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Heat-Escape Behavior in the Rat Following Intrahypothalamic Injection of Acetylcholine[1]

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LAUDENSLAGER, M. L. AND H. J. CARLISLE. *Heat-escape behavior in the rat following intrahypothalamic injection of acetylcholine.* PHARMAC. BIOCHEM. BEHAV. 4(4) 369–373, 1976. – The effects of preoptic/anterior hypothalamic injections of acetylcholine on heat-escape behavior and hypothalamic temperature were investigated in unrestrained rats implanted with bilateral cannulae. Two types of responses were observed, either a fall in hypothalamic temperature coupled with an increase in behavioral responses to escape heat or a rise in hypothalamic temperature associated with little or no change in behavioral heat-escape responses. The fall in hypothalamic temperature observed in one group of rats was significant and was associated with a dose-dependent increase in heat-escape responding. The rise in hypothalamic temperature noted in the other group of rats was nonsignificant, and the associated behavioral responses were variable. Distinct anatomical differences in cannulae loci between the 2 groups were not apparent. It is concluded that acetylcholine activates the heat-dissipating control system of the rat hypothalamus and that the hyperthermic effects of acetylcholine are nonspecific.

ACh Heat-escape Temperature Hypothalamus Hypothalamic temperature

THE preoptic/anterior hypothalamic region (PO/AH) has been implicated in the control of thermal homeostasis by a variety of procedures [14] including the direct application of supposed neurotransmitters (see [13] and [17] for review). The interpretation of these microinjection studies is complicated by a number of factors including between- and within-species differences [23]. For example in the rat, cholinomimetics have been reported to increase core temperature in some studies [1, 2, 3, 21] and to reduce core temperature in other studies [6, 11, 18]. Some of the within-species variability may be attributable, in part, to methodological and procedural differences such as the timing and mode of delivery of the transmitter. The cannulae were inserted and the injection made immediately in some studies [1, 2, 3, 18, 21], while in other work [6,11], the injections were made remotely some time following the insertion of the cannulae. Since core temperature is influenced by handling [7,24], it is conceivable that the changes in temperature attributed to the transmitter are partially a consequence of handling and insertion of the cannulae or an interaction between handling and chemical stimulation. Other variables such as the volume injected, use of restraint, and the dosage of cholinomimetic administered have also been suggested as contributing factors to the within-species variability [2].

The dependent variable in many microinjection studies has been the change in core temperature, usually specified as rectal temperature, but other physiological and behavioral thermoregulatory responses can be measured in order to determine the nature of the thermoregulatory responses which follow the drug treatment. Preoptic/anterior hypothalamic administration of acetylcholine (ACh) is followed by a reduction in heat-seeking behavior [6] and peripheral vasodilation [11] as well as a fall in core temperature. These results suggest that ACh activates complementary physiological and behavioral thermoregulatory response systems so as to facilitate the change in core temperature. Interestingly, in one study reporting a rise in rectal temperature following PO/AH injections of carbachol [4], behavioral responses that attenuated a convective heat stress were increased. Since the behavioral response was one that should lower core temperature, it was considered compensatory to the changes in temperature. This implies that only the physiological control system was directly affected by ACh, whereas the behavioral control system was activated indirectly by the ACh-induced rise in body temperature.

The present study attempts to reconcile some of the contradictory effects of PO/AH cholinergic stimulation on heat-escape responses and body temperature. The animals were given adequate time for behavioral responses and body temperature to stabilize following insertion of the cannulae prior to the injection of ACh. The effects of ACh were thus assessed without unnecessary handling of the animals. Hypothalamic temperature was measured continuously during testing, thus eliminating any effects that insertion of a rectal probe might have on core temperature.

METHOD

Animals

The animals were 13 adult, male Sprague-Dawley rats

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weighing 403–723 g at the time of surgery. Each animal was caged individually in a colony room maintained at 23 ± 1°C, with relative humidity ranging from 40–60%. Food and water were available ad lib except during testing. Animals were tested at approximately the same time of day during the light phase of a LD 12 : 12 light cycle.

