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Thermoregulation in the Rat: Deficits Following 6-OHDA Injections in the Hypothalamus

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MYERS, R. D. AND W. D. RUWE. *Thermoregulation in the rat: deficits following 6-OHDA injections in the hypothalamus.* PHARMAC. BIOCHEM. BEHAV. 8(4) 377–385, 1978. – Bilateral microinjections of 6-hydroxydopamine (6-OHDA) were made in a volume of 0.5–0.75 μl through chronically implanted cannulae into anterior hypothalamic, preoptic loci. Sites were selected at which 1.0 to 12.5 μg of norepinephrine (NE) had previously elicited a fall in the rat's body temperature. After 2.0 to 6.0 μg of 6-OHDA were injected in the same volume at the same loci, a comparable hypothermia ensued. When the rats were exposed repeatedly for one-hour intervals to an environmental temperature of either 35.0°C or 8.0°C, they were unable to thermoregulate against the heat and their colonic temperature rose. In some experiments, the rats also failed to defend adequately against the cold ambient temperature, but mainly following the microinjection of the higher doses of 6-OHDA. The intakes of food and water were generally suppressed; this was accompanied by a transient decline in body weight. Overall, the severity, duration and direction of the thermoregulatory impairment depended upon the anatomical site of injection and the dose regimen of the neurotoxin employed. These results offer further evidence that an intact catecholaminergic pathway within the anterior hypothalamus is required for the rat's physiological control of heat loss in a warm environmental temperature.

Catecholamines and thermoregulation Thermoregulatory deficits Norepinephrine pathway Dopamine pathway
Anterior hypothalamic, preoptic area Ambient temperature 6-OHDA lesion Neurochemical lesion

THE balance in the release of serotonin (5-HT) and norepinephrine (NE) has been proposed to be the neurohumoral mechanism in the anterior hypothalamus whereby an animal regulates its body temperature [11]. Insofar as the catecholamine portion of the theory is concerned, four types of evidence have accrued in support of the idea. First, the application of NE or dopamine (DA) to the anterior hypothalamic, preoptic area (AH/POA) of most species elicits a fall in core temperature [20,29]. Second, the blockade of alpha adrenergic receptors in this region attenuates the actions of the hypothalamically administered catecholamines [28,29]. Third, when an animal is exposed to a warm ambient temperature, there is an enhanced release of NE or DA into push-pull perfusates collected within the AH/POA [23,29]. Fourth, the rostral hypothalamus is richly innervated with noradrenergic and dopaminergic nerve terminals [7, 8, 12, 13, 15, 27, 34, 35].

Recently, the acute pharmacological effect of the neurotoxin, 6-hydroxydopamine (6-OHDA), has been examined by a number of investigators with respect to its central effect on lowering body temperature [4, 14, 25, 30, 31]. However, the role of catecholamines in temperature control has been called into question, since there is reportedly little or no effect of 6-OHDA, injected into the brain, on the rat's subsequent capacity to thermoregulate [37].

The present investigation was undertaken, therefore, because of questions pertaining to the nature of the dose of 6-OHDA, the anatomical locus of action of the neurotoxin, and the time-course of the recovery from a functional lesion induced by 6-OHDA. In this study, we have determined the effect of the 6-OHDA introduced into the catecholamine-sensitive area of the anterior hypothalamus on the thermoregulatory capacity of the rat. Not only the magnitude but also the duration of a catecholamine deficit has been examined.

METHOD

Male rats of the Sprague-Dawley strain weighing between 300–500 g were housed individually in wire mesh cages and maintained on a 12-hr light–dark schedule. Body weight as well as the intakes of powdered Wayne Lab Blox and water, provided ad lib, were measured every day at the same time.

Each rat was anesthetized with sodium pentobarbital (40 mg/kg) given intraperitoneally. Employing surgical procedures described previously [18], two 23 ga guide cannulae, cut to a length of 18 mm from thinwall stainless steel tubing, were implanted bilaterally 2.0 mm above the AH/POA. According to the coordinate system of DeGroot [9], the intended site of injection was in the rostral hypothalamus at: AP, +6.6; Lat, 0.75; Hor, +1.0. Before an

experiment commenced, a 5–7 day postoperative period of recovery elapsed, during which time, food and water intakes as well as body weight stabilized.

Exposure to Different Ambient Temperatures

On the day prior to an intrahypothalamic microinjection, the rats were placed in a chamber divided into eight separate wire mesh compartments. Each compartment measured approximately 12 × 8 × 6 in. and allowed the individual rat to move about freely. The thermoregulatory response of the animals was tested by lowering or raising the temperature of the chamber to 8° C or 35° C, respectively.

The ambient temperature was altered by a stream of either warm air or cold air blown into the chamber. A YSI Model 71A Thermistemp Temperature Controller was used to switch the blowers on or off so as to maintain the given chamber temperature within ±2.0° C. Under either condition, the altered ambient temperature was reached within 5–10 min and then maintained for a full 60 min interval. The sequence of the exposures to the cold or warm temperature was alternated on different days during the course of the series of experiments.

