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Dopamine in the Hypothalamus of the Cat: Pharmacological Characterization and Push-Pull Perfusion Analysis of Sites Mediating Hypothermia

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RUWE, W. D. AND R. D. MYERS. *Dopamine in the hypothalamus of the cat: Pharmacological characterization and push-pull perfusion analysis of sites mediating hypothermia.* PHARMAC. BIOCHEM. BEHAV. 9(1)65–80, 1978.—Within the rostral diencephalon of the cat, 113 sites were examined for their reactivity to 2.33–14.0 μg dopamine (DA) or 2.33–14.0 μg norepinephrine (NE) microinjected in a volume of 0.75 μl. During each experiment, colonic temperature was monitored and additional physiological measures were recorded continuously. In contrast to CSF controls, an intrahypothalamic injection of either catecholamine at circumscribed sites evoked a dose-dependent fall in the cat's body temperature, with NE ordinarily evoking a more profound hypothermic response. The morphological sites of maximum sensitivity were localized in the anterior hypothalamic, preoptic region. At some but not all sites, a prior microinjection of 3.5–7.0 μg phentolamine attenuated the magnitude of the DA-induced hypothermia and delayed its onset. Conversely, at all loci, the pretreatment by the injection of this alpha-adrenergic antagonist markedly reduced the absolute magnitude of the NE-induced fall in the cat's temperature. Similar pretreatment of a reactive hypothalamic locus with a beta-adrenergic receptor blocking agent, practolol (3.5 μg), failed to alter the hypothermia following a microinjection of DA. Either of two DA receptor antagonists, haloperidol (0.04–7.0 μg) or d-butaclamol (0.48–1.47 μg), when given in a sufficient dose, effectively delayed the onset of the DA-hypothermia and reduced its absolute magnitude; however, the NE-induced decline in the cat's temperature was unaffected by DA receptor blockade. Endogenous stores of DA and/or NE in the cat's hypothalamus were radio-labeled with either ^{3}H- or ^{14}C-catecholamines or both, microinjected through the implanted guide tube into an identified amine-sensitive site. By using push-pull cannulae, the site was subsequently perfused for 5 min with artificial CSF at a rate of 25 μl/min with samples collected at 15 min intervals. During either the third or fourth perfusion, the ambient temperature of the cat's chamber of 22–24°C was elevated to 35–45°C and maintained at this level for 15 or 30 min. This environmental warming evoked a release of either DA or NE or both amines from certain circumscribed sites within the cat's rostral hypothalamus. Overall, these results provide pharmacological, physiological and anatomical evidence for a differential role of DA in the hypothalamic mechanism which mediates the heat loss processes.

Dopamine Cat brain Catecholamines Norepinephrine Thermoregulation Hypothalamus Heat challenge Push-pull perfusion Receptor antagonists

ALTHOUGH dopamine was implicated initially in the thermoregulatory mechanism in 1967 [28], this metabolic precursor of norepinephrine, as reviewed recently [29], is not generally considered in monoamine models of thermoregulation. The reason for this is that dopamine injected either into an animal's cerebral ventricle or anterior hypothalamus causes only a moderate decline in body temperature; this is in sharp contrast to the intense hypothermia produced by an equivalent dose of norepinephrine [4, 34, 35]. Nevertheless, a relatively high dose of dopamine given either by the intraventricular or intrahypothalamic route evokes a modest fall in the temperature of the pigeon [6], cat [16, 19, 40] and rat [5,20].

Recently, agents that block dopaminergic receptors have been used to characterize the hypothermia induced by centrally administered DA. For example, when haloperidol is infused intraventricularly or intrahypothalamically, it attenuates the hypothermic response normally evoked by the amine [19,40]. Similarly, pimozide administered systemically or intrahypothalamically reduces the fall in a rat's temperature produced by a central injection of dopamine or its agonist analog, apomorphine [11]. Interestingly, the neurotoxin, 6-hydroxydopamine (6-OHDA), which releases catecholamines from the degenerating nerve terminals, upon its ventricular injection also exerts an immediate hypothermic effect on an animal's body temperature [3,41]. Further, 6-OHDA injected into the anterior hypothalamus of the rat likewise produces an identical type of temperature decline [31].

The purpose of the present experiments was twofold.

First, we attempted to specify pharmacologically and anatomically the precise action of dopamine on body temperature in comparison to that of norepinephrine. In this investigation, a morphological mapping of diencephalic sites sensitive to either or both of the catecholamines was undertaken. Then, identified sites that were reactive to a given catecholamine were examined with respect to the possible antagonism of the hypothermia by alpha- and beta-adrenergic antagonists as well as two dopamine receptor blocking agents.

Second, we determined whether the activity of endogenous DA in hypothalamic pools would change as a result of an environmentally delivered thermal stress. Already it is known that the release of NE from the anterior hypothalamus of the cat is augmented by peripheral warming of the animal, but unaffected by cooling [30]. Further, an enhancement in DA release from diencephalic sites has been demonstrated in the rat as the animal obtains food pellets [22]. In the present experiments, radioactive DA was used to label hypothalamic loci that earlier had been identified as being sensitive to the microinjection of DA.

METHOD

Adult male or female cats weighing from 3.0–5.0 kg were housed individually in a colony room that was maintained at an ambient temperature of 22–24°C and illuminated on a 12 hr light-dark cycle. Each morning, the cats received fresh water and Purina cat chow supplemented occasionally by canned cat food.

Surgical Procedures

Each animal was anesthetized with 20–30 mg/kg sodium pentobarbital injected intravenously into the saphenous vein or one of its superficial branches. Using the stereotaxic coordinate system of Jasper and Ajmone-Marsan [17], an array of two or four 20 ga stainless steel guide tubes was implanted according to surgical procedures described previously [25]. Each guide tube was fitted with an indwelling 23 ga stylet of corresponding length, both of which were beveled at a 45° angle to assure tip conformity. Craniotomy holes were made bilaterally in the calvarium and enlarged with rongeurs to expose the dura.

After the dura was incised, the tips of the guide tubes were lowered always 2.0–4.0 mm above the rostral diencephalon so that damage to the intended sites of microinjection or push-pull perfusion was avoided. Following insertion of three anchor screws into the calvarium, the cannulae were then secured in place with cranioplast cement. A pedestal with a protective cap was placed over the guide tubes and secured to the skull with two additional anchor screws and cranioplast cement. Postoperatively, intramuscular injection of 100 k units of crysticillin were continued daily for a period of 10 days.

Microinjection Procedures

To microinject DA or another compound directly into a given hypothalamic locus, a 28 ga thin-walled stainless steel injector cannula was cut to 55 mm in length and its tip beveled at 45°. It was connected by PE-20 tubing to a Hamilton 50 μl syringe mounted on a Harvard infusion pump, and the tubing was filled with the solution to be injected. Between each injection, the tubing, cannulae and syringes were stored in a 70% ethanol solution. Prior to use, they were flushed repeatedly with 70% ethanol and an artificial CSF consisting of a standard 5-ion solution [26].

Microinjections of a given agonist or antagonist were always made in a volume of 0.75 μl assuring an approximate dispersion of 0.3–1.1 mm in every direction [36]. To allow local infiltration of the solution, the injector needle was kept in place for an additional 30–60 sec, then removed and replaced by the indwelling stylet. The following compounds were used: 3-hydroxytyramine HCl (dopamine, 2.33–14.0 μg [Sigma]); L-arterenol HCl (norepinephrine, 2.33–14.0 μg [Sigma]); haloperidol (0.04–7.0 μg [McNeil]); pimozide (22.0 μg [McNeil]); spiroperidol (22.0 μg [Janssen]); phentolamine HCl (3.5–7.0 μg [CIBA]); practalol HCl (3.5 μg); and d-butaclamol HCl (0.48–1.47 μg [Ayerst]). Doses are expressed as micrograms of the base. Each solution of the compound injected was prepared in either the pyrogen free artificial CSF or glass-distilled water and passed through an 0.22 μm Swinnex millipore filter. The pH of the solution was adjusted to 3.8–4.0 by the addition of 0.1 mg/ml of ascorbic acid.

Physiological Measures

A YSI 401 thermistor probe was inserted 8–10 cm into the colon and held in place by surgical tape wrapped gently around the base of the tail. The cat's colonic temperature was monitored on a YSI tele-thermometer and recorded simultaneously on a multi-point recorder. The baseline temperature of each cat was obtained for at least 60 min before an experiment began. At 5–15 min intervals throughout the course of an experiment, food intakes, ear temperature estimated by gentle palpation, respiratory rate, pupillary dilation, level of arousal and instances of panting, salivation, micturition, miaowing, licking, washing, motor movements, purring, piloerection, shivering, huddling or growling were recorded.

During a series of experiments, each cat was allowed access to its food, which had been weighed carefully, for a seven-hr period throughout the day. The quantity of food consumed following a microinjection was determined by weighing the food dish and its contents at half hour intervals.