Implants and Surgery

The implants have been described in detail previously [5]. Briefly, they consisted of a pair of bilateral 21 ga stainless steel cannula guides bonded with a dental acrylic bridge. Nine animals received medial PO/AH cannula guides, which had an intercannula distance of 2.0 mm. Three animals received lateral PO/AH guides with intercannula distances of 4.0 mm. One animal (No. 29) received lateral hypothalamic guides (intercannula distance = 4.0 mm) as an antomical control. The injection cannulae consisted of 27 ga stainless steel tubing, which protruded a distance of 0.5–1.0 mm below the tips of the guides when fixed in place. When the cannulae were not in place, a 26 ga stainless steel stylette was inserted into each guide. A miniature thermistor (VECO 32A7; 0.33 mm dia.) was affixed to one cannula guide for the recording of hypothalamic temperature (Thy), which was monitored on a Leeds and Northrup potentiometric recorder. Details of this technique of intrahypothalamic temperature recording have been described previously [9].

The animals were anesthetized with sodium pentobarbital (35 mg/kg) and chloral hydrate (160 mg/kg) following pretreatment with atropine sulfate (0.16 mg/kg). The stereotaxic coordinates for the PO/AH guides were 0.5 mm or 0.0 mm rostral to bregma and 7.5–8 mm ventral to the level surface of the skull. Coordinates for the lateral hypothalamic guides were 2.5 mm caudal to bregma and 7.7 mm ventral to the level surface of the skull. The implants were centered an equal distance on either side of the midline and affixed to the skull by means of dental acrylic cement around four stainless steel screws in the cranium. Each animal received an intramuscular injection of 30,000 U of Procaine penicillin G suspension (Crysticillin, E. R. Squibb and Sons, Inc.). One week was allowed for recuperation before any tests were made.

Apparatus

The heat-escape apparatus consisted of a 16.5 × 16.5 × 20.0 cm Plexiglas and plywood box through which temperature controlled air could be circulated. The floor of the chamber was made of acetate tubing, through which cool (5°C) water flowed constantly. Warm (50 ± 3°C) air from a heat gun (Model HG301, Master Appliance Co.) was directed into the chamber through a port beneath the box. The heat gun was connected to an on–off controller (Model 71A, Yellow Springs Instrument Co.), which was activated by a thermistor (Series 403, Yellow Springs Instrument Co.) located in the floor of the chamber. The entire apparatus was located in an environmental chamber maintained at 5 ± 1°C). In the absence of any response by the animal, warm air was forced through the apparatus at a velocity of 21–24 m/min. During this time, a 25 W light illuminated the test chamber from below. A Plexiglas lever, mounted 1.5 cm above the floor, activated programming equipment located in an adjacent room. A press on the lever turned off the heat gun and light and turned on an exhaust blower for 10 sec, thereby drawing 5°C air from the environmental chamber through the apparatus at a velocity of 46–52 m/min. The apparatus was illuminated from the environmental chamber during the reinforcement interval. If the temperature in the heat-escape apparatus was 50°C, a single response by the animal lowered the temperature 10–12°C during the 10 sec reinforcement interval. If the animal continued to respond, the temperature in the box was reduced further, but the relative magnitude of the decrease in temperature was attenuated. For example, if the temperature in the heat-escape apparatus was 30°C, a response lowered the temperature 4–6°C. The total number of responses and reinforcements were counted on electromechanical counters and a print-out counter.

Procedure

Training. Prior to surgery, all animals were trained to escape heat during daily 30–45 min tests. The animals required 7–20 sessions until responding for the cool air reinforcement was stable, at which time session length was extended to 3 hr, and the animals were tested only once or twice a week.

Pre- and post-test rectal temperatures (Tre) were measured with a Model 46 Telethermometer (Yellow Springs Instrument Co.), with the probe inserted 6 cm. Mean Tre averaged 37.2 ± 0.33°C (SD) prior to testing but often rose to as high as 40.6°C during early training tests. When response rates stabilized, post-session Tre averaged 37.4 ± 0.44°C (SD). Surgery was performed when response rates were stable and post-test hyperthermia was absent.