The body temperature of each animal was monitored continuously during an experiment with a YSI 402 thermistor probe inserted 5–8 cm into the colon and held in place with surgical tape wrapped gently around the base of the tail. A 1-hr baseline temperature was obtained prior to an experimental manipulation and monitored thereafter for one to five hours or even longer. On the day following the initial microinjection of 6-OHDA, the rats were tested for their thermoregulatory responses in both warm and cold environmental temperatures. Then, they were retested at subsequent selected intervals for up to 34 days following the injection of the neurotoxin.

Intrahypothalamic Microinjections

To microinject a solution into the AH/POA, a 27 ga

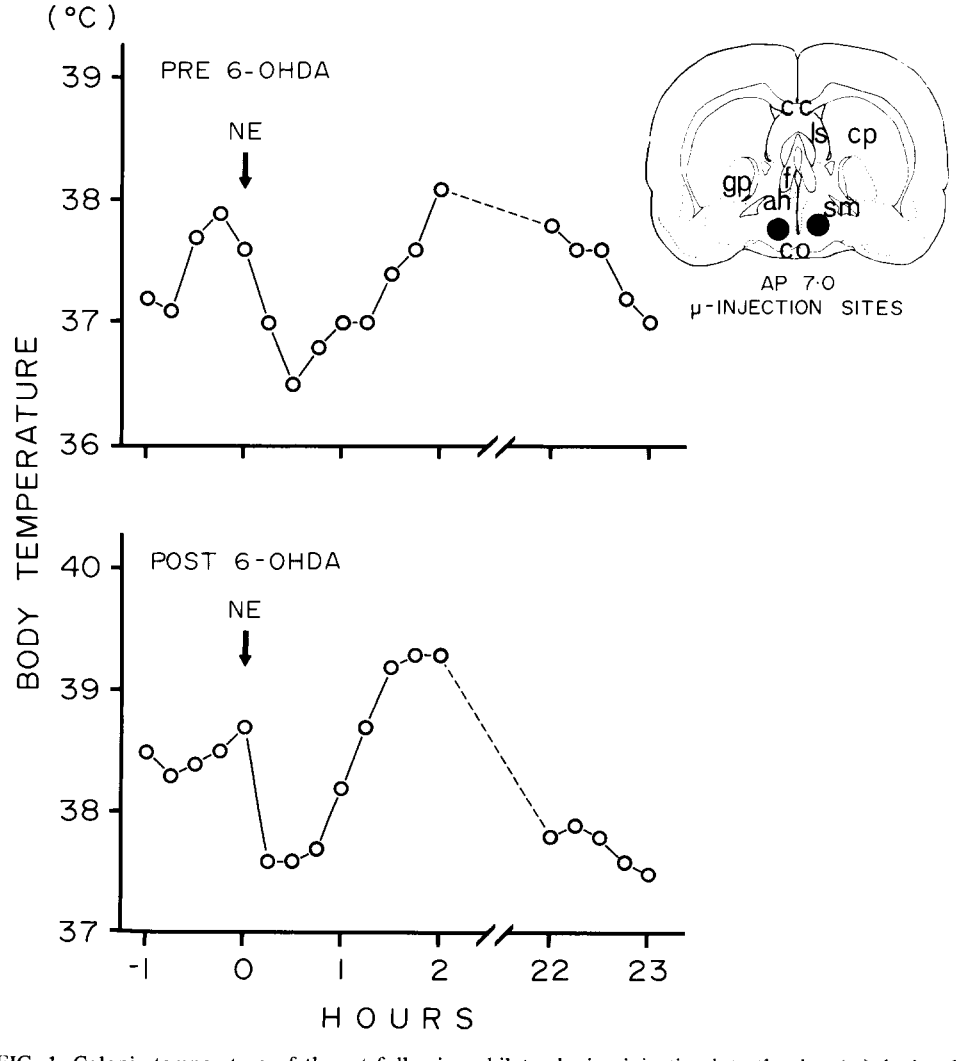

FIG. 1. Colonic temperature of the rat following a bilateral microinjection into the sites (•) depicted in the histological inset. Top: at zero hour, 12.5 μg of NE delivered in 0.5 μl CSF. Bottom: at zero hour, an identical injection of NE after 6-OHDA had been injected in the sites 30 days earlier. Anatomical abbreviations are: ah, anterior hypothalamus; cc, corpus callosum; co, optic chiasm; cp, caudate-putamen nucleus; f, fornix; gp, globus pallidus; ls, lateral septal nucleus; sm, stria medullaris.