Push-Pull Perfusion Procedures

To determine the activity of the endogenous stores of catecholamines within the hypothalamus, sites identified as reactive to DA were perfused using the push-pull technique [23]. As described previously, the inner or push cannula, cut from 29 ga thin-walled stainless steel tubing was inserted into an outer or pull cannula consisting of 23 ga thin-walled stainless steel tubing. The beveled tip of the push cannula was extended 0.5–1.0 mm beyond the tip of the pull cannula. With this separation of the cannulae, it was possible to bathe a sphere of tissue approximately 1.0 mm in diameter [32]. The depth of penetration into cerebral tissue was predetermined by a polyethylene spacer which fit snugly over the outer pull cannula.

The push and pull cannulae were connected by PE-20 and PE-50 tubing, respectively, to calibrated 1.0 ml glass syringes which were mounted on a Harvard infusion-withdrawal pump. The cannula assembly, tubing and syringes were stored in 70% ethanol between experiments and flushed repeatedly with ethanol and artificial CSF prior to an experiment. A minute air bubble introduced into the pull tubing prior to perfusion reflected the perfusion flow rate and volume collected.

Each perfusion site was labeled 20–30 min before the first perfusion with 1.0–1.5 μl of one or more of the following labeled compounds: 1-[7-^{3}H]noradrenaline (8.9 Ci/mM);

(ethylamine-1-^{14}C)dopamine hydrochloride (62.0 mCi/mM) (Amersham/Searle); or dihydroxyphenylethylamine hydrobromide,3,4(ethyl-1-^{14}C) (54.9 mCi/mM) (New England Nuclear). Respectively, the total dose of injected norepinephrine was 0.5 μg/μl/25 μCi, whereas dopamine was 16.0 μg/μl/2.5 μCi.

Successive perfusions were spaced at 15 min intervals with a typical perfusion sequence lasting for 135 min. If the pull side of the system gave signs of occlusion or if the perfusate became discolored, the perfusion experiment was terminated immediately. Three perfusions were completed prior to any experimental manipulation in order to establish a washout curve of declining radioactivity [15,21].

A 50 μl aliquot of each sample of perfusate was transferred by pipette into a counting vial containing 3.0 ml of the scintillation fluor (PCS, Amersham/Searle). The level of radioactivity in CPM of each sample was determined for 10 min in a Packard Tri-Carb 3320 liquid scintillation spectrometer and later converted to DPM using external standard-channels ratio method.

Alteration in Ambient Temperature

To raise the ambient temperature of the cat, streams of hot air from three electrical heat exchangers (General Electric) were gently blown into the test chamber. By this method, it was possible to achieve a temperature elevation of from 13° to 26°C usually within 3 min but no longer than 10 min. The ambient temperature was decreased by 15–24°C over a similar interval by means of a dry ice-ethanol heat exchanger [43]. The animal was exposed to only one of these two conditions during the course of an experiment. The altered ambient temperature was maintained throughout the succeeding one or two perfusions, i.e., 15–30 min.

Histological Analysis of Test Sites

Upon completion of the experiments, each cat was given an overdose of sodium pentobarbital intraperitoneally. After the heart was clamped, saline followed by 10% neutral Formalin was perfused retrograde through the thoracic aorta. The brain was then blocked stereotaxically in the coronal plane and sectioned at 100 μm thickness on a sledge cryotome. Each section was stained for cells and fibers using a modified Weil-Weigert hematoxylin method [44]. The site of each microinjection was localized under light microscopy and mapped anatomically using histological reconstructions.

RESULTS

The present findings reveal that the hypothalamic mechanism underlying catecholamine-induced hypothermia can be selectively differentiated on the basis of neuroanatomical sensitivity and receptor specificity. In the case of either DA or NE, circumscribed anatomical regions of the rostral hypothalamus are involved in the respective decline in the cat's body temperature. However, at least two distinctive types of catecholaminergic receptors mediate the temperature change.

Anatomical Localization of Catecholamine-Sensitive Sites

Within coronal planes AP 11.0 through AP 15.0 of the cat's rostral hypothalamus, 113 sites were examined for their sensitivity to DA. Subsequently, many of these same hypothalamic loci were tested for their reactivity to NE microinjected in the same way. Figure 1 presents a composite anatomical mapping of the sites at which DA or NE or both amines were microinjected. The temperature responses at different loci of the animal's brain were classified according to one of three criteria adopted as follows: (1) *low*, 0.1–0.4°C decrease in colonic temperature within 45 min after a microinjection (●); (2) *medium*, 0.5°C or greater decrease in temperature within 45 min of an injection (□); and (3) *high*, 0.6°C or greater decrease in core temperature within 30 min following a microinjection (■). Sites at which either catecholamine failed to elicit a response also are designated (○).

Of the 113 sites tested, only 19 were found to be catecholamine sensitive. As shown in Fig. 1, these catecholamine-sensitive loci are distributed anatomically in the anterior hypothalamus and the preoptic region, i.e., coronal planes AP 14.0 and 15.0 inclusive. Although nearly all of these 19 sites were reactive to either DA or NE in terms of a hypothermic response, a limited number of sites specifically sensitive to DA alone was discovered.

Characteristics of Catecholamine-Induced Hypothermia

A representative hypothermia following a single microinjection of 7.0 μg of DA is depicted in the upper plate of Fig. 2. The lower plate of Fig. 2 illustrates a typical hypothermia produced by a microinjection of 7.0 μg of NE. The intense and long-lasting hypothermia evoked by either amine was accompanied occasionally by a slight increase in the rate of respiration. This transient shift in respiratory rate, described previously by Myers, Metcalf and Rice [37], subsided usually within the first 15 min following the injection of the amine. Moreover, evidence of peripheral vasodilation during the course of a hypothermic episode was revealed by changes in the color and temperature of the cat's external ear. No other signs of autonomic activation or inhibition were apparent during the course of these experiments.

Figure 3 presents a dose-response analysis obtained following microinjections of artificial CSF, 2.33 μg and 7.0 μg of both DA and NE. The respective amine was injected into the same hypothalamic locus represented in the histological inset, at 24–48 hr intervals. In Fig. 3 (Bottom), it can be seen from the response analysis that doses of 2.33 μg and 7.0 μg of NE were not as efficacious in producing a fall in the cat's temperature as the same doses of DA. However, even though both loci fell in the same coronal plane, the NA site was more dorsally located being adjacent to the anterior commissure. Nevertheless, following the microinjections of the higher concentration of the amine, a much shorter latency, as well as a much more pronounced fall in the cat's body temperature were clearly evident. In fact, at most of the catecholamine-reactive loci in the hypothalamus, a dose of 2.33 μg of NE was equipotent to 7.0 μg of DA in eliciting hypothermia.

The existence of specific DA receptors for heat loss in the rostral hypothalamic area of the cat was suggested by several experiments. At the site depicted at the inset of Fig. 4, two consecutive microinjections of 2.33 μg of NE failed to elicit a hypothermic response, even though this dose ordinarily evoked a substantial temperature decline (Fig. 3) in homologous loci. However, a third microinjection of 7.0 μg of DA produced a fall in the cat's core temperature of 0.6°C within 45 min (Fig. 4). The apparent insensitivity of this site to NE was again verified 24 hr later.