Microinjections. Injections were made with microliter syringes (Series CGV, Precision Sampling Co.) attached to the cannulae with polyethylene tubing (PE 20, Clay-Adams Co.). The syringes were mounted in an infusion pump (Model 975, Harvard Apparatus Co.) set to dispense a 1.0 μl volume over a 14 sec interval. All microinjection equipment was soaked in 70% ethanol when not in use. Solutions were prepared fresh the day of an injection in glassware oven baked at 200°C for 3 hr. The cannulae, tubing, and microliter syringes were filled by backflushing through an autoclaved, double Millipore filter assembly (Swinnex-13 adapter, 0.22 μm pore dia., Millipore Corporation). Acetylcholine chloride (Sigma Chemical Co.), dissolved in sterile 0.9% NaCl (Abbott Laboratories), was injected bilaterally in concentrations of 15.0, 25.0, and 50.0 μg/μl in a 1.0 μl volume. Concentrations of ACh are calculated as the salt. Control injections consisted of bilateral injections of sterile 0.9% NaCl in a 1.0 μl volume.

The animals were allowed to respond for cool air reinforcements for 60 min on the day of a microinjection. The cannulae were inserted, and an additional 15–20 min were allowed for Thy and response rates to stabilize. The microinjections were then made from outside the environmental chamber without disturbing the animal. The animal was observed closely through a window, and any significant changes in behavior were recorded for 60 to 90 min following the injection. Each animal received at least one microinjection of each concentration of ACh and the control solution in a randomized order. Statistical comparisons (2-tailed *t* tests) were made between the effects noted following 0.9% NaCl injections and the effects observed following each concentration of ACh.

Histology

At the conclusion of the study, the animals were given a

lethal dose of sodium pentobarbital (100 mg/kg) and perfused through the aorta with 0.85% NaCl followed by neutralized 10% Formalin. Frozen sections were cut at 35 μm through the cannulae tracks, and every fourth or fifth section was retained for staining with cresyl violet stain.

RESULTS

Two general patterns of responses occurred following PO/AH injection of ACh. Some rats consistently showed a fall in Thy and an increase in heat-escape responding. Other animals showed either an increase or a decrease in Thy and variable behavioral responses. For the purpose of analysis, the animals were divided into 2 groups depending on the reliability of the change in Thy. If Thy always fell in response to ACh, the animal was assigned to the hypo group (n = 6). If on any occasion Thy rose in response to ACh, the animal was assigned to the hyper group (n = 6). The mean change in Thy and the precent change in behavioral response rates for both groups are shown in Fig. 1. The change in Thy was the difference between Thy prior to ACh delivery and Thy 10 min following the injection. The percent change in behavioral response rates was calculated as the percent increase or decrease in responses during the 10 min interval following the injection compared with the number of responses made during the 10 min interval preceding the injection.

The decrease in Thy observed in the hypo group following administration of all concentrations of ACh was significantly different from the change in Thy following control 0.9% NaCl injections (t tests, $p<0.05$ or less). In addition the hypo group showed a significant increase in heat-escape responses and in reinforcement rates following all concentrations of ACh injected compared to the changes observed following 0.9% NaCl injections (t tests, $p<0.05$ or less). The dose-dependent increases in Thy of the hyper group were not significantly different from the NaCl control injections, and changes in behavioral responses were similarly insignificant (t tests, $p>0.05$). Changes in reinforcement rates in the hyper group were not different from 0.9% NaCl injections except at the 50 μg dose where mean reinforcement rates decreased 10.1% (t = 4.58, df = 5, $p<0.01$). The anatomical control animal, with cannulae directed toward the lateral hypothalamus, showed small but variable changes in Thy and behavioral response rates as indicated in Fig. 1.

