Pharmacology Biochemistry and Behavior, Vol. 1, pp. 159–165. ANKHO International Inc., 1973. Printed in the U.S.A.

Effects of Intrahypothalamic Injections of Adrenergic and Cholinergic Substances on Behavioral Thermoregulation and Associated Skin Temperature Levels in Rats[1]

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(Received 24 July 1972)

AVERY, D. D. AND P. E. PENN. *Effects of intrahypothalamic injections of adrenergic and cholinergic substances on behavioral thermoregulation and associated skin temperature levels in rats.* PHARMAC. BIOCHEM. BEHAV. 1(2) 159–165, 1973.–Intrahypothalamic injections of carbamylcholine chloride (carbachol) and L-arterenol bitartrate hydrate (noradrenaline) in rats led to hyper- and hypothermia respectively, and differentially affected bar holding to escape heat. Cholinergic stimulation increased bar holding and adrenergic stimulation decreased the same response. In a second experiment it was found that skin temperatures associated with response onset and response offset were different following the neurochemical injections. Peripheral temperatures were maintained at lower levels with carbachol and were elevated with noradrenaline.

Behavioral thermoregulation Skin temperature Carbachol Noradrenaline Intrahypothalamic injections Heat escape

IT IS now well-known that injections of monoaminergic and cholinergic compounds into either the cerebral ventricles or the medial preoptic area of the hypothalamus of many species affect physiological thermoregulatory processes as reflected by changes in core temperature. In the rat, the species used in the following experiments, injections of cholinergic subtances (acetylcholine and carbachol) have been observed to increase colonic temperature while injections of noradrenaline decreased core temperature whether application was via the ventricular route [14] or directly in the preoptic area [3, 4, 5].

The foregoing findings, along with the many studies which have been designed to explore the effects of changes in thermophysiology on behavioral thermoregulation, provide the basis for the present experiments. Experimental manipulations other than intracerebral injections which affect core temperature and thermoregulatory behavior include ventromedial hypothalamic lesions and subsequent obesity [10], preoptic lesions [9], desalivation [11], heating and cooling of the hypothalamus [2,17] and clipping of fur [8]. The usual behavioral observation following these imposed impairments in ability to regulate body temperature was that thermoregulatory responding changed in a compensatory direction. For example, Satinoff [17] observed that local cooling of the preoptic areas of the hypothalamus of rats, maintained in either a cold or neutral environment, caused an increase in bar pressing which turned on a heat lamp, and Lipton [10] found that increases in depot fat insulation in the rat led to increments in responding to escape heat. Our first experiment was designed to determine if similar compensatory behavioral shifts would occur in the heat escape situation following changes in core temperature caused by intrahypothalamic injections of cholinergic and adrenergic substances.

The results of the first experiment were positive, and these findings along with considerations from an earlier study [12] prompted a second experiment. From the earlier work it was concluded that behavioral thermoregulatory responses were at least partially dictated by peripheral factors. Our second experiment was conducted to decide if peripheral sensory factors were also determinants of behavioral shifts following intrahypothalamic chemical injections.

EXPERIMENT 1

Beckman and Carlisle [7] injected acetylcholine into the preoptic area in rats and found that the rate of responding

[1] This research was supported by Biomedical Sciences Support Grant No. 1-S05-FR07127-01.

leading to heat reinforcement in the cold decreased. In addition, Beckman [6] has shown that intrahypothalamic injections of norepinephrine leads to opposite effects in that behavioral situation: an increase in response rates. In Experiment 1, similar neurochemical manipulations and behavioral measures were employed in the heat escape situation for comparison with results obtained in the heat reinforcement studies.

METHOD

Animals

Six adult male Charles River rats (CD Strain) were used. They were maintained in standard rodent cages located in a constantly illuminated temperature controlled (23.0 ± 0.5°C) room. Water was continuously available. Food intake was restricted during behavioral testing so that the animals' body weight varied less than 5% from the weight recorded at the time of initiation of the experiments. This procedure was used to reduce variability in responding due to changes in body weight [10].