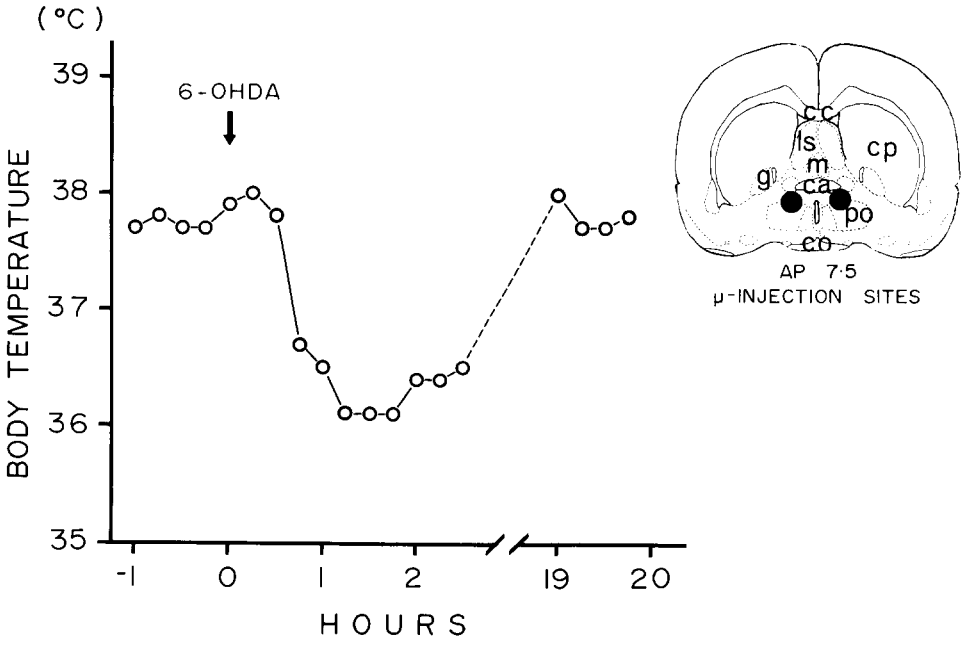

FIG. 2. Colonic temperature of the rat following a bilateral microinjection into AH/POA sites (•) as depicted in the histological inset. At zero hour, 3.0 μg of 6-OHDA given in 0.75 μl CSF. Anatomical abbreviations as in Fig. 1, ca, anterior commissure; m, medial septal nucleus; po, preoptic area.

injector cannula was connected by PE-50 tubing to a 50 μl Hamilton syringe mounted on an infusion pump. The tip of the injector cannula was positioned below the tip of the guide tube and a microinjection made during an interval of 30–60 sec. The injector cannula was kept in place for 60 sec and then a permanent indwelling stylet was reinserted into the guide tube [18].

In order to determine the sensitivity of a specific hypothalamic locus to a catecholamine, 1.0 to 12.5 μg of NE (L-Arterenol HCl; Sigma Chemical) were microinjected unilaterally in a volume of either 0.5 or 0.75 μl. Loci were tested at successive 0.5 mm depths below the tip of the guide cannula. A given site was considered responsive if NE produced a decline in the rat's body temperature of 0.5°C or more within 30 min following its injection. After at least one day had elapsed, 2.0 to 6.0 μg of 6-hydroxydopamine (2,4,5-trihydroxyphenethylamine hydrobromide, 6-OHDA; Sigma Chemical) were microinjected bilaterally into catecholamine-reactive sites. Again, the temperature of each rat was observed for a comparable period of time following the injection.

The carrier vehicle consisted of an artificial cerebrospinal fluid (CSF) to which 0.1 mg/ml of ascorbic acid had been added to reduce the pH and retard auto-oxidation of the amine [19]. Each solution was prepared in pyrogen-free glassware immediately prior to use. The tubing and syringe were stored in 70% ethanol and flushed repeatedly with ethanol and artificial CSF prior to use. The dose of each compound is expressed as the amount of the free base.

Histological Analysis

At the conclusion of the experiments, the sites of microinjection were verified. After the rat was given an overdose of sodium pentobarbital, the heart was clamped and 0.9% normal saline followed by 10% Formalin was perfused retrograde through the thoracic aorta. The brain was washed, blocked and sectioned on a Lipshaw cryostat at 60 μm. Subsequently, the sections were stained for cells and fibers according to Wolf [39], and examined under light microscopy.

RESULTS

Depending upon the hypothalamic locus of injection and the total dose of 6-OHDA administered, two distinct patterns of thermoregulatory incapacitation became apparent during the course of the experiments. Those animals which had received a single low dose of 6-OHDA in the critical region of the AH/POA developed a thermoregulatory impairment mainly in response to heat, which was moderate and transient in nature. A second group of animals in which a second microinjection of a higher dose of the neurotoxin was given, suffered a severe thermoregulatory impairment upon exposure not only to heat but in several cases in response to the cold air temperature. Other rats in which microinjections were in sites outside of the AH/POA failed to develop any thermoregulatory deficit.

NE and 6-OHDA Hypothermia

A bilateral microinjection of NE into sites in the rat's AH/POA, depicted in the histological inset of Fig. 1, caused a marked hypothermia. As illustrated in Fig. 1, the fall in body temperature (top) of a representative rat began within 5 min after the NE injection and continued to 1.1°C below the baseline level. When microinjected into the same hypothalamic sites 30 days after 6-OHDA had been applied similarly, NE evoked an almost identical fall in the rat's body temperature (Fig. 1, bottom). Each of the hypothermic responses was characterized by a rapid reduction in