Pharmacological Characterization of Catecholamine Hypothermia

When a control microinjection of artificial CSF was given

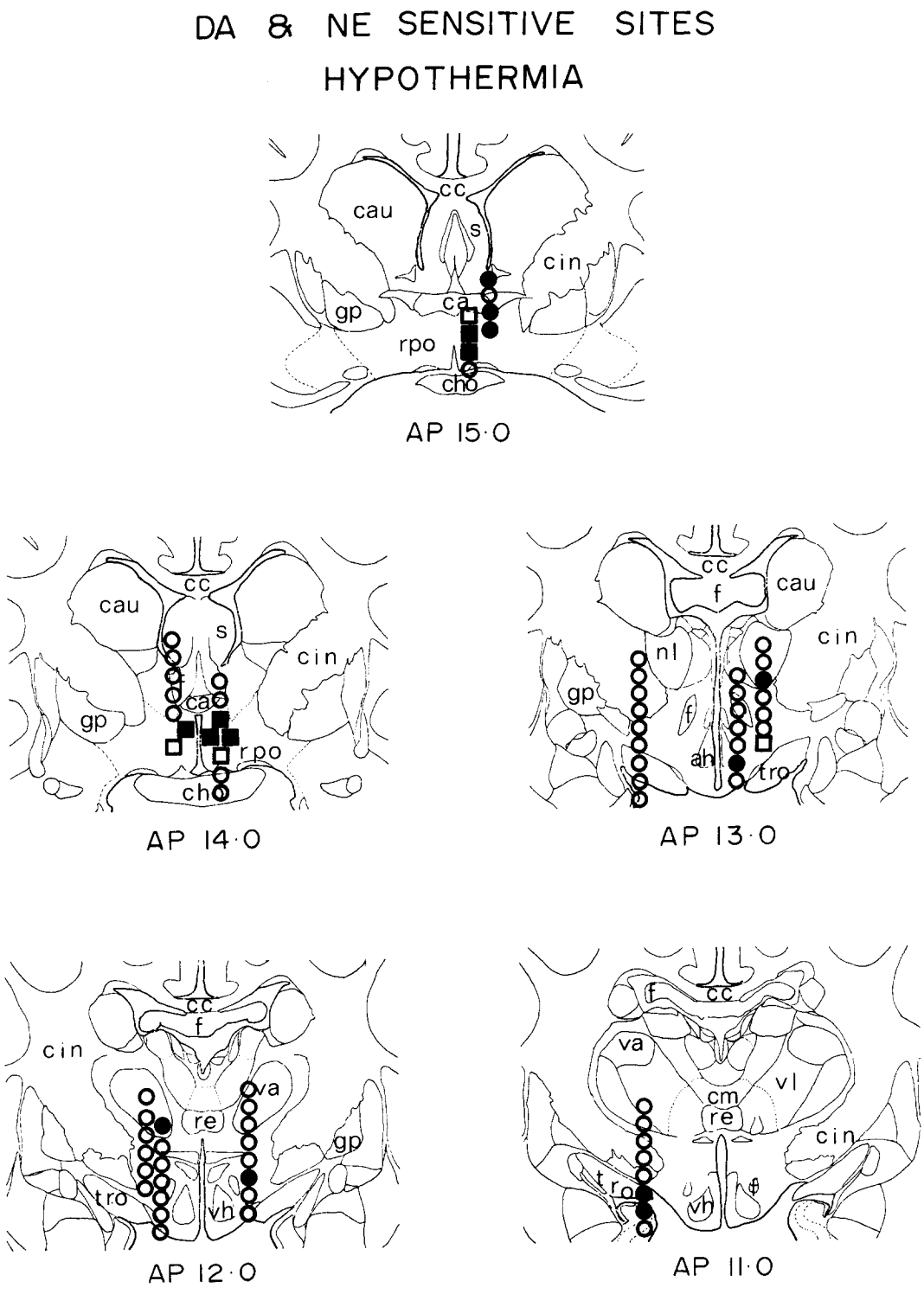

FIG. 1. Composite anatomical mapping in the cat of sites within coronal planes AP 11.0 through AP 15.0 at which DA and NE were microinjected. Exclusion of some sites was necessary because of homologous anatomical overlap. Closed circle (●) indicates that the colonic temperature decreases 0.1° to 0.4°C within 45 min after the microinjection; open square (□) denotes a temperature decline of 0.5°C or greater within 45 min; closed square (■) indicates 0.6°C or greater fall in temperature within 30 min; open circle (○) depicts site at which neither catecholamine elicited hypothermia. Anatomical abbreviations are: ah, anterior hypothalamic area; ca, anterior commissure; cau, caudate nucleus; cc, corpus callosum; cho, optic chiasm; cin, internal capsule; cm, central medial nucleus of the thalamus; f, fornix; gp, globus pallidus; nl, reticular nucleus of the thalamus; re, nucleus reuniens of the thalamus; rpo, preoptic area; s, septal region; tro, optic tract; va, ventral anterior nucleus of the thalamus; vh, ventromedial hypothalamic nucleus; vl, ventral lateral nucleus of the thalamus.

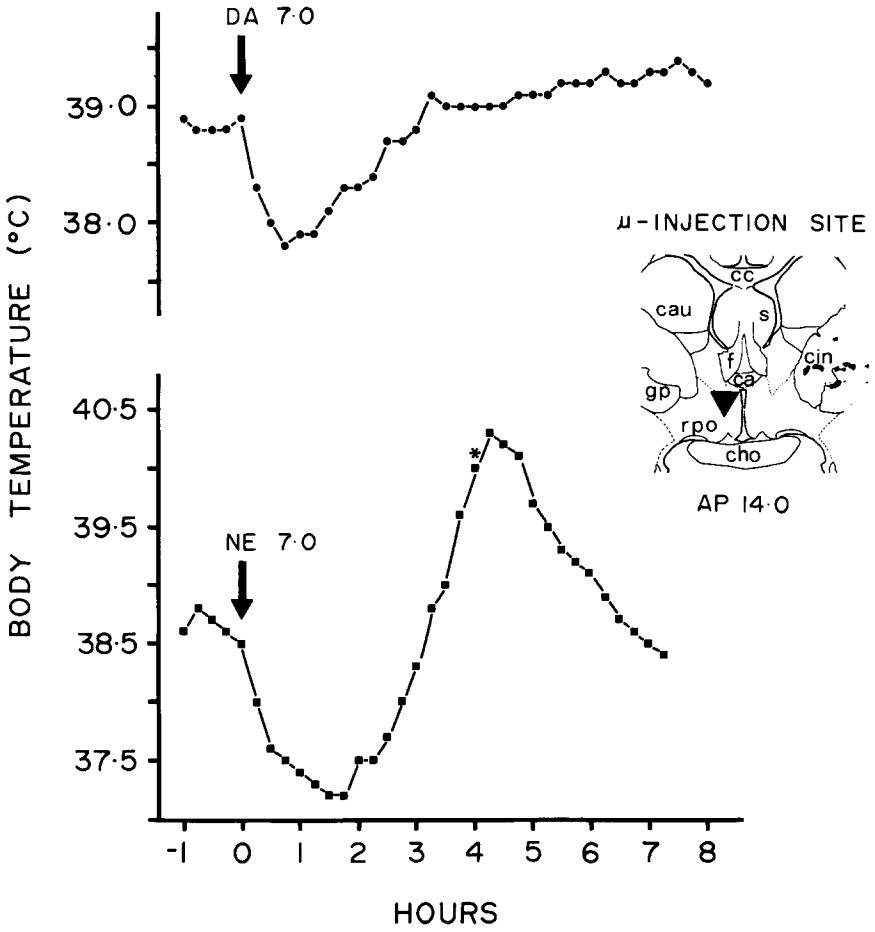

FIG. 2. Hypothermia evoked by 7.0 μg DA microinjected at zero hour into the preoptic area (histological inset −▼) (Top) and by 7.0 μg NE microinjected (Bottom) in the same site in the same cat. Asterisk denotes the intramuscular administraton of Dipyrone (1.25 mg in 0.25 ml). Anatomical abbreviations as in Fig. 1.

20–30 min prior to DA or NE, neither the latency nor the absolute magnitude of the hypothermic response to either amine was altered. However, receptor blocking agents modified the temperature changes considerably.

Adrenergic receptor antagonism. Following the initial control experiments in which CSF was microinjected into each amine sensitive site, phentolamine (PHT) was microinjected in a dose of either 3.5 or 7.0 μg. Then 20–30 min later, the monoamine was given in the reactive site in a dose that reliably produced at least 0.6°C decline in the cat's body temperature. Figure 5 illustrates the ineffectiveness of a low dose of this alpha-adrenergic receptor antagonist in altering the fall in temperature produced by 7.0 μg of DA at a reactive locus. In contrast to the hypothermia produced in the cat by a single microinjection of DA, the low dose of phentolamine failed to affect significantly the magnitude of hypothermia recorded at 30 min ($t=1.39$; $df=8$; $p<0.125$), 45 min ($t=0.79$; $df=8$; $p<0.25$) or the nadir of the cat's response ($t=0.62$; $df=8$; $p<0.30$). However, following pretreatment of the locus with the higher dose of phentolamine, the usual temperature decline after 7.0 μg of DA was reduced significantly. This is shown in Fig. 5 (Middle). After 7.0 μg of DA was injected, the hypothermia reached in 30 min ($t=2.61$; $df=8$; $p<0.025$), in 45 min ($t=2.39$; $df=8$; $p<0.05$) or overall was markedly attenuated by the higher dose of phentolamine ($t=2.24$; $df=8$; $p<0.05$).

Phentolamine always reduced the intensity of the fall in the cat's body temperature drastically ($t=3.67$; $df=5$; $p<0.001$) after a similar microinjection of NE. This potent action of the alpha-adrenergic antagonist in blocking NE hypothermia is illustrated in Fig. 5 (Bottom).

At some sites determined previously to be insensitive to NE, neither 3.5 nor 7.0 μg of phentolamine, preinjected earlier, attenuated the heat loss evoked by the DA microinjection in the cat's hypothalamus. The response to 7.0 μg of DA alone as well as to the same dose of the amine preceded by 7.0 μg of phentolamine is illustrated (for a representative experiment) in Fig. 6 (Top and Bottom, respectively). The magnitude of the DA-induced hypothermia, 30 or 45 min after microinjection of the agonist alone, was not significantly different from the level that it reached following phentolamine pretreatment ($t=1.4$; $df=2$; $p<0.20$, and ($t=0.70$; $df=2$; $p<0.30$), again suggesting a receptor specificity for DA hypothermia that does not involve alpha-adrenergic receptors.