Surgery

At the time of surgery, each animal weighed between 375 and 425 g. Double walled cannulae, directed towards the medial preoptic area of the hypothalamus, were implanted into the brains of the animals. The cannula assembly has been described in detail previously [3,4]. Briefly, it consisted of an outer guide cannula constructed from 22-gauge stainless steel tubing, an inner injection cannula, constructed from 30-gauge stainless steel tubing, and a dummy cannula, constructed from 30-gauge stainless steel wire, which was in place at all times except during injections. A nylon cap covered the cannula orifice, thus preventing, or at least minimizing heat transfer from the radiant energy source during behavioral testing. The cannula assembly was unilaterally implanted in each animal under sodium pentobarbitol (45 mg/kg) and chloral hydrate (150 mg/kg) anesthesia, according to stereotaxic coordinates at 7.5 mm anterior to the interaural line, 1.0 mm lateral to the midline, and 7.5 mm below the surface of the dura.

Apparatus

Detailed descriptions of the operant, heat-escape apparatus have appeared earlier [9, 10, 12]. The behavioral chamber consisted of a Plexiglas cylinder which was situated in a plywood manifold box. The floor was constructed of glass rods and the manipulandum was a solid glass rod extending into the chamber and situated above the grid floor. This rod was connected to a microswitch bolted to the outside of the chamber.

A fan (200 cfm) was situated at one end of the manifold box and drew ambient air through the top of the chamber and exhausted air from the manifold box. The apparatus was located in a temperature controlled room (23.0 ± 0.5°C). A 250 W red bowl heat lamp was situated above the behavioral chamber. The rate of heating was 200 W. throughout the experiments and was obtained by reducing the voltage to the heat lamp.

Procedure

After at least a one week postsurgery recovery period, the animals were trained to depress the bar to terminate heat during 7--10 daily trials of 15 min each. During these trials the intensity of the heat lamp was 250 W and the exhaust fan only operated during a response. Thus, during the time that the bar was depressed, the heat lamp was off and ambient air was drawn through the behavioral chamber. Thereafter, and throughout the experiments, the sessions were 30 min, the intensity of the heat lamp was 200 W, and the fan was continuously operating irrespective of behavioral responses. Presession and postsession measures of colonic temperature, number of heat escape responses, and duration of bar holding were recorded for all training and experimental sessions. Colonic temperature was measured by inserting a YSI 402 thermistor probe 6 cm beyond the anal orifice. The probe was held in place until body temperature, indicated on a YSI telethermometer (Model 46), became stable (45–60 sec).

The injection procedure has been described previously [3, 4, 5]. On injection days an animal was removed from its home cage, colonic temperature was measured, and the drug was injected. The two substances used were carbamylcholine chloride (carbachol), 8 μg, and L-arterenol bitartrate hydrate (noradrenaline), 25 μg. Each was dissolved in pyrogen free, normal saline and all injections were 0.5 μl. Both doses represent the highest concentrations tested in earlier work and were chosen so as to maximize temperature changes in a short time period following administration [3,4]. After injection an animal was returned to its home cage and colonic temperature was measured 15 min later. If at this time, following noradrenergic stimulation, colonic temperature had fallen at least 0.3°C, the animal was placed in the heat escape chamber, and the session began. If colonic temperature had not fallen 0.3°C, the animal was returned to its home cage for another 15 min at which time colonic temperature was again measured. This procedure was continued for 45 min if necessary; if the criterion of a drop in body temperature of 0.3°C was not reach at this time, the animal was not tested. The same procedure was used for injections of carbachol with a criterion of a 0.5°C increase in colonic temperature. On baseline days the dummy cannula was manipulated. On the basis of previous experiments, showing no difference between sham stimulation and injections of saline on body temperature, it was deemed unnecessary to inject saline [3].

Each animal received three injections of each substance, administered in a random sequence, except for animals AH3, AH4, and AH6. AH4 died of thermal stress during the shaping procedure. Because of cannula dislodgement, AH3 received only one application of noradrenaline and AH6, one application each of both substances. There were at least four days between injections and each injection session was preceded by a baseline session 24 hr earlier. The experiments occurred at the same time each day.