Regardless of the group, changes in Thy were related to changes in reinforcement rates. The correlation coefficients (Pearson r) between the change in Thy and reinforcement rate were -0.735 (df = 24, $p<0.0005$) for the hypo group and -0.524 (df = 23, $p<0.01$) for the hyper group. In contrast, only the hypo group demonstrated a statistically significant negative correlation between the change in Thy and the response rates ($r = -0.437$, $df = 24$, $p<0.025$).

Figure 2 illustrates the change in Thy and response rates following a 15 μg ACh microinjection. In the upper panel, responses increased 91.7% following the administration of ACh in a hypo animal; the total fall in Thy was 0.45°C. As response rate increased, the animal became more active. This increase in activity included sniffing at the walls of the chamber, occasional grooming, and pushing at the lid of the apparatus. Activity declined during the second 10 min interval, and the animal assumed a sprawled posture to the side of the lever. Hypothalamic temperature rose approximately 0.4°C above the preinjection baseline during the

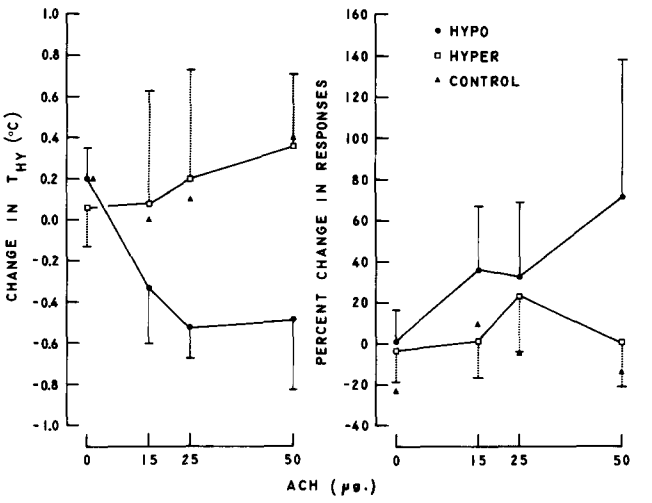

FIG. 1. Mean change in Thy and percent change in behavioral response rate for the hypo (●) and the hyper (□) groups. The vertical lines and brackets indicate one standard deviation. The animal with cannulae implanted in the lateral hypothalamus is indicated by ▲. The dose of 0 μg ACh indicates the effects observed following injections of 0.9% NaCl.

recovery period. The lower panel of Fig. 2 shows the effect of a similar treatment for an animal from the hyper group. Response rate decreased 25.5% concomitant with a 0.4°C increase in Thy after the administration of ACh. The animal maintained a sprawled posture to one side of the lever during the period when response rate was depressed. As Thy declined during the second 10 min interval, response rates increased.

Observation of the animal following injections of ACh revealed there was a tendency for 4 of the 6 animals of the hypo group to increase activity. The increase in activity was characterized by grooming, exploration of the test box, pushing at the lid to the box, and occasional circling responses. Response rates were typically elevated throughout the period of increased activity. If the animals were away from the vicinity of the lever when a reinforcement ended, they quickly returned and pressed the lever. This is a significant observation because it indicates that the increase in response rate was not related to inadvertent activation of the lever during the period of increased activity. Two of the 6 animals of the hyper group increased activity following injections of ACh, while the other 4 often assumed a sprawled posture to one side of the lever. The sprawling response was most prominent following injections of higher concentrations of ACh.

The location of the cannulae tips for the animals are shown in Fig. 3. One animal from the hypo group died before a histological analysis was performed. Since a 1.0 μl volume diffuses over a region approximately 1.9 mm in dia. [19], it can be assumed that a large region of the medial and lateral preoptic area, the medial forebrain bundle, anterior hypothalamus, and diagonal band of Broca were affected by the injections. There was no obvious anatomical distinction between the 2 groups.

