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The Effects of Intrahypothalamic Hemicholinium-3 on Muricide, Irritability and Feeding

BYRON C. YOBURN[1] AND MURRAY GLUSMAN

Department of Behavioral Physiology, New York State Psychiatric Institute and Department of Psychiatry, College of Physicians and Surgeons Columbia University, New York, NY 10032

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YOBURN, B. C AND M. GLUSMAN. *The effects of intrahypothalamic hemicholinium-3 on muricide, irritability and feeding.* PHARMACOL BIOCHEM BEHAV 20(6) 829–833, 1984.—Male Long-Evans rats that consistently killed mice when food deprived were injected unilaterally in the lateral hypothalamus with 20 μg and 30 μg of the acetylcholine synthesis inhibitor hemicholinium-3 (HC-3) and saline in a counterbalanced order. Rats were evaluated 1, 2, 3, 24, 48 and 72 hours post-injection for effects on muricide, irritability and feeding. HC-3 suppressed muricide and feeding and produced a trend toward reduced irritability during the first three hours post-injection These data indicate that modulation of cholinergic systems in the lateral hypothalamus influences several behaviors and not any one behavior specifically

Hemicholinium-3 Muricide Irritability Handling Interspecific aggression Feeding Acetylcholine Rats

MODULATION of cholinergic function in the hypothalamus has been shown to alter muricidal (mouse-killing) aggressive behavior. Direct injections of the cholinergic agonist carbachol into the lateral hypothalamus will facilitate muricide [2, 7, 15, 21, 24]. Conversely, intrahypothalamic injections of the cholinergic antagonists atropine [1,21] and d-tubocurarine [23] will suppress muricidal behavior.

These findings strongly suggest that acetylcholine is a primary neurochemical substrate for muricide in the rat. However, there are complications in adopting this hypothesis. Although it is clear that intrahypothalamic carbachol does modulate muricide, this effect is far from specific. Carbachol stimulation of the hypothalamus has been demonstrated to concurrently increase handling difficulty (irritable aggression) [24], and, in separate experiments, to induce drinking [11, 12, 14, 22] and affect temperature regulation [12]. Furthermore, it has been found that the muricidal behavior of carbachol-stimulated rats differed from that of unstimulated killers [7], and that in low doses carbachol will inhibit muricide [16]. With regard to anticholinergic hypothalamic stimulation, both atropine and d-tubocurarine suppress feeding in addition to inhibiting muricide [1,23]. These data suggest that cholinergic agonists and antagonists simply activate and inhibit, respectively, many behaviors, and that there is no specific control of muricide by cholinergic mechanisms. On the other hand, it is possible that in the rat there does exist a cholinergic substrate for muricidal behavior, but that other behaviors are also controlled by the level of cholinergic function. In this case it is not that muricide is nonspecifically modulated, but that many behaviors are specifically affected.

All of the above studies of intrahypothalamic drug effects on muricide have employed agents that primarily act upon postsynaptic receptors. In an effort to determine if these results extend to a drug that exerts presynaptic effects by depleting acetylcholine levels, the present experiment evaluated the effects of intrahypothalamic hemicholinium-3 (HC-3) upon muricide, irritability and feeding. Intraventricular HC-3 has been shown to deplete brain levels of acetylcholine [9] probably by inhibiting high affinity transport of choline into the nerve terminal [10].

METHOD

Subjects

Subjects were seven male, experimentally-naive, Long-Evans rats between 3 and 6 months of age that had consistently exhibited muricide during muricide screening, postsurgical testing and saline posttests (see below). Animals were housed in 21×24×20 cm plastic cages with metal tops. The bottoms of the cages were filled with a thin layer of wood chips, that was changed regularly, but never within 24 hours of a test session. All rats had ad lib access to water and were fed approximately 20 g of lab chow per day except where noted.

[1]Requests for reprints should be addressed to Byron C Yoburn, Ph d, Department of Pharmacology, Rm. E-321, Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

Male and female white mice of various ages served as targets

Initial Muricide Screening

Upon arrival in the laboratory, rats were given free access to food for 1 week following which they were placed on a 23 hr food deprivation schedule for 7 days. Ad lib access to food was allowed at the same time each day for one hour. Water was present at all times

Following 1 week of 23 hr food deprivation animals were tested for mouse killing. A mouse was placed in the animal's home cage and the rat was monitored for muricide for 20 min. Mice were removed as soon as they were killed. We typically find 50–80% of rats exposed to this procedure exhibit muricide.