Histology

Each animal was killed via standard 10% formal-saline procedures at the conclusion of the experiments. Frozen brain sections, 20 μ thick, were cut and stained using the Luxol-fast blue procedure. The locations of the cannula tips for each animal are depicted in Fig. 1. The placements for all animals were found to be located in the medial preoptic area of the hypothalamus between 7.2 mm and 7.8 mm anterior to the interaural line. Animals AH5 and AH6 had placements that were on the ventral border of the POA and

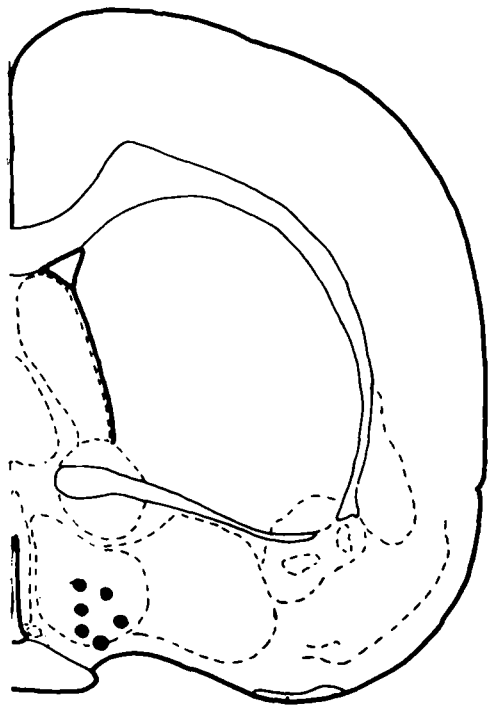

FIG. 1. Locations of cannula tips for each of the six animals used in Experiment 1 (A-P + 7.6, after Pellegrino and Cushman, 1967).

1.0 mm to 1.25 mm lateral to the midline. Placements for the other four animals were between 0.5 and 1.0 mm below the dorsal extent of the POA. On the basis of Myers' [13] data with respect to diffusion of centrally injected solutions, it can be concluded reasonably that injections at each site extended to some of the same brain areas in all subjects.

RESULTS

Body Temperature

Mean (±S.E.) preinjection, presession, and postsession colonic temperatures for baseline sessions and carbachol injection sessions in which the criterion for body temperature change was met are presented for each animal in Table 1. At the time of initiation of behavioral testing the average increase in body temperature associated with injections of 8 μg. of carbachol was 0.66°C. This resulted in a significant average increase of 0.58°C in presession colonic temperature as compared with the previous day's baseline presession measure (t = 18.28, $p<0.01$, Student's t). Postsession colonic temperatures were also significantly higher following injections than for baseline sessions with a mean increase of 0.27°C (t = 9.85, $p<0.01$).

Similar measures for the noradrenergic condition are also presented in Table 1. Injections of 25 μg of noradrenaline led to a mean decrease of 0.7°C at the time of behavioral testing. Thus, presession colonic temperatures following such injections were significantly lower than comparable measures for baseline sessions with a mean of 0.35°C (t = 7.55, $p<0.01$). The same pattern appeared in postsession measures with a mean decrease in postsession colonic temperature of 0.30°C following noradrenergic injections as compared with baseline (t = 5.73, $p<0.01$).

Behavior

Neither carbachol (t = 0.04, $p>0.10$) nor noradrenaline (t = 0.10, $p>0.10$) affected number of responses. Mean duration of bar holding to escape heat, on the other hand, was significantly altered compared to baseline days after injections of either substance. As shown in Fig. 2, each animal increased the amount of time spent bar holding to escape heat after cholinergic stimulation (t = 12.42, $p<0.01$) and, as shown in Fig. 3 decreased bar holding following injections of noradrenaline (t = 5.72, $p<0.01$). Cholinergic stimulation led to an overall average increase of 2.41 min and noradrenaline, to an overall average decrease of 1.85 min per 30 min session.