DISCUSSION

Injections of ACh into the region of the PO/AH were followed by a reliable and statistically significant reduction

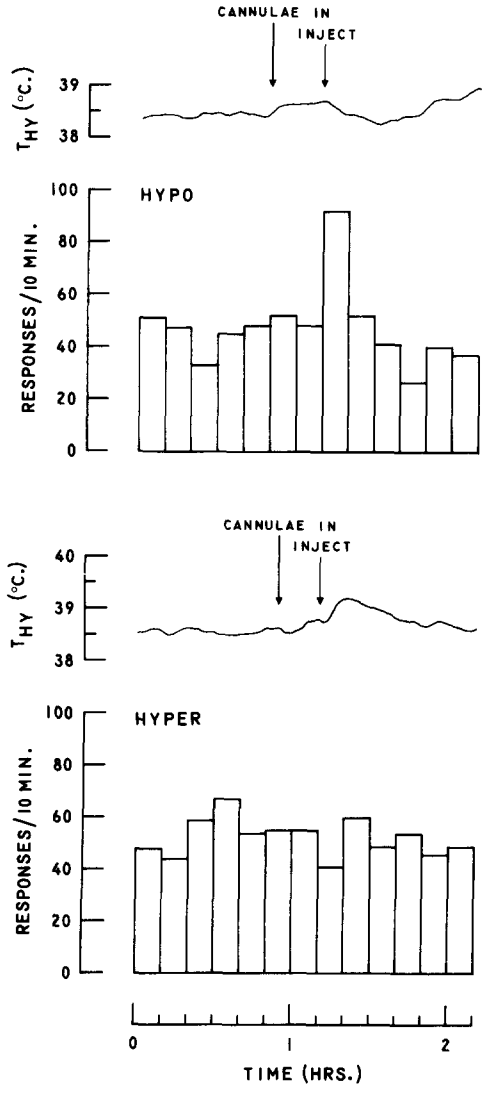

FIG. 2. Change in Thy and behavioral response rates following 15 μg ACh. The upper panel shows the effects noted in an animal from the hypo group, and the lower panel shows the effects noted in an animal from the hyper group.

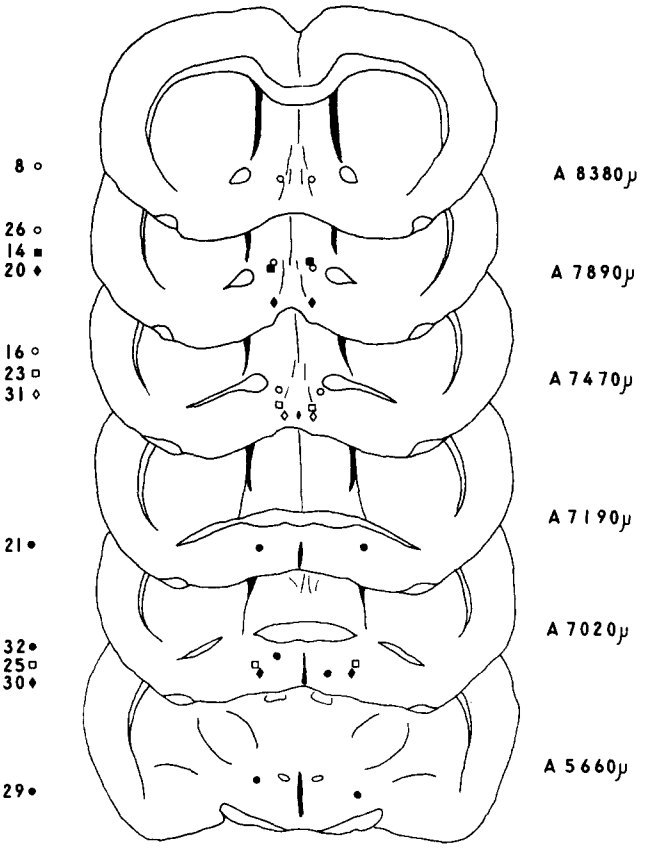

FIG. 3. Location of the cannulae tips in animals from the hypo (filled symbols) and hyper (open symbols) groups. Coronal levels are based on line tracings made from the atlas of König and Klippel [15]. The location of the cannulae tips in the anatomical control animal (No. 29) are also indicated.