Surgery and Postsurgical Muricide Testing

Rats were allowed free acess to food for a minimum of one week prior to surgery. They were then stereotaxically implanted under anesthesia (50 mg/kg sodium thiopental, IP, 0.4 mg/kg atropine sulfate, SC) with a 22-ga guide cannula (Plastics Products) aimed at the right lateral hypothalamus. Animals were allowed to recover for at least 4 days following surgery prior to return to a 20 g food/day feeding regimen. They were exposed to this restricted feeding schedule for at least one week prior to a postsurgical muricide test in which a white mouse was placed in the home cage and the latency to kill was determined. All seven rats killed within 5 min.

Drug Injection Procedure

Rats were injected using a 28-ga internal injection cannula (Plastics Products) inserted into the implanted guide cannula and connected by a polyethylene tubing connector (Plastic Products) to a 1.0 μl syringe (Hamilton) mounted on a microdrive. The tip of the internal cannula extended approximately 0 5 mm beyond the tip of the guide cannula. Injections of 0.9% saline solution and 20 μg and 30 μg of HC-3 (Sigma) dissolved in saline were given in a counter-balanced order with at least 6 days between injections. These doses were based on pilot experiments that indicated that lower concentrations produced minimal behavioral effect. Injection volume was 0.5 μl infused at 0.5 μl/min. The injection cannula was left in place for 1 min following the infusion Animals were tested once at each dose

Muricide and Handling Testing

Rats were tested 1, 2, 3, 24, 48 and 72 hr following each injection. Each test consisted of a muricide test followed by a handling test [24]. During muricide testing, a mouse was introduced into the home cage and the latency to sniff, attack and kill the mouse was recorded. Mice were removed immediately upon being killed. If the rat failed to attack or kill within 5 min, the mouse was removed and a latency of 300 sec was recorded.

One min following the termination of the muricide test (either by a kill or removal of the mouse) animals were rated for handling difficulty. The test consisted of four trials: (1) picking up the rat by the base of the tail with tongs, (2) picking up the rat by the nape of the neck with tongs; (3) probing the mouth area of the rat with tongs; (4) picking up the rat with a gloved hand. For each trial the rat was scored as follows 0=no vocalization; 1=vocalization; 2=escape attempt with or without vocalization; 3=bite attempt with escape attempt, with or without vocalization; 4=bite attempt without escape attempt, with or without vocalization. One number was assigned for each of the four trials and the sum of the numbers was the handling score for that post-injection test All testing was conducted by a single experimenter who was aware of treatment condition Rats were fed at least 1.5 hr following testing.

Feeding Tests

In order to evaluate HC-3 produced alterations in a nonaggressive behavior, ten rats were tested in a separate feeding experiment. Five of the rats were mouse killers from the previous muricide and irritability studies. The remaining five had exhibited muricide during an initial muricide screening test as described above but had occasionally failed to exhibit muricide in subsequent tests These five rats had all been screened and implanted with a cannula as described above. All ten rats were maintained on 15 g chow/day during feeding tests

Prior to formal feeding tests all rats were trained in 12 trials to eat 3, 45 mg food pellets stuck to a length of masking tape attached to the wiregrid cage top The latency to eat the 1st and 3rd pellet was recorded over the final pretraining trails All rats ate the 3 pellets within 3 min during the last two trials.