TABLE 1

MEAN (± S.E.) COLONIC TEMPERATURES FOR EACH ANIMAL FOR BASELINE AND EXPERIMENTAL SESSIONS ASSOCIATED WITH INTRAHYPOTHALAMIC INJECTIONS

		Baseline		Injection		
Drug	Animal	Presession	Postsession	Preinjection	Presession	Postsession
Carbachol	AH 1	37.80 ± 0.26	38.80 ± 0.10	37.85 ± 0.22	38.45 ± 0.32	39.25 ± 0.45
	AH 2	38.10 ± 0.36	39.05 ± 0.05	38.45 ± 0.10	39.10 ± 0.10	39.40 ± 0.20
	AH 3	38.67 ± 0.14	38.90 ± 0.17	38.50 ± 0.29	38.83 ± 0.06	38.70 ± 0.06
	AH 5	38.35 ± 0.05	39.34 ± 0.15	38.70 ± 0.33	39.40 ± 0.37	39.55 ± 0.30
	AH 6*	38.20	38.70	37.90	38.90	39.50
Noradrenaline	AH 1	37.87 ± 0.17	38.40 ± 0.20	38.23 ± 0.09	37.37 ± 0.38	38.07 ± 0.45
	AH 2	37.75 ± 0.32	39.45 ± 0.32	39.05 ± 0.15	37.95 ± 0.15	39.15 ± 0.22
	AH 3*	38.20	38.90	39.00	38.60	39.40
	AH 5	38.43 ± 0.27	39.10 ± 0.05	38.60 ± 0.35	37.43 ± 0.29	38.40 ± 0.52
	AH 6*	38.90	38.40	38.70	38.40	38.60

*Only one injection was made as explained in the text.

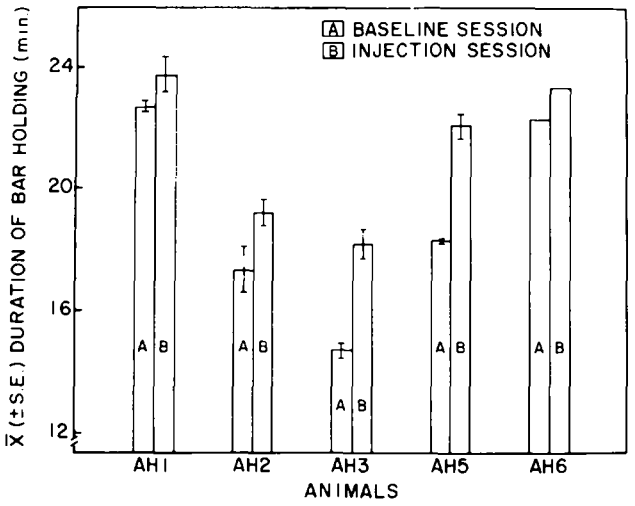

FIG. 2. The effects of intrahypothalamic injections of carbamylcholine chloride, 8 μg, on heat-escape behavior are shown for each animal. Each bar represents the mean (± S.E.) duration in min the bar was depressed to escape heat during the 30 min sessions. Note that each animal increased its duration of bar holding following injections.

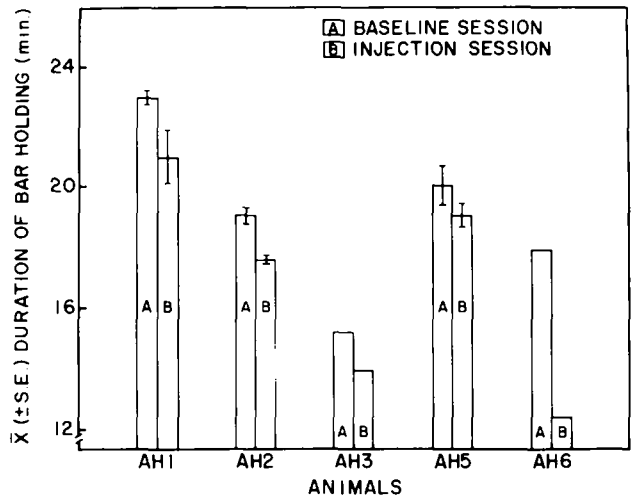

FIG. 3. The effects of intrahypothalamic injections of noradrenaline bitartrate hydrate, 25 μg, on heat-escape behavior are shown for each animal. Each bar represents the mean (± S.E.) duration in min the bar was depressed to escape heat during the 30 min sessions. Note that each animal decreased its duration of bar holding following injections.