in Thy and an increase in behavioral responses to escape heat stress in one group of rats. A second group of rats, which received similar treatments, showed unreliable and insignificant increases in Thy with no change in behavioral response rates. The increase in heat-escape responses and reduction in Thy observed in the hypo group are consistent with the interpretation that ACh activates behavioral and autonomic thermoregulatory heat-dissipation responses [6,11] but conflicts with the conclusion that ACh activates heat-production mechanisms [1, 2, 3, 4, 21].

It is possible that the reduction in Thy observed in the hypo group may be related to such nonspecific factors as 1) the production of a depolarizing block within the PO/AH, 2) actions outside of the PO/AH, or 3) alterations in hypothalamic blood flow. The first possibility can be partially dismissed since the production of a depolarizing block within the PO/AH with potassium chloride results in a rise in core temperature [10]. A 1.0 μl volume may diffuse over a region 1.9 mm in dia. [19], and it has been suggested [2] that the hypothermic effects of ACh may be due to diffusion to the lateral hypothalamus, medial forebrain bundle, or the ventricular system. A reduction in Thy related to effects occurring in the lateral hypothalamus can be discounted because the anatomical control animal (No. 29) showed no reduction in Thy and little change in behavioral response rates following injections of ACh. The reduction in Thy in the hypo group was immediate and could not be a function of diffusion into the ventricular system as it has been demonstrated that the diffusion of a 1.0 μl volume of ^{3}H-ACh into the ventricles is negligible 30 min following hypothalamic injections [20]. The reduction in Thy was typically complete 30 min following the injection in the present study, and Thy was returning to pre-injection levels. Finally, hypothalamic injections of norepinephrine and 5-hydroxytryptamine have been demonstrated to alter hypothalamic blood flow and consequently Thy [22]. Increased hypothalamic blood flow might result in a fall in Thy and cannot be eliminated as a factor contributing to the change in Thy, but it cannot account for the increase in behavioral heat-escape responses. In other words, if behavior was compensating for an ACh-induced reduction in Thy, heat-escape responses should decrease since experimental reduction of Thy results in a decrease in heat-escape responses [16].

Several reviewers [8,17] have suggested that the exogenous administration of various transmitter substances may be acting to alter the setting of the hypothalamic set-point about which body temperature is regulated [12]. If core temperature was to fall following the administration of some agent and if this fall was accompanied by a reduction in heat production in a cold environment, decreased vasomotor tone at thermoneutrality, and an increase in evaporative heat loss in a warm environment, a shift in the hypothalamic set-point is probable. The behavioral effects of PO/AH administration of ACh are compatible with this hypothesis. Acetylcholine reduces heat-seeking responses in a cold environment [6], evokes peripheral vasodilation concomitant with the assumption of sprawled postures at thermoneutrality [11], and, as demonstrated in the present study, facilitates heat-escape responses in a warm environment. Thus, the reduction in Thy following the administration of ACh appears to be a regulated phenomenon in which the set-point about which body temperature is regulated has been lowered.

Four of the 6 animals of the hyper group were observed to assume a sprawled posture following injections of ACh. Sprawling is a behavioral heat-dissipation response, and it also competes with lever press responses. Since ambient temperature rapidly rose to 50°C in the absence of a response by the animal, inhibition of the lever-press response was a sufficient condition to elicit a rise in core temperature. Therefore, sprawling was not an effective heat-dissipation response in the present study. Perhaps ACh lowered the set-point in the hyper group, but the sprawling response was inadequate to reduce Thy.

In summary, the present study supports the view that ACh lowers body temperature by the activation of thermoregulatory heat-dissipation responses. This may be related to a lowering of the set-point about which body temperature is regulated. Although the hyper group showed an average increase in Thy, this change was nonsignificant and may be a nonspecific effect of the ACh or the injection procedure.

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