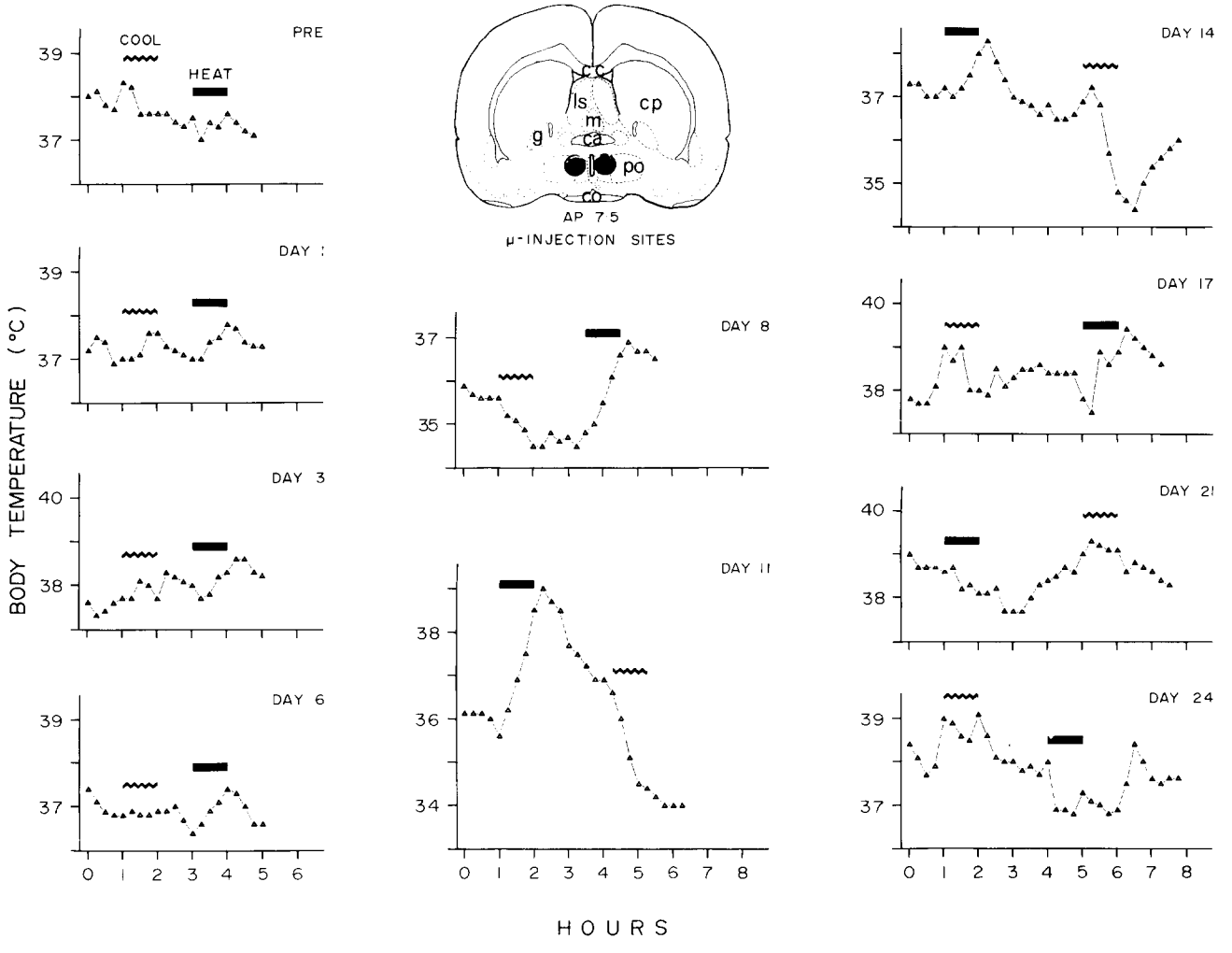

FIG. 3. Body temperature of a given rat recorded in a series of experiments carried out over 24 days at intervals indicated on each graph. The animal was alternately exposed to ambient temperatures that were cool 8.0°C (〰), or hot, 35.0°C (▬), for a 1-hr interval: before 6-OHDA (PRE), 24 hrs after microinjection of the neurotoxin (Day 1) and at 2–3 day intervals thereafter (Days 3–24). Prior to Day 1, 2.0 μg of 6-OHDA were microinjected into the AH/POA sites (•) depicted in the histological inset. Prior to Day 8, 6.0 μg of 6-OHDA were microinjected again in the same sites. Anatomical abbreviations as in Fig. 2.

the rat's basal colonic temperature which lasted from 15–30 min. After an interval of 1–2 hr, the animal's temperature either returned to its preinjection level or an overshoot occurred (Fig. 1, bottom) which subsided within 24 hr. Of the loci retested for NE sensitivity following 6-OHDA, only when the amine was injected into the AH/POA did a fall in temperature occur. Thus, NE had virtually no effect when the amine was injected into sites that lay outside of this thermosensitive region. Moreover, the NE-induced hypothermia was not any more intense after 6-OHDA microinjections.

When 3.0 μg of 6-OHDA was delivered again bilaterally in the AH/POA (Fig. 2, insert) in a volume of 0.75 μl, a sustained fall in the rat's body temperature occurred once again. Although the decline was nearly 2.0°C, the latency of the response, as illustrated in Fig. 2, was somewhat longer than that following the microinjection of NE into an homologous site.