Pretreatment of the DA-sensitive sites with a beta-adrenergic receptor blocking agent also did not interfere with the hypothermia following the microinjection of DA.

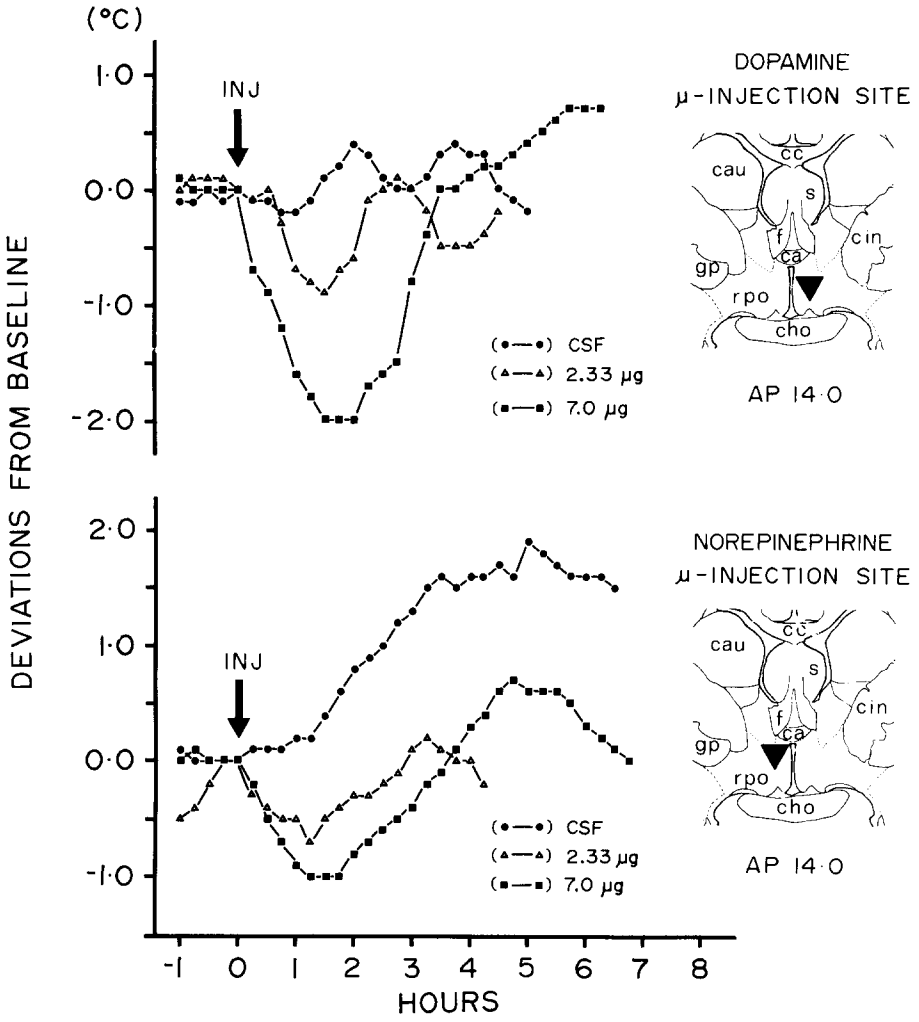

FIG. 3. Dose-dependent changes in colonic temperature of two cats following catecholamine microinjection (INJ) into preoptic loci (histological insets −▼) of: (Top) at zero hour (arrow), 0.75 μl of artificial CSF (●—●); 2.33 μg DA (△—△), or 7.0 μg DA (■—■); (Bottom) at zero hour (arrow), 0.75 μl of artificial CSF (●—●); 2.33 μg NE (△—△); or 7.0 μg NE (■—■). Anatomical abbreviations as in Fig. 1.

Figure 7 depicts the characteristic temperature fall following a 7.0 μg injection of DA alone (Top) and when preceded by a microinjection (Bottom) of 3.5 μg of practolol (PRAC), a beta-adrenoreceptor blocking agent.

Antagonism of DA hypothermia: haloperidol. Haloperidol, a DA receptor blocking agent, exerted differential effects on the temperature response produced in the cat by the two catecholamines. Figure 8 (Top) illustrates two experiments in which microinjections were given into an amine-reactive hypothalamic locus of 7.0 μg of DA alone and of 7.0 μg of DA preceded by 0.04 μg of haloperidol (HAL). The low dose of haloperidol did not affect the degree of hypothermia at any stage of the response. However, when the concentration of the antagonist was raised, the magnitude in the temperature decline was reduced significantly after DA was injected in the same 7.0 μg dose. As portrayed in Fig. 8 (Middle), 7.0 μg of haloperidol clearly altered the development of the hypothermia as well as the absolute magnitude of the fall in temperature. Within 30 and 45 min following the microinjection of the agonist, the response was significantly reduced ($t=3.79$; $df=2$; $p<0.05$).

As illustrated in Fig. 8 (Bottom), a low dose of the dopaminergic antagonist did not prevent the hypothermic response to NE. When the catecholamine was microinjected in the cat at an homologous site at AP 14.0, without prior treatment with haloperidol, the hypothermia corresponded to that seen following the microinjection of DA. Except for a slight delay in onset, NE microinjected also in a dose of 7.0 μg, at a site pretreated with haloperidol, produced a nearly identical response in terms of the latency, duration and magnitude. Interestingly, the antagonist did not alter the cat's resting temperature.

Antagonism of DA hypothermia: d-butaclamol. To clarify further the receptor specificity of the catecholamines in the heat loss response, DA-reactive sites were also pretreated with a somewhat more specific and potent dopaminergic receptor blocking agent [12], d-butaclamol (BUTAC). Figure 9 (Top) depicts the changes in body temperature following a single injection of 7.0 μg of DA and the same dose preceded by only 0.48 μg of d-butaclamol, both microinjected at the same hypothalamic site in the preoptic area. At this low dose, d-butaclamol had no effect on the onset or overall

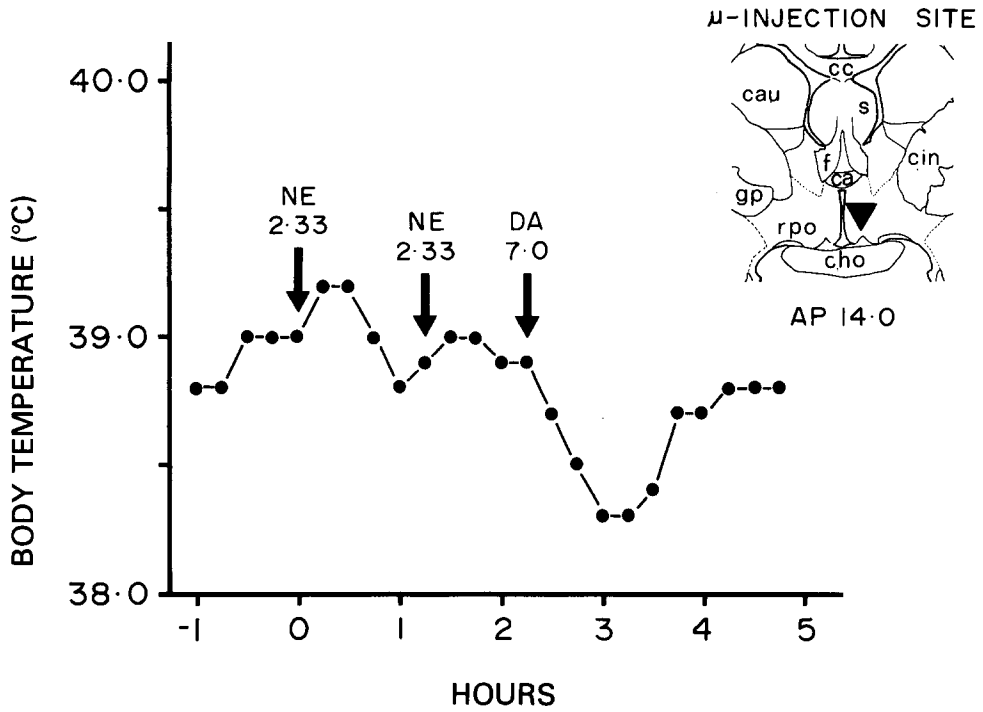

FIG. 4. Colonic temperature of the cat following successive microinjections (arrow) into preoptic area (histological inset −▼) of 2.33 μg NE; 2.33 μg NE 75 min later; and 7.0 μg DA 135 min after zero hour. Anatomical abbreviations as in Fig. 1.

magnitude of the two temperature responses. However, as illustrated in Fig. 9 (Bottom), a higher dose of 1.47 μg of the blocking agent microinjected in the same site prior to 7.0 μg of DA abolished the hypothermic response.

