTHALAMUS OF ANIMALS WITH MEDIAL HYPOTHALAMIC NA DEPLETION AND OF NORMAL ANIMALS. STANDARD DEVIATIONS ARE IN PARENTHESES

		Lidocaine			Tolazoline		
Group	N	Preinjection Baseline	Increase in Reactivity	Frequency of Muricide	Preinjection Baseline	Increase in Reactivity	Frequency of Muricide
NA Depleted	7	1.1($\pm$ 0.7)	6.9($\pm$ 3.3)	3/7	1.1($\pm$ 1.0)	5.9($\pm$ 3.7)	3/7
Control	5	0.7($\pm$ 1.1)	7.9($\pm$ 0.7)	3/5	1.1($\pm$ 0.7)	5.0($\pm$ 2.5)	4/5

Biochemical Analysis

The level of NA in the hypothalamus was 2.07 (± 0.24) μg/g brain in the vehicle injected control group (N=3). There was a 49% reduction or 1.06 (± 0.10) μg/g in the 6-OHDA-injected animal (N=6).

DISCUSSION.

Each aspect of the data seems to reinforce the conclusion that the neural system in the medial hypothalamus which inhibits reactivity and muricide is not noradrenergic. To begin with, the depletion of NA by the 6-OHDA should itself have increased reactivity and muricide if the neural system in question is noradrenergic. The NA depletion does constitute a lesion [16]. As such, if the lesion involves the medial hypothalamic system inhibiting reactivity and muricide there should be an increase in each of these dimensions of behavior. It must be borne in mind that the 6-OHDA lesion was in the very same location where the lidocaine and tolazoline infusions produced an increase in reactivity and muricide. Further, the behavioral testing was begun at 48 hr following the 6-OHDA injection, thereby allowing very little time for compensatory physiological and biochemical changes to occur. While the NA depletion for the entire hypothalamus is far from complete, we assume that the depletion is very high at the actual 6-OHDA injection site.

The absence of a behavioral difference between normal and NA depleted animals in response to the lidocaine infusion into the medial hypothalamus also argues that the system modulating reactivity and muricide is not noradrenergic. Given that the 6-OHDA injection is made into the precise medial hypothalamic site where lidocaine is subsequently infused, it seems only a remote possibility that the system is substantially damaged by the 6-OHDA and that this is not reflected in some measure by a difference in the effect of lidocaine in the experimental and control groups. If the system is totally incapacitated by the NA depletion, the lidocaine infusion should produce no effect at all.

Congruent with the above findings, tolazoline infusion produces the same effect in both normal and NA depleted animals. It would appear that the effect of the tolazoline does not depend on the integrity of a NA transmitter system. This seems to warrant both the conclusion that the neural system mediating reactivity and muricide is not noradrenergic and that the antagonism of tolazoline in the medial hypothalamus is not specific to α-adrenergic receptor sites.

EXPERIMENT 4

Lack of Interaction between the Adrenergic Antagonist Tolazine and the Adrenergic Agonist Clonidine

The noradrenergic antagonist tolazoline does not seem to be exerting a pharmacologically specific action in the present experiments. This shows most conclusively in its indifference to the depletion of NA at the infusion site (Experiment 3). Further, the observation that lidocaine continues to induce hyperreactivity and muricide following NA depletion suggests that the neural system modulating these behaviors is not noradrenergic and, consequently, implies that the action of tolazoline must be nonspecific in the present instance.

The pharmacological specificity of tolazoline was further examined by determing whether its actions would be antagonized by the noradrenergic agonist clonidine. The effectiveness of this substance centrally has recently received important support [47]. In the present experiment, animals were first screened with the general blocking agent lidocaine to determine which animals had cannulas that were properly placed for the induction of reactivity and muricide. On the following day, the animals giving effects with lidocaine were infused with tolazoline alone or with a mixture of tolazoline and clonidine.

METHOD

Animals

Thirty male hooded rats were used.

Surgical Procedure

These animals had cannulas implanted into the medial hypothalamus and were then tested in accordance with the procedure described in Experiment 1. The screening of the animals resulted in the selection of 11 animals to be injected with the adrenergic drugs. These animals were selected because they showed both an increase in reactivity of 5 points or more and exhibited muricide during lidocaine infusion.