During formal feeding tests, rats were injected as described above for muricide testing with saline or 30 μg HC-3. Three rats received saline and 30 μg HC-3 injected one week apart. The remaining seven rats were injected with either saline or 30 μg HC-3. The latency to eat the first and third pellets was recorded in two trials (3 pellets/trial) spaced 15 seconds apart at 1, 2, 3, 24, 48 and 72 hr post-injection If the rat failed to eat within 5 min, the trial was terminated and a latency of 300 sec was recorded. The mean latency to eat the first and third pellets during the two trials was calculated for each post-injection test. Rats were fed their daily ration of 15 g chow at least 1.5 hr following testing

Data Analysis

All latency data were converted to $\log_{10}$ (X+1) prior to evaluation by analysis of variance. Irritability data was evaluated using the Friedman nonparametric analysis of variance

Histology

At the end of testing rats were sacrificed by an overdose of thiopental and perfused with 0 9% saline followed by 10% formalin. The brains were removed and 40 micron frozen sections were cut and stained with neutral red and luxol fast blue The sections were examined and the location of the cannula tips were determined and mapped onto schematic brain sections [13]

RESULTS

Muricide and Irritability Testing

Hemicholinium-3 produced a dose-dependent increase in the latency to attack and kill mice. Figure 1 presents the time course for the latency to attack and kill following 20 μg and 30 μg HC-3 and saline injections. Two-way analyses of variance (dose × post-injection trial) revealed a significant dose ×post-injection trial interaction for attack, $F(10,85)=4.93$, $p<0$ 01 and kill latencies, $F(10,85)=4.00$, $p<0.01$ Analyses

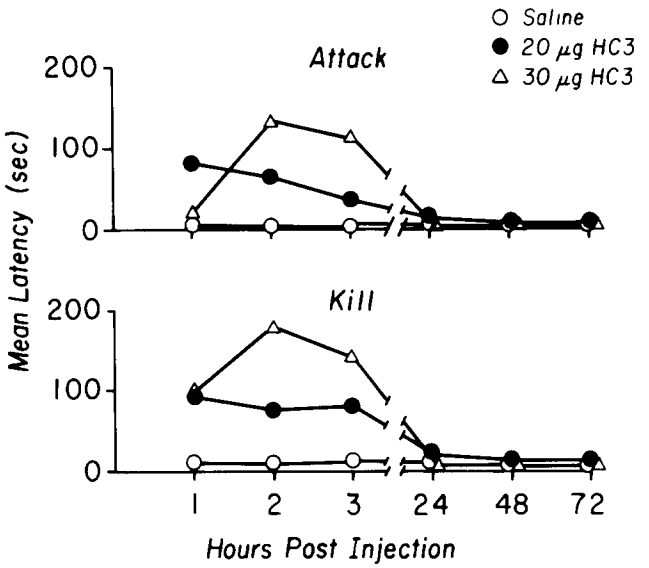

FIG 1 Time course of the effects of HC-3 (20 μg and 30 μg) and saline injections on the mean latency to attack (upper panel) and kill (lower panel).

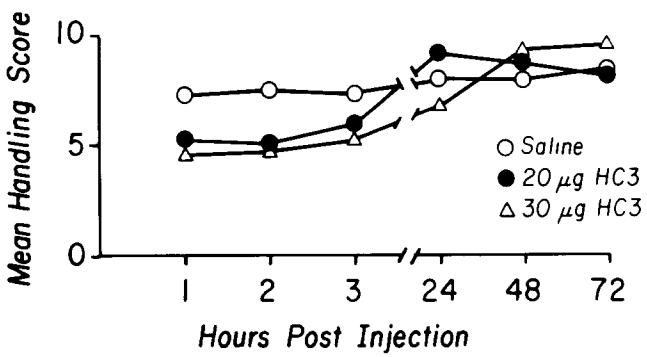

FIG 2 Time course of the effects of HC-3 (20 μg and 30 μg) and saline injections on the mean handling score

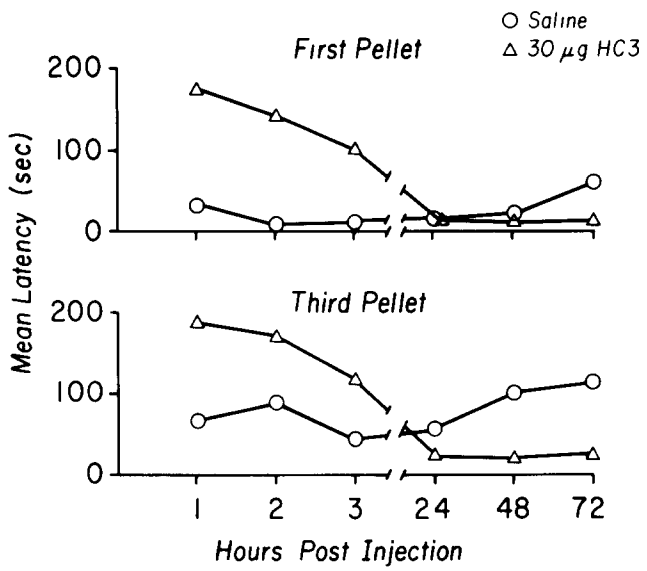

FIG. 3 Time course of the effects of HC-3 (30 μg) and saline injections on the mean latency to eat the first (upper panel) and third (lower panel) pellet during the feeding test