Given in the lower concentration at a hypothalamic site in another cat, d-butaclamol exerted a highly potent but differenential effect on the characteristic catecholamine hypothermia. As illustrated in Fig. 10 (Top), the intense fall in temperature caused by 7.0 μg of DA microinjected into the anatomical site designated in the inset, was prevented by a preinjection of the site with only 0.48 μg of d-butaclamol. However, when the experiment was repeated (Fig. 10, Bottom) two days later, but in this case 2.33 μg of NE was microinjected at the same anatomical locus, the resultant noradrenergic hypothermia was identical to that produced by 7.0 μg DA. Again the DA antagonist failed to affect the animal's baseline temperature.

As portrayed also in Fig. 10 and in some of the preceding figures, a rebound hyperthermia or overshoot [14] often occurred in the cat following a prolonged period of hypothermia induced either by DA or NE.

DA Release During Heat Exposure

In 21 push-pull perfusion experiments, nine anatomically distinct sites located between coronal planes AP 11.0 and AP 15.0 in the cat's rostral hypothalamus and preoptic region were examined. Earlier, their catecholamine-sensitivity had been demonstrated by virtue of a decline in body temperature following a microinjection of DA and/or NE.

After 2.5 μCi ^{14}C-DA and 25 μCi ^{3}H-NE were microinjected into the site depicted in the inset of Fig. 11, a rapid decline in body temperature to 1.9°C below its baseline occurred which was accompanied by a four-fold increase in the cat's respiratory rate. Approximately 45 min after the microinjection, the cat began to shiver vigorously as its body temperature began to rise.

At the end of the third push-pull perfusion, the ambient temperature of the environmental chamber was increased to 40°C while the animal shivered to regain its basal body temperature. During the course of the perfusion sequence, the typical decline in rate of release of NE (open bars) failed to change in successive perfusions. Similarly, the output of DA (closed bars) also declined which is similar to the normal washout curve of radioactivity with repeated push-pull perfusions during control experiments [30]. The values of DPM for the third perfusion ranged from 209 to 2079 and 441 to 6271 for ^{14}C-DA and ^{3}H-NE, respectively.

If the labeling procedure did not affect the cat's colonic temperature, changes in output of amine were observed. Figure 12 depicts the results of an experiment in which a preoptic site in the diencephalon of the cat (inset) was again double-labeled with the same amount of ^{14}C-DA and ^{3}H-NE. In response to the warm air temperature of 35°C, hyperthermia began as the cat sat up initially and respired at more than twice its baseline rate. Upon termination of the heat challenge, its respiratory rate returned to normal.

During this period of elevated ambient temperature, the amount of ^{3}H-NE radioactivity (open bars) collected in the sample perfusate (PERF No. 4) was nearly 50% above that in the sample immediately preceding the warming interval. Within this site, however, a small increase of only 8% in ^{14}C-DA activity (closed bars) was detected. In the subsequent sample, the heat-evoked release of hypothalamic DA and NE remained elevated as reflected by the relatively high proportion of radioactivity in both perfusates.

If the interval was increased to 30 min during which time the chamber air was heated, the evoked release of both of the catecholamines was substantially augmented. Figure 13 illustrates the enhanced release of both ^{14}C-DA (closed bars) and

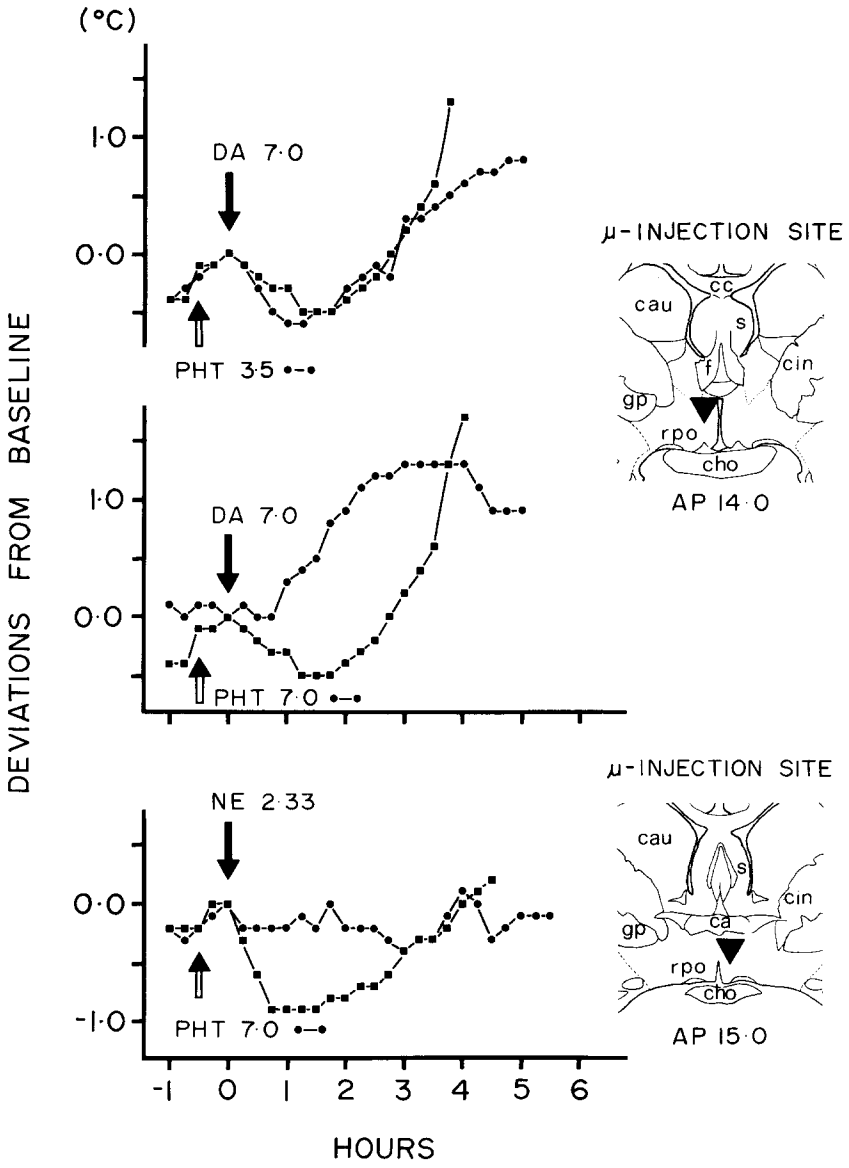

FIG. 5. Colonic temperature of two cats following microinjection into preoptic sites (histological insets −▼) of (Top) at zero hour, 7.0 μg DA (■—■) or 7.0 μg DA after site pretreated (open arrow) with 3.5 μg phentolamine (●—●); (Middle) at zero hour, 7.0 μg DA (■—■) or 7.0 μg DA after site pretreated (open arrow) with 7.0 μg phentolamine (●—●); (Bottom) at zero hour, 2.33 μg NE (■—■) or 2.33 μg NE after site pretreated (open arrow) with 7.0 μg phentolamine (●—●). Anatomical abbreviations as in Fig. 1.

^{3}H-NE (open bars) which occurred when the temperature of the chamber was raised to 40°C. An almost three-fold, concomitant increase in the cat's respiratory rate persisted intermittently during the experiment, with a peak tachypnea occurring during perfusion number 8. Although the usual washout curve of declining radioactivity continued into the first perfusion after the interval of heating began, the release of both ^{14}C-DA and ^{3}H-NE remained relatively stable or above that seen at the beginning of the warm air treatment.

A preliminary thin-layer chromatographic analysis carried out on five samples of perfusate showed a substantial rise in the level of HVA during the period when the cat was exposed to the warm air challenge. This suggests that the metabolic activity of DA within the preoptic area is enhanced concomitantly while the animal is thermoregulating in response to heat.

Evocation of Feeding

Of the 113 microinjection sites examined within the diencephalon of the cat, dopaminergic stimulation evoked voracious feeding consistently at six highly circumscribed loci. An anatomical reconstruction of the histological sites of greatest sensitivity to DA (squared circle) is portrayed in Fig. 14. Sites at which the catecholamine failed to elicit feeding are indicated also by an open circle. Within coronal planes AP 14.0 and 15.0, sensitive sites were located immediately ventral to the anterior commissure in isolated areas of the anterior hypothalamus and the preoptic region.