DISCUSSION

The major point to be considered is the comparability of the present findings with results from similar experiments in the heat reinforcement situation. Our heat-escape results are, as should be expected, directly opposite. When animals were working for heat in the cold, cholinergic stimulation decreased [7] and adrenergic stimulation increased responses which led to heat reinforcement [6]. We found cholinergically induced increases and adrenergically induced decreases in bar holding to escape heat. Thus, in both the heat-escape and the heat reinforcement situations, injections of neurohumors affected behavioral thermoregulatory responding in the same direction. That is, cholinergic injections led to behavioral responses which reduced response contingent heat loads and adrenergic injections, the reverse.

However, the behavioral results reported by Beckman [6,7] are indeed paradoxical. He found that injections of acetylcholine and noradrenaline into the preoptic area of rats produced respectively decreases and increases in hypothalamic temperature. If, as earlier studies have indicated, beginning with the reports of Weiss and Laties [18], behavioral thermoregulation is compensatory, one would have expected in Beckman's experiments that hypothermia would have led to increases in responding for heat in the cold and that rises in core temperature would have decreased response rate. Recall that he found just the opposite.

It could be argued that the differences between Beckman's results and our findings are a function of injection or experimental procedures such as volume used (0.5 μl in the present experiment; 1.0 μl in the earlier studies), concentration of the neurochemicals, or our use of a criterion of temperature change before behavioral testing. But the consistency in thermoregulatory behavior between the two situations does not support such an interpretation. A more reasonable explanation may be found in the indices of core temperature used: in the heat reinforcement studies hypothalamic temperature was the index of body temperature; in the present experiments colonic temperature was measured. It could be that although brain and rectal temperatures have been found to parallel each other [1], this not always the case, particularly when hypothalamic manipulations are involved. For example, Satinoff [17] observed that local colling of the anterior hypothalamic-preoptic area in rats elevated rectal temperature as much as 3.1°C. Perhaps intrahypothalamic neurochemical injections similarly affect brain and colonic temperatures differentially. Even in view of these inconsistencies, there can be no doubt that in both the heat reinforcement and the heat escape situations, cholinergic and adrenergic stimulation activate respectively behavioral heat loss and behavioral heat conservation mechanisms.

Other features of the present data deserve comment. First, the changes in core temperature imposed by both cholinergic and adrenergic injections did not represent just simple alterations in physiological heat loss and heat production mechanisms, but led to changes of a motivational nature in heat-escape behavior. Whether an animal was made hyperthermic by injections of carbachol or hypothermic by injections of noradrenaline, behavioral compensation was evidenced in shifts of duration of holding the bar to escape heat. Second, the observation that neither substance altered the number of responses would seem to indicate that the effects of injections on duration were not the result of simple changes in activity level or in ability to respond.

One other point warrants discussion. It might be argued that had the animals been tested immediately following injection the behavioral results would have been opposite. For example, one might expect that if cholinergic injections raise the thermoregulatory set point then an animal would work to augment its body temperature until the new level is

reached and with a lowered set point following noradrenaline, work to reduce body temperature. Thus, the present findings might be a function of thermoregulation at a different set point after temperature had already stabilized at this new level. However, this does not seem plausible in view of earlier work. In our previous experiments [3, 4, 5] we found that cholinergically induced hyperthermia typically did not reach asymptote until three hours after injection, and maximum reductions in core temperature following adrenergic injections usually occurred two hours later. Thus, the present findings would seem to indicate that neurochemical injections disrupt normal thermoregulation and that animals attempt to reduce the imposed changes via behavior when appropriate thermal contingencies are made available.