of simple effects indicated a significant effect of dose at 1, 2 and 3 hours post-injection for attack, $F(2,102) \geq 3.20, p < 0.05$ and kill, $F(2,102) \geq 3\ 85, p < 0.05$, while there was no significant effect of dose 24, 48 or 72 hr post-injection for either measure, $F(2,102) \leq 0.96, p > 0.05$. A significant post-injection trial effect was observed at 20 μg and 30 μg for latency to kill, $F(5,85) \geq 4.01, p < 0.05$ and at 30 μg for latency to attack, $F(5,85) = 3.14, p < 0.05$. There was no significant effect of post-injection trial following saline for latency to kill, $F(5,85) = 0.18, p > 0.05$ or following saline or 20 μg HC-3 for latency to attack, $F(5,85) \leq 0.82, p < 0.05$. There were no other statistically significant effects.

In contrast to the effects on the actual interspecific aggressive behavior, we found no evidence that HC-3 affected the latency to approach and sniff the mouse. Two-way analysis of variance indicated that there was no effect of drug dose, $F(2,17) = 0.81$, $p > 0.05$ or post-injection trial, $F(5,85) = 1.93$, $p > 0.05$ and no interaction, $F(10,85) = 1.86$, $p > 0.05$.

There was a trend for HC-3 to produce a decrease in the handling score (Fig. 2). The overall mean handling score during the first 3 hours post-injection was 7.6, 5.5 and 4.9 for the saline, 20 μg and 30 μg tests, respectively. Friedman two-way analysis of variance for ranked data indicated that this trend approached significance, $\chi^2(2) = 6.08$, $p < 0.07$ There was no evidence of any difference in mean handling score 24–72 hr post-injection ($\bar{x} = 8.0, 8.8, 8.8$ for saline, 20 μg, 30 μg, respectively, $\chi^2(2) = 0.58, p > 0.20$).

Feeding Test

Figure 3 presents the results from the feeding tests following saline or 30 μg HC-3 injections. Two-way analyses of variance indicated a significant post-injection trial effect for the latency to eat the first and third pellets, $F(5,55) \geq 2.58$, $p < 0.05$ and a significant dose × post-injection trial interaction for both measures, $F(5,55) \geq 3.24, p < 0.05$. Analyses of simple effects revealed a significant effect of dose at 1 and 2 hr post-injection for latency to eat the first pellet, $F(1,66) \geq 6.26, p < 0.05$ and at 1 hr post-injection for latency to eat the third pellet, $F(1,66) = 4.04, p < 0.05$. For both measures there was a significant effect of post-injection trial at the 30 μg dose, $F(5,55) \geq 5.92, p < 0.05$. No other effects were significant.

Histology

Figure 4 is a schematic reconstruction of the cannula tip locations for rats from the muricide and supplemental feeding studies. Injection sites were located primarily in the lateral hypothalamus. Visual inspection of the sections showed that histological damage was approximately 1 mm in diameter at the cannula tip.

DISCUSSION

In the present experiment, the acetylcholine synthesis inhibitor HC-3 injected into the lateral hypothalamus was found to suppress muricide and feeding, and tended to suppress irritability. These results are consistent with those reported for anticholinergic stimulation of comparable sites in