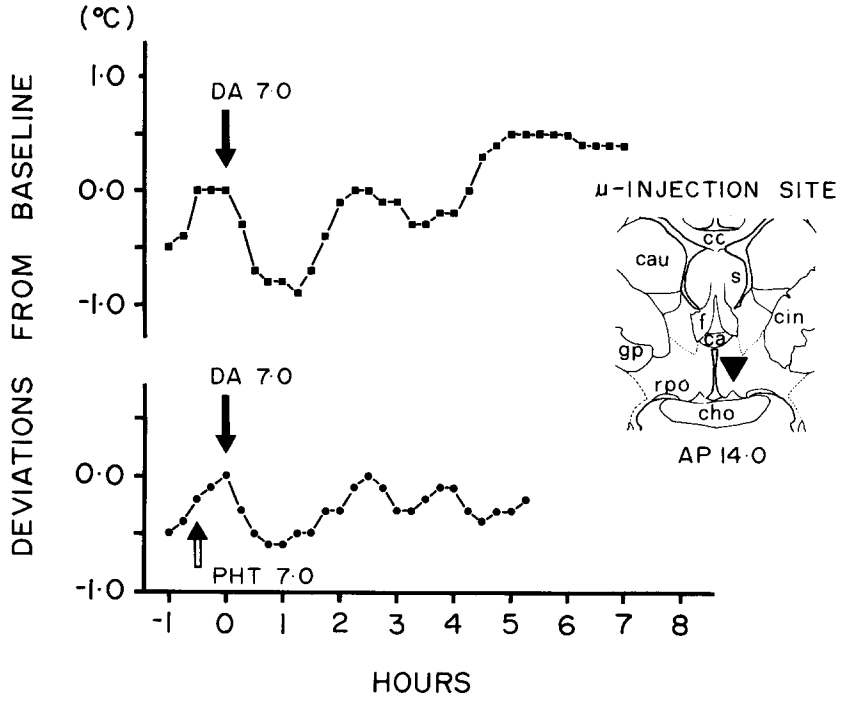

FIG. 6. Colonic temperature of the cat following a microinjection into preoptic site (histological inset −▼) at which NE failed to evoke a hypothermia: (Top) at zero hour, 7.0 μg DA (■—■); (Bottom) at zero hour, 7.0 μg DA after site pretreated (open arrow) with 7.0 μg phentolamine (●—●). Anatomical abbreviations as in Fig. 1.

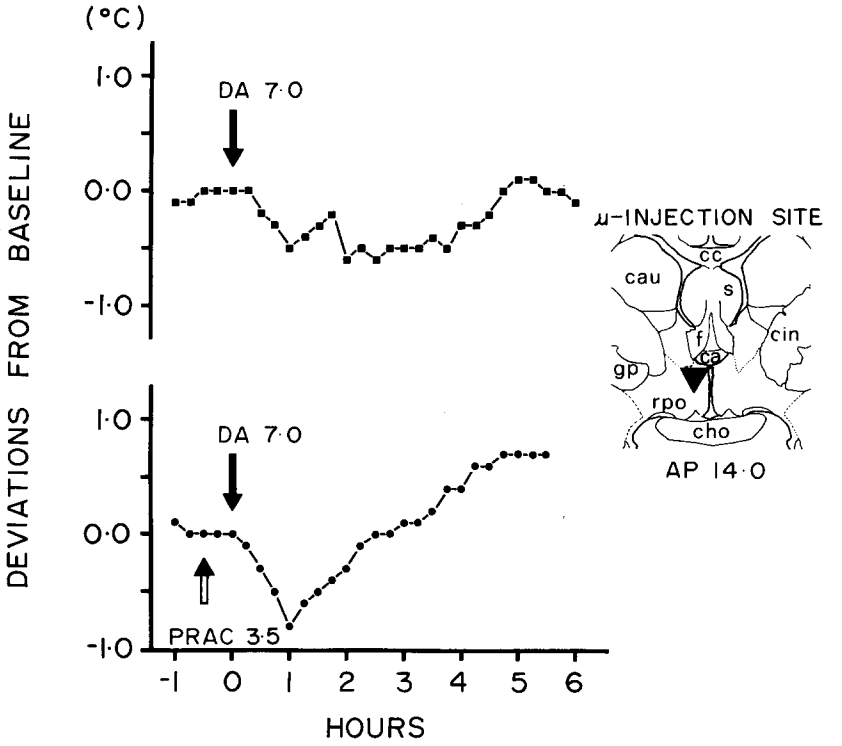

FIG. 7. Colonic temperature of the cat following microinjection into preoptic area (histological inset −▼) of (Top) at zero hour, 7.0 μg DA (■—■); and (Bottom) at zero hour, 7.0 μg DA after site pretreated (open arrow) with 3.5 μg practolol (●—●). Anatomical abbreviations as in Fig. 1.

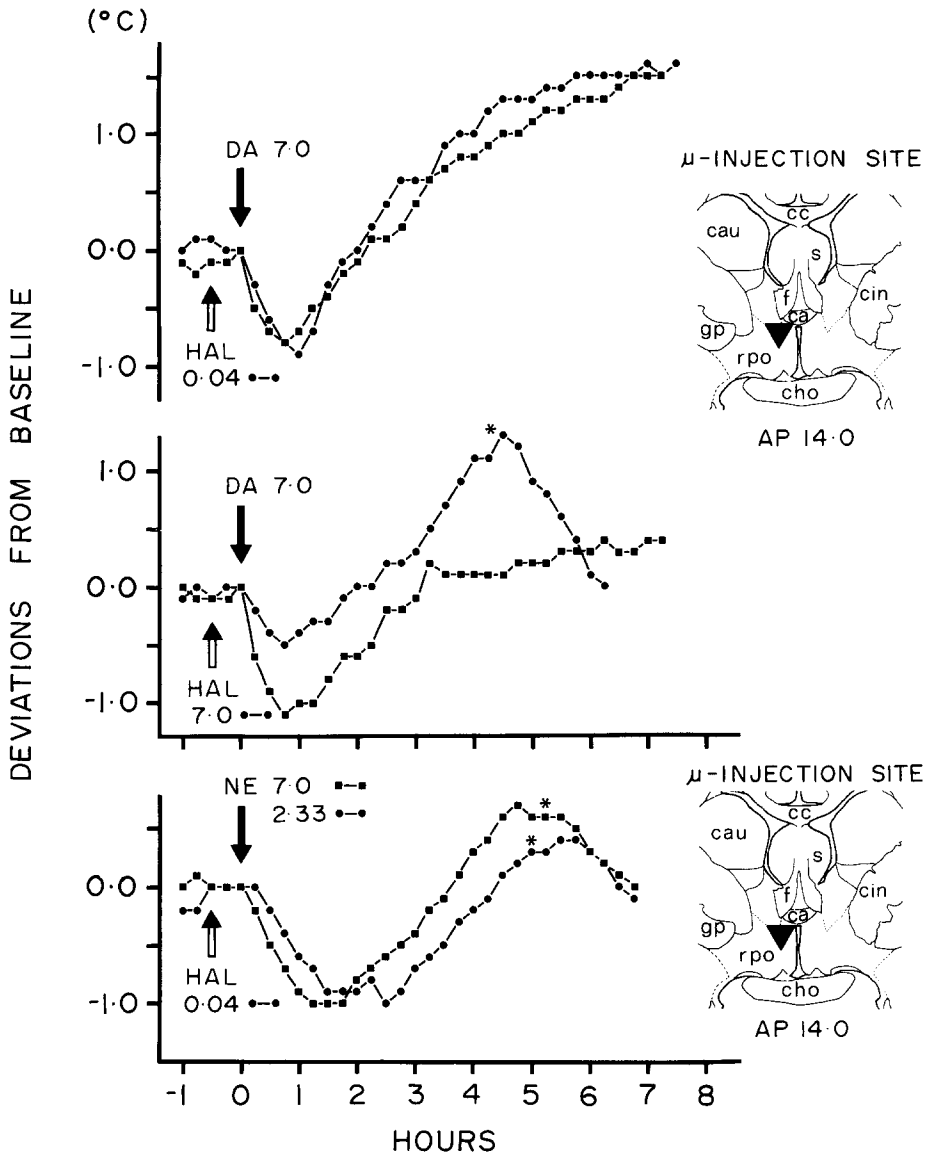

FIG. 8. Colonic temperature of two cats following microinjections into preoptic sites (histological insets −▼) of: (Top) at zero hour, 7.0 μg DA (■—■) or 7.0 μg DA after site pretreated (open arrow) with 0.04 μg haloperidol (●—●); (Middle) at zero hour, 7.0 μg DA (■—■) or 7.0 μg DA after site pretreated (open arrow) with 7.0 μg haloperidol (●—●), with acetaminophen, 30 mg/kg, administered intraperitoneally (asterisk); (Bottom) at zero hour, 7.0 μg NE (■—■) or 2.33 μg NE after site pretreated (open arrow) with 0.04 μg haloperidol (●—●), with acetaminophen, 30 mg/kg, administered intraperitoneally (asterisk). Anatomical abbreviations as in Fig. 1.