Responses to Warming and Cooling

The general time-course of thermoregulatory impairment caused by a bilateral microinjection of 6-OHDA into the sites depicted in the histological inset is presented in Fig. 3 for a representative rat. Prior to the intrahypothalamic administration of 6-OHDA (PRE), the thermoregulatory capacity of the rat was unaffected during a one-hour period of either warming or cooling. However, on the day following the microinjection of the neurotoxin (Day 1), the temperature of the animal increased by 0.5°C when it was exposed to warm air for the same interval. This impairment became progressively more marked, and six days after the 6-OHDA injection, the rat's temperature rose more than 1.0°C in response to the peripheral warming. On Day 8, a displacement of 2.0°C occurred following the warm challenge. In all tests except on Day 8, exposure to cold air caused only a slight fluctuation in the rat's temperature.

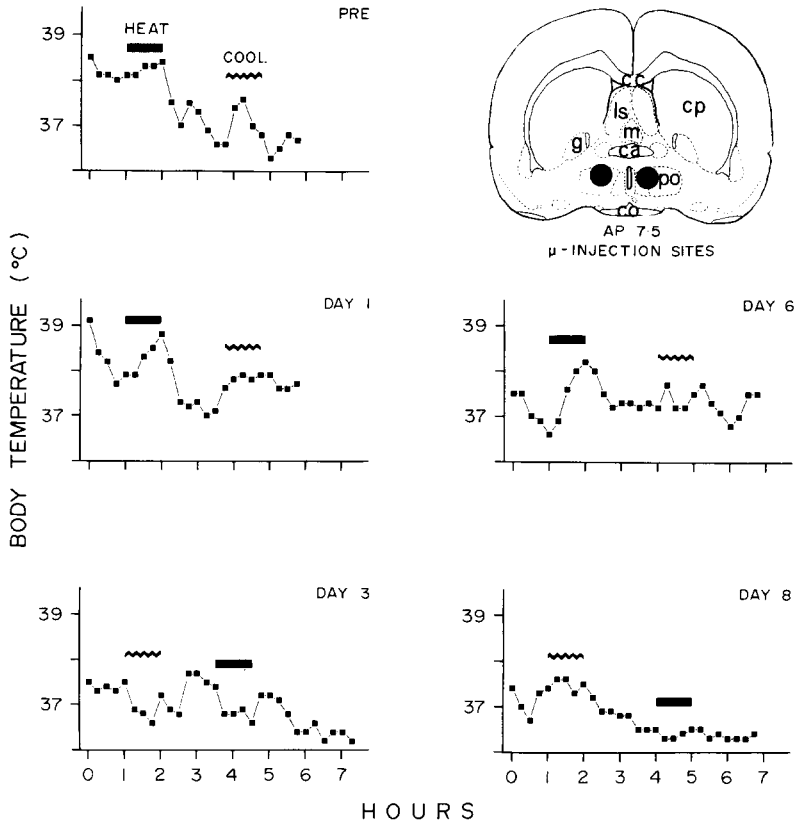

FIG. 4. Body temperature of a rat alternately exposed to cold, 8.0°C (〰), or to heat, 35.0°C (▬), for a 1-hr interval before 6-OHDA (PRE), 24 hrs after the microinjection of 6-OHDA (Day 1) and at 2–3 day intervals thereafter (Days 3–8). 6-OHDA was microinjected in a dose of 3.0 μg in the AH/POA (●) as depicted in the histological inset. Anatomical abbreviations are as in Fig. 2.

The thermoregulatory deficit was accentuated by a second set of microinjections of 8.0 μg of 6-OHDA given at the same sites. On Day 11, either peripheral heating or cooling displaced the rat's body temperature above or below the baseline level by more than 2.0°C. This is shown in Fig. 3. Thereafter, the impairment began to abate. In fact, within one week after the second microinjection of 6-OHDA, the animal thermoregulated adequately when exposed to either of the peripheral thermal challenges, and by Day 21 it had essentially recovered.

The temporal nature of the deficit caused by a single injection of 6-OHDA is illustrated in Fig. 4. When 6-OHDA was injected into an homologous site in the AH/POA (inset), in a dose of 3.0 μg in 0.5 μl, only a moderate and transitory thermoregulatory deficit occurred. As depicted in Fig. 4, the rat's temperature increased only slightly during exposure to the heat on Day 3 and was not affected by the exposure to the cold air. On Days 1 and 6, however, the one hour interval of warming caused an increase in colonic temperature of between 1.0°C and 2.0°C; again, cooling was without effect.

If 6-OHDA was microinjected in an identical volume and concentration into a diencephalic area that was totally insensitive to NE, no evidence of a thermoregulatory deficit was obtained. Figure 5 illustrates the sequence of heating and cooling at intervals during a 24-day period following microinjection of the neurotoxin in sites as indicated in the inset for a rat in which the catecholamine was without effect.