EXPERIMENT 2

The exact mechanisms by which neurochemical injections affect thermoregulatory behavioral responses are not discernable from Experiment 1. However, several mechanisms seem plausible. First, as previously pointed out, animals may be responding to imposed changes in the thermoregulatory set point and thus may simply be regulating core temperature around this new level. Second, the neurochemical injections may be directly altering efferent pathways, or third, there may be a peripheral sensory basis for the behavioral changes. Of these three possibilities the third one is most plausible in view of previous work by Lipton, Avery and Marotto [12] who contended that the control of thermal behavior is based on peripheral sensory mechanisms. They found that skin temperatures of normal animals were maintained at a relatively constant level over a wide range of radiant energy intensities in the operant heat-escape task, although the skin temperature preferance range shifted as a function of ambient temperature. Thus, Experiment 2 is addressed to the question of a similar peripheral basis for changes in thermoregulatory behavior following manipulation of core temperature via intrahypothalamic injections.

METHOD

Animals and Surgery

Three adult male Charles River rats (CD Strain) were used. Maintenance and surgical procedures were the same as in Experiment 1. At the time of surgery, each animal weighed between 350 and 400 g.

Apparatus and Procedure

The same apparatus and behavioral shaping procedures utilized in Experiment 1 were used in Experiment 2. After the animals' performance had stabilized, skin temperature measurement procedures, similar to previous experiments [12], were initiated. These measurements were made with YSI thermistor probe with reflective back (No. 425). The probe was held in place over the shaved scapulae located at the dorsal midline, 5--10 mm posterior to the point of the withers, by an elastic harness. Restraint was minimal because of the flexibility of the lead wire from the probe. Temperatures at initiation and termination of each bar press were recorded by an observer from a YSI telethermometer (Model 46). Three to five sessions occurred prior to injections to allow the animals to adapt to the skin temperature probe.

Injection procedures were identical to those employed in Experiment 1. Again the substances used were carbamylcholine chloride (carbachol), 8 μg, and L-arterenol bitartrate hydrate (noradrenaline), 25 μg, dissolved in pyrogen free, normal saline and injected in 0.5 μl volumes. The criteria for colonic temperature changes used in Experiment 1 were again applied. Two of the animals received three injections of each substance, administered in a random sequence.

Pre- and postsession colonic temperatures, number of responses and duration of bar holding during the 30 min sessions, and skin temperatures at initiation and termination of each behavioral response were measured.

Histology

The cannula of one animal became dislodged before the completion of the experiments and data from this subject was not used in the statistical analysis. The animals were perfused via standard 10% formal-saline procedures at the end of the experiments. Frozen brain sections were microscopically examined and microphotographed (10x) with a Polaroid MP3 camera according to procedures similar to those outlined by Powell [16]. Placements were found to be located in approximately the same area of the POA, 1.0 mm lateral to the midline and midway between the optic chiasm and anterior commissure.

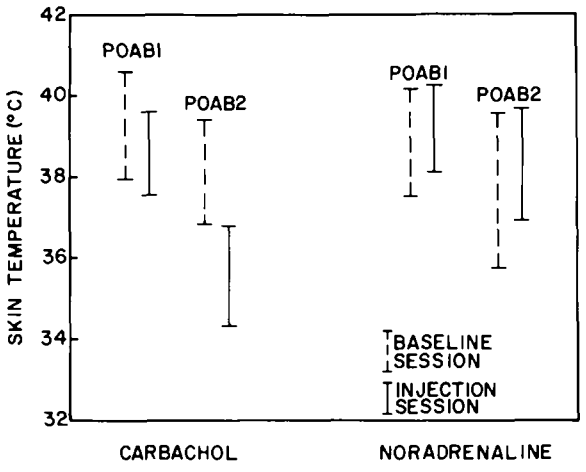

FIG. 4. The effects of intrahypothalamic injections of carbachol and noradrenaline on skin temperature (mean) associated with initiation (top of each bar) and termination (bottom of each bar) of heat-escape responses. Each mean is for three sessions. Note that for both animals carbachol lowered and noradrenaline raised the regulated range of skin temperatures, but that neither substance appreciably altered the absolute width of this temperature range.