Five of the animals were then infused with tolazoline (12.5 μg/μl). The remaining 6 were infused with a mixture of tolazoline (12.5 μg/μl) and clonidine (0.15 μg/μl). The use of a lower dose of tolazoline than in Experiment 2 was intended to allow more optimal conditions to observe an antagonism of tolazoline's effect by clonidine (see Table 3). The procedures for drug infusion and behavioral testing were as described in Experiment 1 except that a maximum of 3 instead of 4 μl were infused into each hemisphere.

Following behavioral testing, the brains were removed, sectioned, and stained with thionin.

RESULTS

The increase in reactivity induced by tolazoline alone was 8.4 (Table 6). In the animals infused with tolazoline and clonidine, the increase in reactivity was 7.3 ($t=0.8, p>0.20$). All 5 animals injected with tolazoline killed mice as compared to 4 out of 6 animals injected with the tolazoline-clonidine mixture ($p>0.10$).

Histological examination of the brains of the animals in this experiment showed that the placements were similar to those found to elicit muricide and hyperreactivity in Experiment 1.

DISCUSSION

The clonidine does not appear to substantially counteract the effect of tolazoline. In concern for the possibility that the clonidine might be inducing some unexpected behavior change that was interacting in an unpredictable way with tolazoline, 2 animals which had been infused with lidocaine in the screening phase were injected with the same quanitities of clonidine as were mixed with tolazoline. Clonidine alone did not induce a behavior change in either of these animals while with lidocaine both had shown increases in reactivity although not muricide.

The absence of an attenuating effect of clonidine suggests that under the present experimental conditions the effect of tolazoline is not specific to noradrenergic neurons. Since we did not do a dose response study, however, the possibility

TABLE 6

A COMPARISON OF THE BEHAVIORAL EFFECTS OF A MEDIAL HYPOTHALAMIC INFUSION OF TOLAZOLINE ALONE OR TOLAZOLINE PLUS CLONIDINE. STANDARD DEVIATIONS ARE IN PARENTHESES

Group	N	Lidocaine			Drug		
		Preinjection Baseline	Reactivity Increase	Frequency of Muricide	Preinjection Baseline	Reactivity Increase	Frequency of Muricide
Tolazoline + Clonidine	6	0.5(± 1.2)	9.5(± 2.8)	6/6	0.5(± 0.6)	7.3(± 1.3)	4/6
Tolazoline	5	0.6(± 0.5	11.5(± 3.4)	5/5	0.2(± 0.4)	8.4(± 2.8)	5/5

that the concentration of clonidine is not appropriate can not be ruled out. In our choice of the dose, we were concerned by the report of Svensson, Bunney and Aghajanian [47] that at high concentrations clonidine does not mimic the effect of norepinephrine. Stark and Montel [46] have also shown a change in the effect of clonidine with increasing concentrations. Further, the clonidine was infused with about the minimum effective dose of tolazoline. The dose of clonidine used here was lower (0.9 μg as compared to 3.0 μg) than that which others [10,49] have used to produce excitatory effects which were blocked by α-adrenergic antagonists (and in some cases other agents as well [49]). However, the higher doses used by others (3.0 μg) may well be producing nonspecific effects or effects at some distance from the injection site since about the same dose injected intravenously is sufficient to alter the firing of locus coeruleus cells in rats anesthetized with chloral hydrate [47].

GENERAL DISCUSSION

Alpha adrenergic but not β-adrenergic, dopaminergic, or cholinergic antagonists induce mouse killing and hyperreactivity when infused into the medial hypothalamus. However, except for the finding that tolazoline and phentolamine induce increased reactivity and muricide, the experiments do not support the conclusion that a medial hypothalamic noradrenergic system is involved in the inhibitory modulation of these behaviors. These is no increase in reactivity or muricide following a 6-OHDA lesion which depletes the dorsal and ventral noradrenergic bundles and which results in a whole brain NA deficit of 76%. Depletion of NA to this extent also does not diminish the induction of reactivity and muricide by intrahypothalamic infusion of the general blocking agent lidocaine or the α-adrenergic antagonist tolazoline (Table 1). More persuasive is the subsequent finding that depleting NA levels by 6-OHDA injections directly into the medial hypothalamus does not increase reactivity or muricide nor does it decrease the effectiveness of intrahypothalamic infusions of lidocaine or tolazoline in inducing reactivity and muricide (Table 2). The absence of some effect in these two experiments strongly suggests that the neural systems which are being affected by lidocaine and tolazoline are not noradrenergic. This conclusion holds regardless of whether the action of the lidocaine or tolazoline in this instance is on synaptic interfaces or fibers of passage, since 6-OHDA should destroy either. In the absence of some effect of NA depletion in Experiments 2 and 3, the lack of effect with clonidine in Experiment 4 was not surprising.