A statistical analysis of the maximal deflections in body temperature revealed several facts. Prior to the microinjection of 6-OHDA, the regulatory response to either thermal challenge was similar in both the extra-hypothalamically injected control rats as well as in those in which the neurotoxin was microinjected subsequently into the AH/POA, that is for tests conducted in the heat, $t(11) = 0.18$; $p > 0.30$, or in the cold, $t(11) = 1.35$; $p < 0.125$. However, when compared to the non AH/POA injected controls, the rats in which 6-OHDA was microinjected directly into the AH/POA showed a greater deviation in colonic temperature in response to peripheral warming, $t(11) = 2.20$; $p = 0.05$. Following exposure to the cold, the thermoregulatory response of the animals in which the neurotoxin had been microinjected within the AH/POA did not differ significantly from the extra-hypothalamic control injected rats, $t(11) = 1.37$; $p < 0.10$. Figure 6 illustrates these two types of injection loci: Top, section shows that the 6-OHDA lesion sites are in the AH/POA dorsal to the

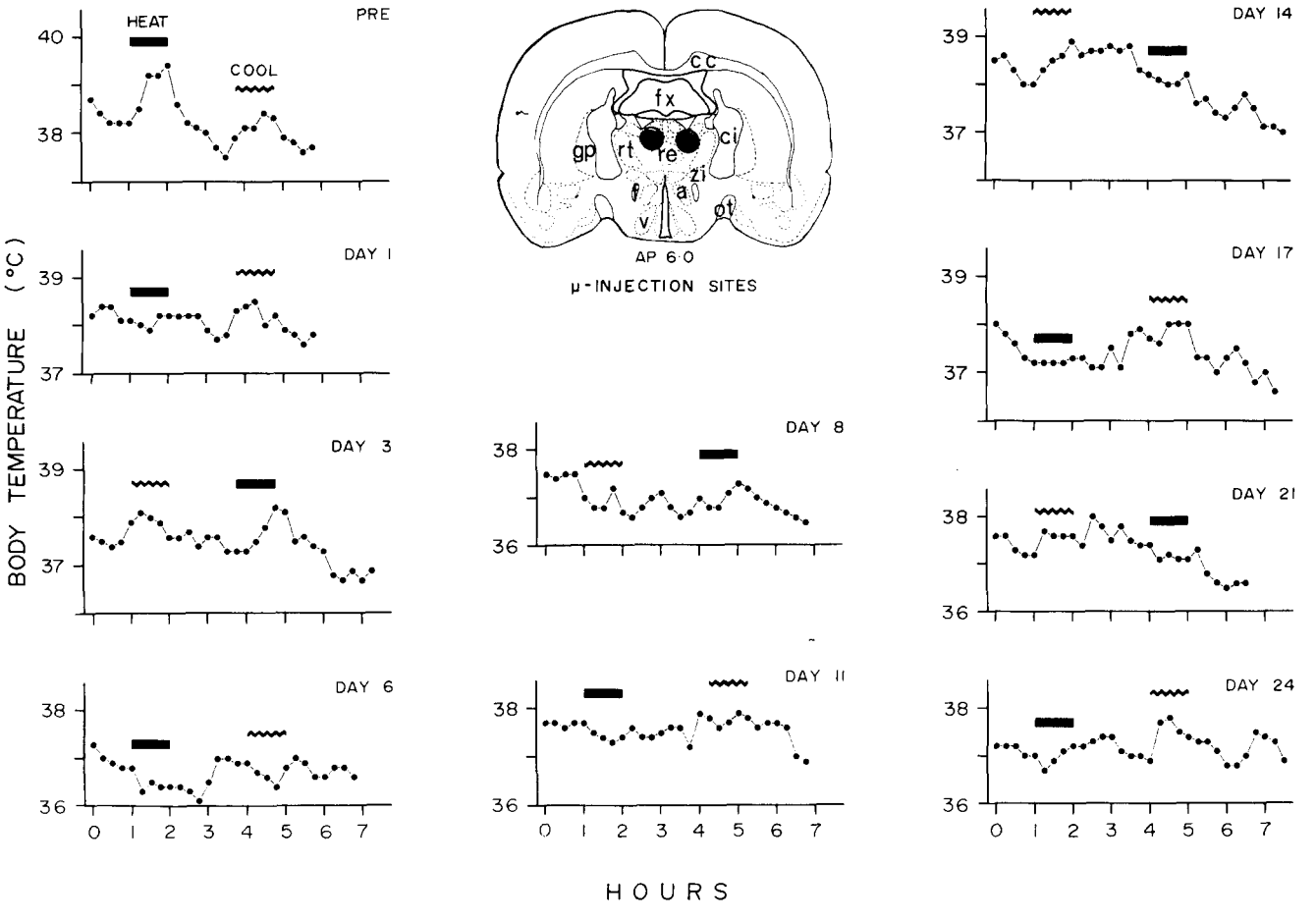

FIG. 5. Temperature of the rat alternately exposed to cold, 8.0°C (ww), or to heat, 35.0°C (▬), for a 1-hr interval before 6-OHDA (PRE), 24 hr after the neurotoxin (Day 1) and at 2–3 day intervals thereafter (Days 3–24). 3.0 μg of 6-OHDA were microinjected into the sites in the thalamus (•) as depicted in the inset. Anatomical abbreviations are ci, internal capsule; fo, columns of the fornix; fx, fornix; ot, optic tract; re, reuniens nucleus of the thalamus; rt, reticular nucleus of the thalamus; v, ventromedial hypothalamus; zi, zona incerta.

optic chiasm; Bottom, section depicts the 6-OHDA microinjection loci in a region medial to the anterior commissure within the rostral portion of the preoptic area.