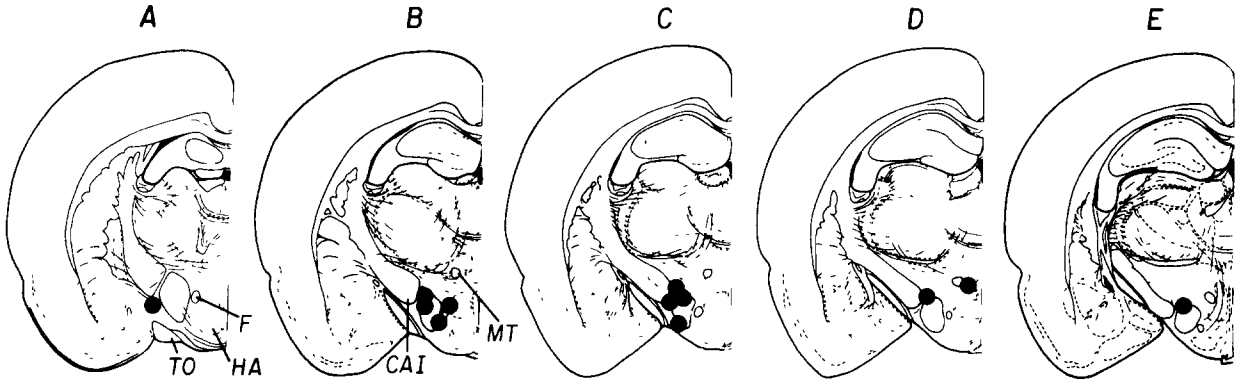

FIG 4 Location of cannula tips from histological analysis for rats in muricide, handling and feeding tests Sections A-E correspond to Figs 30b, 33b, 34b, 36b, and 38b respectively from [13] F=fornix, HA=anterior hypothalamic nucleus, TO=optic tract, CAI=internal capsule, MT=mamillothalamic tract

the lateral hypothalamus, in which suppression of feeding and muricide also were noted [1,23]. The present findings are the logical inverse of stimulation studies in which the cholinergic agonist carbachol injected into similar areas of the lateral hypothalamus facilitated muricide [2,24] and irritability [24] Thus, lateral hypothalamic administration of cholinergic agonists facilitate muricide and irritability, while cholinergic antagonists suppress muricide, irritability and feeding. In general, the sites that mediate these effects are scattered within the lateral hypothalamus

These findings suggest that there is a role for lateral hypothalamic cholinergic mechanisms in muricide. However, the specificity of these effects to interspecific aggression is open to question It is clear that both presynaptic and postsynaptic modulation of lateral hypothalamic cholinergic function produce a variety of effects and the suppression observed in this experiment may represent a nonspecific behavioral effect. On the other other hand, the failure of HC-3 to suppress the sniff measure in the muricide test argues against a pervasive behavioral suppression The sparing of this investigative behavior indicates that it is not dependent upon cholinergic activity in the lateral hypothalamus. On the other hand, attacking, killing, and feeding which were suppressed 1-3 hr post-injection are behaviors that are related to cholinergic activity. The trend for HC-3 to suppress irritability suggests that this behavior is modulated by cholinergic mechanisms as well. The concerted variation in these behaviors (attack, killing, feeding, irritability) might be expected since food deprivation can induce and facilitate muricide [18, 19, 24], although feeding and muricide have been dissociated by a number of measures [3, 6, 18, 20] Furthermore, food deprivation and forms of irritable aggression [17] such as shock-elicited ([4], however see [5]) and schedule-induced aggression [8] have been found to covary

We do not have direct evidence that lateral hypothalamic injections of HC-3 actually depleted acetylcholine. However, the time course of the behavioral effects parallel the demonstrated time course for the depletion of acetylcholine following intraventricular administration [10]. In those experiments, whole brain acetylcholine levels were depleted maximally between 1 and 8 hr following intraventricular HC-3. In addition, whole brain acetylcholine depletion was associated with a behavioral hyperreactivity with a time course consistent with decreased levels of acetylcholine. Although our findings represent a generally opposite behavioral effect (a trend towards hyporeactivity on the handling test), these differences could be the result of widespread versus local neurotransmitter depletion.

In summary, our experiments demonstrated that HC-3, when applied intrahypothalamically, produced effects similar to that exerted by similarly administered anticholinergics and opposite to cholinergic agonists. The results of this and other studies indicate that modulation of lateral hypothalamic cholinergic systems influences a variety of behaviors including drinking [11, 12, 14, 22], temperature regulation [12], muricide, irritability and feeding

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