In 30 experiments in which DA was microinjected in doses ranging from 2.33 to 14.0 μg, a significant increase in food consumption occurred which was nearly double that consumed following microinjection of a control solution of artificial CSF (5.7 g versus 3.3 g). The onset of feeding was almost immediate, with most responses occurring within less than three minutes after the DA microinjection. Within the one-half hour interval following a microinjection, the amount of food that the cat consumed was as much as 25 g. Although NE in a similar dose range caused spontaneous feeding in several instances, the cat's feeding response was not as vigorous nor was the amount eaten in response to NE significantly greater than that following a CSF injection (4.8 g versus 3.3 g).

Histological Analysis

Figure 15 presents a representative histological section illustrating the morphological appearance of a catecholamine-sensitive site in the anterior hypothalamic, preoptic area, designated by the arrow, upon completion of a series of the experiments. From sections such as these the reconstructions of the maps were drawn. In this case, the locus of microinjection was one mm deeper than the more dorsal site of push-pull perfusion.

DISCUSSION

Within a circumscribed region of the rostral hypothalamic, preoptic area, DA causes a dose-dependent fall

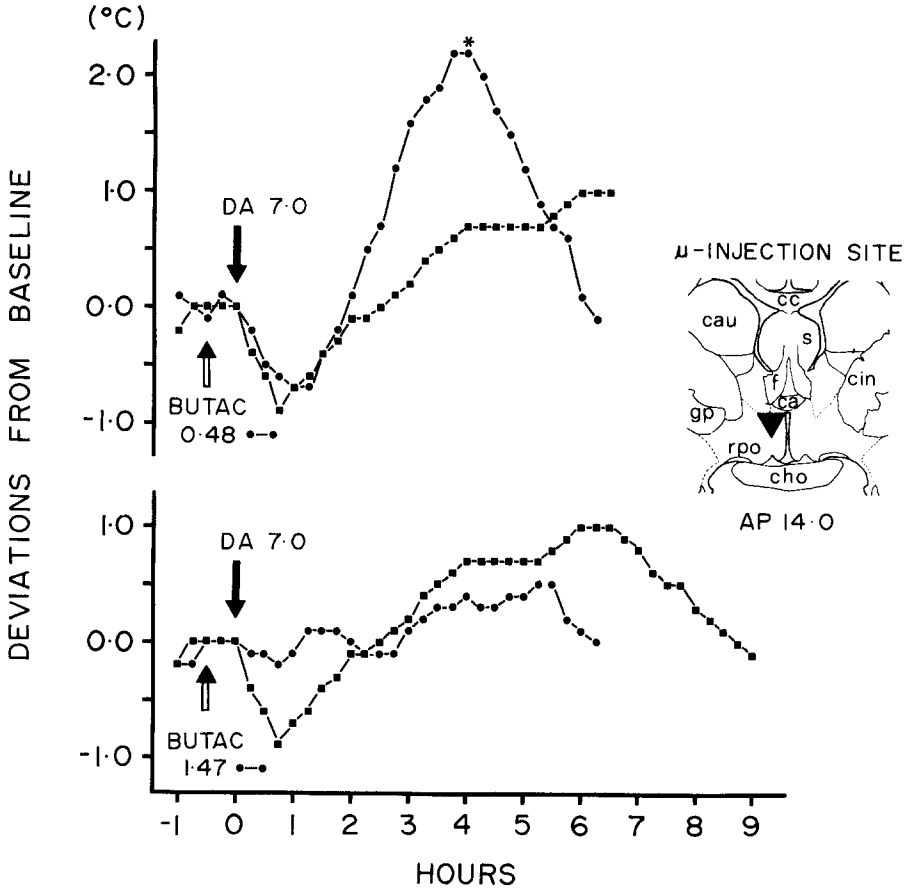

FIG. 9. Colonic temperature of the cat following microinjection into preoptic site (histological inset −▼) of: (Top) at zero hour, 7.0 μg DA (■—■) or 7.0 μg DA after site pretreated (open arrow) with 0.48 μg d-butaclamol (●—●), with acetaminophen, 30 mg/kg, administered intraperitoneally (asterisk); and (Bottom) at zero hour, 7.0 μg DA (■—■) or 7.0 μg DA after site pretreated (open arrow) with 1.47 μg d-butaclamol (●—●). Anatomical abbreviations as in Fig. 1.

in the cat's body temperatre. Of particular significance is the close morphological concordance between the pharmacological localization of the catecholamine-sensitive sites and the presence of catecholamine terminals. Based on histochemical fluorescence studies, this area of the cat's ventral forebrain is among the most densely supplied regions of the entire diencephalon [10,39]. Another region equally rich in catecholamine-containing fibers and terminals lies proximal to the para- and peri-ventricular nuclei of the anterior hypothalamus [7], again a region in which dopamine application elicited a hypothermic response of maximum magnitude.

Similarly, the immediacy and the intensity with which the cat feeds in response to a DA microinjection would lend support to the concept of a dopaminergic mediation of ingestive behavior [21,28]. Since the areas of the cat's hypothalamus which exhibit maximum sensitivity to DA correspond to brain-stem areas implicated previously in the feeding mechanism of other species [2,45], DA could exert a direct effect on an as yet unidentified component of the neural circuit for feeding in the cat [28].

DA Versus NE Hypothermia

At many hypothalamic loci, a microinjection of NE ordinarily produces a consistently more intense fall in temperature than DA, even when the concentration of the latter amine is three times higher. However, as shown in the results, 7.0 μg of DA is often equally as potent as the same dose of NE. In both instances, peripheral vasodilatation as reflected by flushing of the pinna and a warm ear temperature is always a cardinal physiological characteristic of the hypothermic response. In some experiments, a transient increase in the cat's respiratory rate also occurs; however, the posture adopted by the cat following the intrahypothalamic microinjection of either catecholamine coupled with tachypnea, resembles the responses described earlier by Cooper and co-workers [9], indicative of the heat loss response. Therefore, the results of the present experiments and those of others [11,16] suggest that dopaminergic hypothermia is due at least partially to the activation of the heat loss pathway postulated to originate in catecholamine-containing neurons in the anterior hypothalamus [29]. Further support of this view stems from the fact that adrenergic blocking agents as well as dopaminergic antagonists, when injected directly into the thermosensitive zone of the hypothalamus, fail to cause an increase in the cat's body temperature. Such a hyperthermic response would be expected if the catecholamine receptors were involved solely in the blockade of the heat production pathway [28], even at a euthermic temperature.

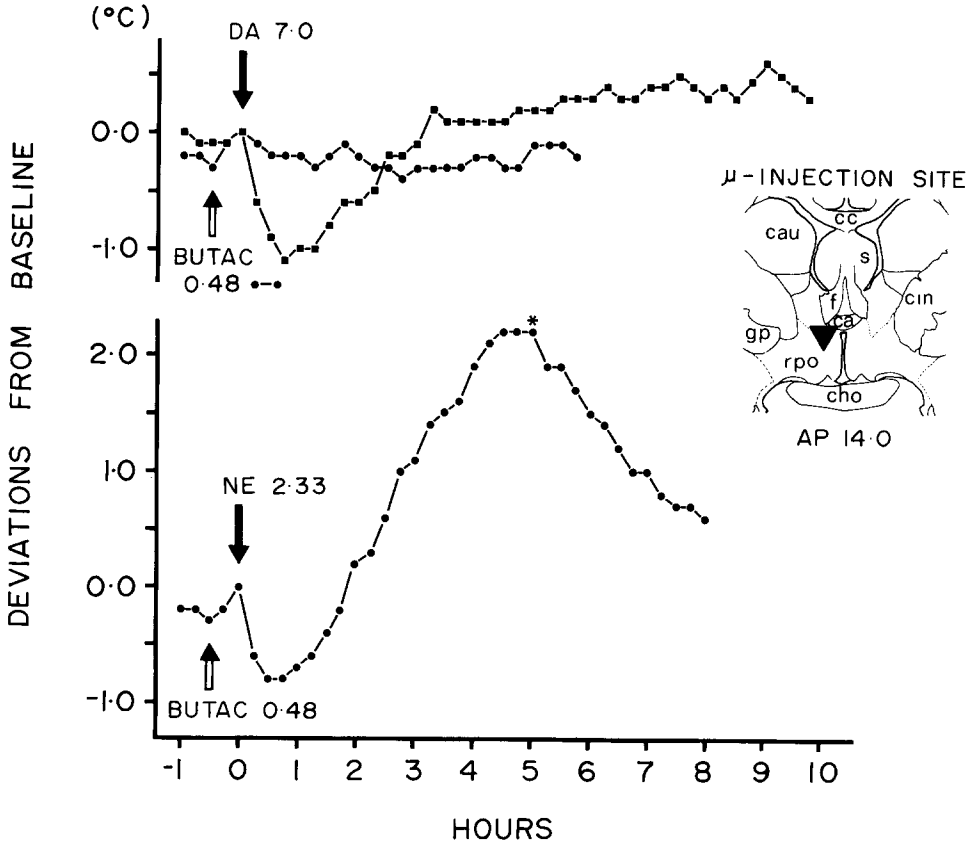

FIG. 10. Colonic temperature of the cat following a microinjection into preoptic site (histological inset -▼) of (Top) at zero hour, 7.0 μg DA (■—■) or 7.0 μg DA after site pretreated (open arrow) with 0.48 μg d-butaclamol (●—●); and (Bottom) at zero hour, 2.33 μg NE after site pretreated (open arrow) with 0.48 μg d-butaclamol (●—●). Dipyrone (1.25 mg/0.25 ml) administered intramuscularly (asterisk). Anatomical abbreviations as in Fig. 1.