RESULTS

Body Temperature and Behavior

The changes in colonic temperature observed in Experiment 1 following injections of carbachol were replicated in Experiment 2. Presession measures of body temperature were significantly higher, as compared with baseline, following injections ($\bar{X}$ increase = 0.62°C, t = 16.76, $p<0.01$), as were comparable postsession measures ($\bar{X}$ increase = 0.10°C, t = 4.08, $p<0.02$). Again cholinergic

stimulation did not affect number of responses ($t = 0.003$, >0.10), but did increase duration of responding ($\bar{X}$ increase = 4.91 min, $t = 6.85$, $p<0.01$).

With one exception, injections of noradrenaline also led to the same changes in colonic temperature and behavioral measures as occurred in Experiment 1. Presession colonic temperatures were lowered by an average of 0.75°C ($t = 8.72$, $p<0.01$) following injection as compared with baseline presession measures. Postsession temperatures were not significantly altered ($t = 1.13$, $p>0.10$) although following four of the six injections, colonic temperature was below baseline presession measures. As in Experiment 1, noradrenergic injections did not change number of responses ($t = 0.009$, $p>0.10$), but did decrease the duration of responding ($\bar{X}$ decrease = 2.02 min, $t = 2.63$, $p<0.10$).

Skin Temperature

As shown in Fig. 4, injections of both carbachol and noradrenaline had significant effects on mean skin temperature at response initiation and termination. In each session following cholinergic stimulation, the average skin temperature observed with response onset was lower than the average skin temperature observed with response onset in baseline sessions ($\bar{X}$ decrease = 1.77°C, $t = 6.10$, $p<0.01$). Although there was greater variability at response offset, the mean skin temperature following injections of carbachol was again lower than for baseline sessions ($\bar{X}$ decrease = 1.48°C, $t = 2.12$, $p<0.10$). Injections of noradrenaline, on the other hand led to opposite effects. Average skin temperature was higher in injection sessions than in baseline sessions at both response initiation ($\bar{X}$ increase = 0.55°C, $t = 2.97$, $p<0.05$) and response termination ($\bar{X}$ increase = 1.30°C, $t = 9.09$, $p<0.01$).

DISCUSSION

The answer to the major question to which Experiment 2 was addressed, i.e., is there at least partially a peripheral basis for changes in response pattern, would seem to be unequivocally yes. Injections of both substances led to levels in skin temperature at both response onset and response offset that appear adaptive given the imposed shifts in core temperature. Under conditions of cholinergic hyperthermia both the initiation and termination of responses occurred at significantly lower skin temperatures than were observed in baseline sessions. With noradrenergic hypothermia, the reverse was true: response onset and response offset occurred at significantly higher skin temperatures.

Skin temperature levels associated with baseline sessions corresponded well with what was observed in an earlier experiment [12]. The mean skin temperature at response onset was 39.9°C, and at response offset, 37.0°C. In the previous experiment a similar band width (mean temperature at initiation of bar press minus mean temperature at release of bar press) between 2.0° and 3.0°C was found. Injections of either substance did not appreciably disrupt this constancy in the band width, but shifted the entire temperature range; carbachol (band width = 2.5°C) lowered it, and noradrenaline (band width = 2.3°C) raised it. Similar shifts occurred in the heat-escape situation when ambient temperature was either above or below thermoneutrality [12]. Thus, it would appear that changes in both ambient temperature and body temperature shifted the skin temperature preference band within which thermoregulatory responses occurred, but neither of these thermal manipulations appreciably altered the width of the band.

In addition, the changes in colonic temperature and behavior measures associated with injections of both substances in Experiment 2 closely paralleled similar measures in Experiment 1. However, the absolute level of duration of bar holding for all conditions was lower in the present experiment than in the first study. This was probably due to effects associated with the skin probe harness; a similar phenomenon was observed in earlier experiments [12]. Even so, the consistency in the direction of the change in duration of responding associated with applications of both noradrenaline and carbachol add further credence to the initial observations, and further support the contention that neurochemically induced shifts in body temperature prompt compensatory behavioral thermoregulation.