Since previous evidence has shown that α-adrenergic antagonists were effective in inducing increased reactivity when infused ventral to the anterior septum we have done pilot work to determine whether NA depletion alters that effect. Injections of 6-OHDA into the region ventral to the anterior septum did not increase reactivity in three animals, nor was the hyperreactivity induced by infusion of lidocaine or tolazoline ventral to the anterior septum attenuated by NA depletion of the dorsal and ventral noradrenergic bundles (N=6) in comparison to control animals (N=4). It would appear that the neural system in the region ventral to the anterior septum is also not noradrenergic even though that system responds to tolazoline much as does the one in the medial hypothalamus [4].

The evidence that two of the major inhibitory systems controlling reactivity and muricide (the septal and medial hypothalamic systems) do not appear to be noradrenergic is reason for caution in the interpretation of other less direct evidence for a noradrenergic involvement in the inhibitory control of these behaviors. For example, a number of studies have shown that intraventricular injections of 6-OHDA cause increased reactivity [12, 36, 48]. In tht light of the present evidence that the medial hypothalamic and septal systems are probably not noradrenergic, perhaps more consideration should be given to the possibility of damage to noncatecholaminergic neurons due to the use of very large doses of 6-OHDA with intraventricular injections. Prolonged electrical stimulation of the amygdala produces rage and a fall in NA levels [20, 24, 39]. The decreased levels of NA have been interpreted as being involved in the occurrence of the rage but there are alternatives, such as the possibility that the fall of NA levels represents a consequence rather than a cause of the rage behavior. The suppression of mouse killing by systemic administration of amphetamines and tricyclic antidepressants has been interpreted in terms of a facilitation of activity in a noradrenergic inhibitory system [28, 29, 42]. However, it is difficult to rule out conclusively indirect effects such as a general suppression of behavior, the induction of competing responses, or a suppression (direct or indirect) of the excitatory system controlling aggression.

The evidence that tolazoline and phentolamine did not have a specific blocking action is not entirely surprising. The results of some experiments using these agents intracranially can be accounted for by assuming a nonspecific blocking action [4, 14, 18, 26, 30, 31, 33, 45, 49]. Many transmitter antagonists appear to produce nonspecific blocking at higher concentrations [13, 17, 19, 21, 25, 37, 43, 44]. Our use of a slow infusion procedure would appear to facilitate uptake of

the pharmacological agent in the vicinity of the infusion needle tip and may in that way raise the effective dose at the needle tip and increase the likelihood of a nonspecific effect. It should be noted that the duration of the block produced by tolazoline and phentolamine in the present experiment was short. This should be a useful fact for distinguishing the nonspecific effects found here from specific effects which may be occurring in other experiments. Subsequent experiments have confirmed that haloperidol, atropine, propranolol, and hydralazine can also induce nonspecific blocking effects when infused intracranially [1]. However, except for tolazoline, phentolamine, and to a slight extent haloperidol, the doses of the other agents required to produce a nonspecific a nonspecific blocking action is considerably higher than that used in the present experiment [1].

To summarize, the results from the infusion of various transmitter antagonists suggest that α-adrenergic but not β-adrenergic, cholinergic, or dopaminergic transmitter substances might be involved in the medial hypothalamic neural systems modulating reactivity and attack. The failure of 6-OHDA lesions to induce a behavior change or to alter the effect of tolazoline injections shows that the behavioral effects induced by the infusion of the α-adrenergic antagonist tolazoline are nonspecific. In the wake of these negative findings regarding some of the more well known transmitter substances, further work elucidating the pharmacology of the medial hypothalamic neural system modulating reactivity and attack will have to evaluate the possibility that synapses relevant to the control of these behaviors are not present in the medial hypothalamus as well as the possibility that some lesser known transmitter substance is involved.

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