Food and Water Intakes

The body weights of both 6-OHDA injected groups, i.e., the AH/POA and the extra-hypothalamic rats, did not differ before surgery, $t(11) = 0.048$; $p>0.30$, nor prior to administration of the 6-OHDA, $t(11) = 0.149$; $p>0.30$. However, after the 6-OHDA injections, a significant decline in weight, $t(118) = 2.235$; $p<0.025$, and in both food and water intakes occurred only in those rats in which 6-OHDA had been given in the AH/POA. Dietary supplements of palatable Sustagen biscuits and liquid Sustagen were required to maintain the weight of the latter animals. As the rats recovered from their thermoregulatory impairment, their normal feeding and drinking patterns resumed.

DISCUSSION

The present findings further substantiate the supposition that a catecholaminergic pathway within the rostral hypothalamus mediates the heat loss mechanism in an animal's defense against a warm environment [22]. Although the dose of amine and anatomical site are crucial [6], norepinephrine microinjected within this area of the brain induces a decline in the animal's body temperature [1,17]. Veale and Whishaw [38] have demonstrated that norepinephrine evokes hypothermia when it is microinjected into an area extending from the lateral hypothalamic region to the mid-portion of the rostral hypothalamus. Our experiments not only confirm this finding but reveal that the area sensitive to the catecholamine extends even more anteriorally, encompassing coronal plane AP 7.5 which passes through the preoptic area.

When injected at a norepinephrine-sensitive site, 6-OHDA produces a fall in the rat's body temperature, the characteristics of which are similar to those seen following a microinjection of NE into the same locus. This result corresponds to a series of studies in which 6-OHDA infused by the intraventricular or intracisternal route likewise elicits a dose-dependent hypothermia [4, 25, 30]. Taken together, these findings suggest that at least one site of action of 6-OHDA, given by either route, is the anterior hypothalamic, preoptic area. Interestingly, we find no evidence of a localized supersensitivity to norepinephrine following the