In summary, intrahypothalamic injections of carbachol and noradrenaline affected body temperature differentially: carbachol induced hyperthermia; noradrenaline lowered colonic temperature. These changes in body temperature provided a motivational influence on thermoregulatory behavior in the heat-escape situation. Duration of bar holding to escape heat changed in a direction dictated by the imposed alterations of core temperature, i.e., cholinergically indiced hyperthermia and adrenergically induced hypothermia resulted in increased and decreased bar holding respectively. In addition, skin temperatures associated with the behavioral responses were affected by the neurochemical injections. Carbachol lowered the preference band within which skin temperature was regulated and noradrenaline raised the preference band.

REFERENCES

1. Abrams, R. and H. T. Hammel. Hypothalamic temperature in unanesthetized albino rats during feeding and sleeping. *Am. J. Physiol.* **206**: 641–646, 1964.
2. Adair, E. R., J. U. Casby and J. A. J. Stolwijk. Behavioral temperature regulation in the squirrel monkey: Changes induced by shifts in hypothalamic temperature. *J. comp. physiol. Psychol.* **72**: 17–27, 1970.
3. Avery, D. D. Hyperthermia induced by direct injections of carbachol in the anterior hypothalamus. *Neuropharmacology* **9**: 175–178, 1970.
4. Avery, D. D. Intrahypothalamic adrenergic and cholinergic injection effects on temperature and ingestive behavior in the rat. *Neuropharmacology* **10**: 753–763, 1971.
5. Avery, D. D. Thermoregulatory effects of intrahypothalamic injections of adrenergic and cholinergic substances at different environmental temperatures. *J. Physiol.* **220**: 257–266, 1972.
6. Beckman, A. L. Effect of intrahypothalamic norepinephrine on thermoregulatory responses in the rat. *Am. J. Physiol.* **218**: 1596–1604, 1970.
7. Beckman, A. L. and H. J. Carlisle. Effect of intrahypothalamic infusion of acetylcholine on behavioral and physiological thermoregulation in the rat. *Nature* **221**: 561–562, 1969.
8. Carlisle, H. J. Heat intake and hypothalamic temperature during behavioral temperature regulation. *J. comp. physiol. Psychol.* **61**: 388–397, 1966.

9. Lipton, J. M. Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiol. Behav.* 3: 165 169, 1968.
10. Lipton, J. M. Effects of high-fat diets on caloric intake, body weight and heat-escape responses in normal and hyperphagic rats. *J. comp. physiol. Psychol.* **68**: 507–515, 1969.
11. Lipton, J. M. and D. R. Marotto. Effects of desalivation on behavioral thermoregulation against heat. *Physiol. Behav.* **4**: 723 727, 1969.
12. Lipton, J. M., D. D. Avery and D. R. Marotto. Determinants of behavioral thermoregulation against heat: Thermal intensity and skin temperature levels. *Physiol. Behav.* **5**: 1083–1088, 1970.
13. Myers, R. D. Injections of solutions into cerebral tissue: Relation between volume and diffusion. *Physiol. Behav.* **1**: 171–174, 1966.
14. Myers, R. D. and T. L. Yaksh. Feeding and temperature responses in the unrestrained rat after injections of cholinergic and aminergic substances into the cerebral ventricles. *Physiol. Behav.* **3**: 917–928, 1968.
15. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 21–22, 1967.
16. Powell, E. W. A rapid method of intracranial electrode localization using unstained frozen sections. *Electroenceph. clin. Neurophysiol.* **17**: 432–434, 1964.
17. Satinoff, E. Behavioral thermoregulation in response to local cooling of the rat brain. *Am. J. Physiol.* **206**: 1389–1394, 1964.
18. Weiss, B. and V. G. Laties. Behavioral thermoregulation. *Science* **133**: 1338–1344, 1961.