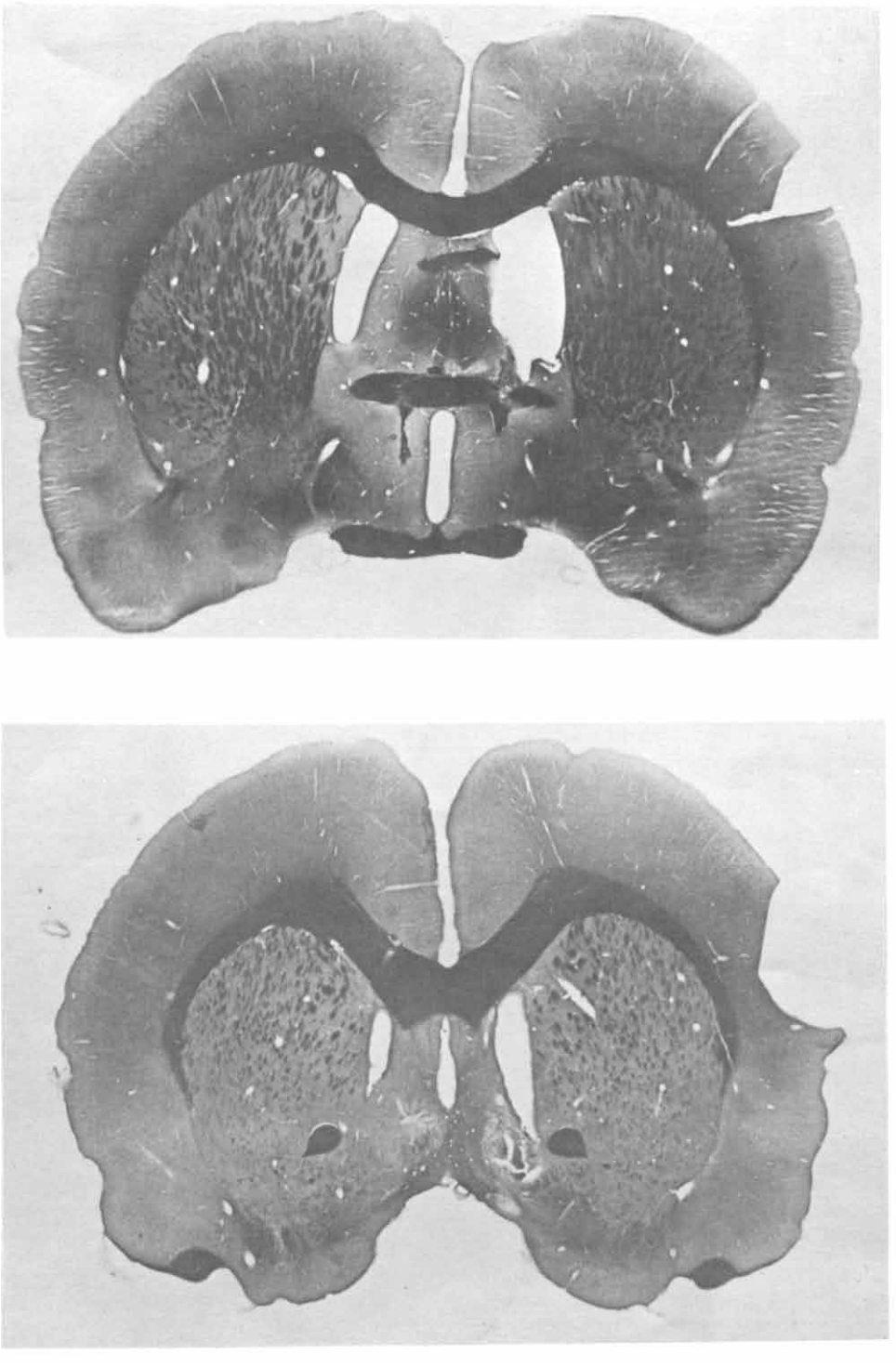

FIG. 6. Representative histological sections. Top: sites in the AH/POA dorsal to the optic chiasm in which 6-OHDA produced a profound thermoregulatory impairment. Bottom: lesion sites in an area medial to the anterior commissure and the preoptic area in which 6-OHDA had relatively no effect on the thermoregulatory capacity of the rat.

neurotoxic infusion. That is, upon retest, the hypothermia evoked by norepinephrine is not notably greater than the temperature decline observed prior to the 6-OHDA microinjection. Such an absence of supersensitivity has been reported also after 6-OHDA is infused at sites at which norepinephrine evokes feeding [24]. In addition, after the serotonergic neurotoxin, 5,6-DHT, is microinjected at an anterior hypothalamic site at which 5-HT evokes thermogenesis in the rat, again there is no evidence of a heightened sensitivity upon the reinjection of 5-HT [21].

The incapacitation produced by 6-OHDA of the entire thermoregulatory mechanism has been described previously. For example, after 6-OHDA is microinjected in a high dose into the hypothalamus of the rabbit, the animal no longer is able to defend against locally applied warm or cold stimulation of the thermosensitive zone [40]. Although the same sort of regulatory deficit occurs in the rat when it is exposed to a warm or cold ambient temperature, the impairment is a time-dependent, transient one from which the rat recovers. Presumably, the specific pattern of deficit depends on the number of catecholamine terminals that are destroyed; however, the depletion of catecholamine stores may persist for several weeks following the administration of the neurotoxin [36]. In all likelihood, the loss of the animal's capacity to thermoregulate is related to an initial non-specific action of a sufficiently high dose of 6-OHDA in destroying aminergic nerve endings [5, 26, 32] including serotonergic terminals. A disseminated zone of necrosis surrounding the immediate region of the microinjected droplet is caused by 6-OHDA [10]. In fact, the 6-OHDA-induced degeneration of catecholamine neurons is generally considered to be dose-dependent, with high doses causing generalized tissue destruction [3,16]. Non-specific trauma, retrograde damage along the cannula shaft as well as along blood vessels and myelin tracts can ostensibly be avoided if a lower dose of the neurotoxin and a reasonable injection volume are employed [2].

From an anatomical standpoint, if 6-OHDA is not microinjected at a locus containing noradrenergic endings, the animal then thermoregulates quite adequately in either the warm or cold environment. Similarly, if the neurotoxin is delivered into the brain substance in a sufficiently large volume, so that the bulk of the injected solution effluxes into the cerebral ventricle, little or no impairment in thermoregulation would be expected. The reason is simply that the dose of 6-OHDA ultimately reaching the noradrenergic nerve terminals is ineffective. Either of these two possibilities could explain why thermoregulation may not always be affected by 6-OHDA when the neurotoxin is injected into the hypothalamus [37]. If a lower dose of 6-OHDA is infused repeatedly, then norepinephrine depletion may be even more marked than that following a single dose [3]. This could determine, in part, why the second 6-OHDA treatment caused such a severe incapacitation in the thermoregulatory responses of our animals to warm or cold ambient temperatures.

As described previously, the intake of food in the 6-OHDA-treated rat declines substantially. This result underscores the integrated functional nature of the catecholaminergic pathways within the rostral region of the hypothalamus. In the rat, monkey and other species, norepinephrine infused into the anterior hypothalamic area can evoke eating and in some cases a simultaneous fall in body temperature [20]. Moreover, it is probable that a functionally intact catecholamine system is required in the diencephalon for a normal pattern of regulated food intake [33].

Finally, our results with the 6-OHDA correspond well with those in which 5,6-DHT has been applied to the diencephalon in the same way. Following the microinjection of this serotonergic neurotoxin into homologous loci of the rat's anterior hypothalamus, the animal exhibits a marked impairment in its physiological defense against a cold ambient temperature [21]. In this case, the mechanism for thermogenesis is not activated following the 5-HT lesion to the self-same area. Overall, these observations provide further support for the concept that a balance in the presynaptic release of 5-HT, norepinephrine and possibly dopamine within the AH/POA constitutes one neurochemical component underlying the mechanism whereby thermoregulation is achieved [20].

ACKNOWLEDGEMENTS

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