Although the precise neurochemical events underlying the catecholamine hypothermia are unknown, there are several possible alternatives which would account for the heat-dissipating action exerted by DA on the rostral hypothalamus. First, DA could serve as the metabolic substrate for the synthesis of NE [42], and as such produce a fall in the cat's temperature as it subsequently raises the endogenous level of the amine [29]. The histological observations of the frequent anatomical overlap of DA with NE-sensitive sites in the hypothalamus favors this alternative. Conversely, the fact that haloperidol or the stereospecific neuroleptic, d-butaclamol, given in a much lower dose, blocks the DA-induced hypothermia but fails to interfere with the NE-induced decline in temperature would tend to negate such a conclusion. In this connection, our latter finding corroborates the results of Kennedy and Burks [19] who obtained the same results with an intraventricular infusion of haloperidol.

Second, further specificity of action of DA is documented in that phentolamine does not alter the DA-induced temperature decline, which is in contrast to its attenuation of NE hypothermia. Although a higher dose of the alpha-adrenergic antagonist is sufficient to affect the dopaminergic hypothermia, it is known that the drug in a relatively high concentration may act nonspecifically on receptor sites [1,13].

Third, endogenous DA within the hypothalamus may influence other amine containing neuronal systems [46] such as those served by serotonergic or cholinergic pathways. Already, experimental evidence suggests that a catecholamine or its metabolic precursor can alter the content of serotonin in the CNS [18,38]. That DA and acetylcholine may likewise be linked functionally at the neuronal level has also received some preliminary experimental support [8].

Release of Catecholamines During Thermoregulation

The heat-induced release of both DA and NE, from certain circumscribed sites within the hypothalamus of the cat, provides direct physiological evidence of the involvement of the monoamines in hypothermia. The mechanism could involve the activation of a heat-loss pathway, an active inhibition of a separate heat-production pathway, or both actions [9, 24, 27].

Previously, it was shown that in a cat placed in a warm temperature of 40°C, sites in the anterior hypothalamus and preoptic region of the diencephalon release radio-labeled NE [30]. Exposure of the same animal to a cold temperature of 10°C or to the normothermic environment has no effect on the steady-state release of NE which simply follows a slope of declining radioactivity [30,33]. Although in the present study an increase in the ambient temperature to 35–45°C evoked the release of both DA and NE from the anterior hypothalamus, as might be expected the proportional efflux of the amines is not necessarily the same in each site. Within

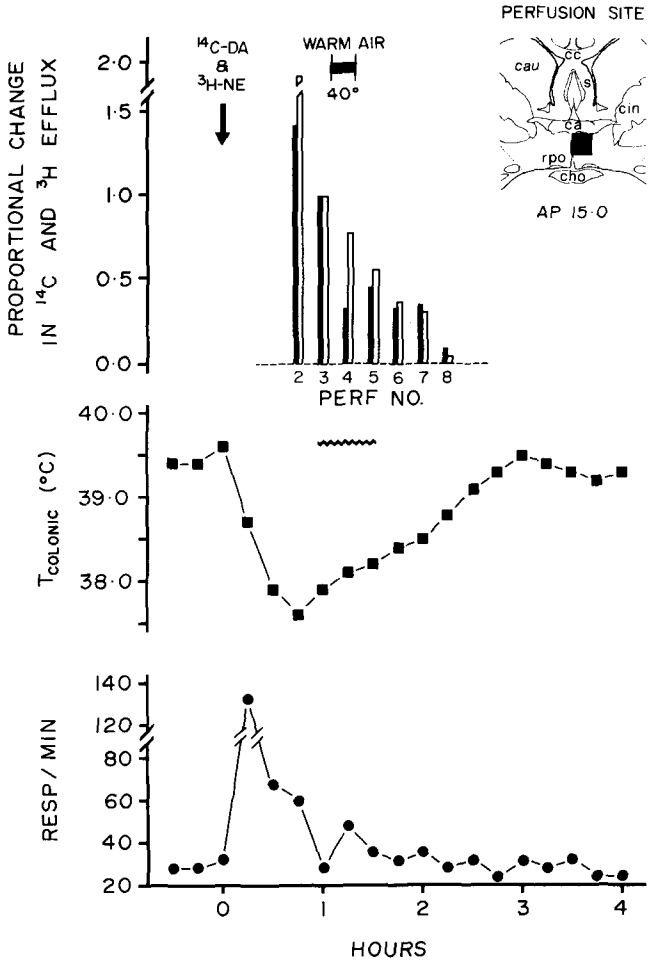

FIG. 11. Proportional efflux (Top) of ^{14}C-DA (solid vertical bars) and ^{3}H-NE (open vertical bars) in successive push-pull perfusates collected at a rate of 25 μl/min within preoptic site (histological inset—■)) of the cat. At the arrow, 2.5 μCi ^{14}C-DA and 25 μCi ^{3}H-NE were microinjected into this site. Cat's ambient temperature elevated to 40°C (solid horizontal bar) by warm air, during 15 min interval. Colonic temperature (Middle) is °C and respiratory rate (Bottom) is in resp/min. Shivering is denoted by zig-zag line. Anatomical abbreviations as in Fig. 1.

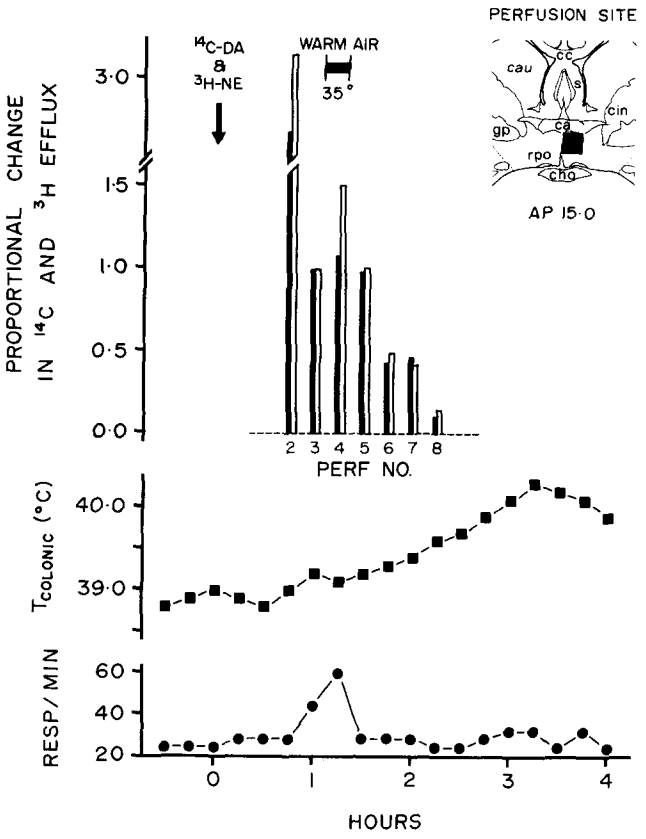

FIG. 12. Proportional efflux (Top) of ^{14}C-DA (solid vertical bars) and ^{3}H-NE (open vertical bars) in successive push-pull perfusates collected at a rate of 25 μl/min within preoptic site (histological inset—■) of the cat. At the arrow, 2.5 μCi ^{14}C-DA and 25 μCi ^{3}H-NE were microinjected into this site. Ambient temperature elevated to 35°C (solid horizotal bar) by warm air during 15 min interval. Colonic temperature (Middle) is °C and respiratory rate (Bottom) is in resp/min. Anatomical abbreviations as in Fig. 1.

some perfusion sites, the output of NE exceeded that of DA, whereas at others, DA release was greater than that of NE during the heat challenge.

Since the efflux of either catecholamine does not occur when the cat is actively producing body heat, as reflected by vigorous shivering and signs of peripheral vasoconstriction, this suggests that the two amines are subserving different components of the physiological mechanism underlying the heat-loss process.

CONCLUSION

From the present results, it is clear that within the population of thermosensitive neurons in the anterior hypothalamus, DA activity would seem to play an important role in the thermoregulatory mechanism of the cat. Although NE is already implicated in the heat loss function [29], the precise physiological part played by DA is not as yet established. However, one could envisage that DA underlies one of the many diencephalic components of the heat dissipating process such as the activation of vasodilation, a motor response.

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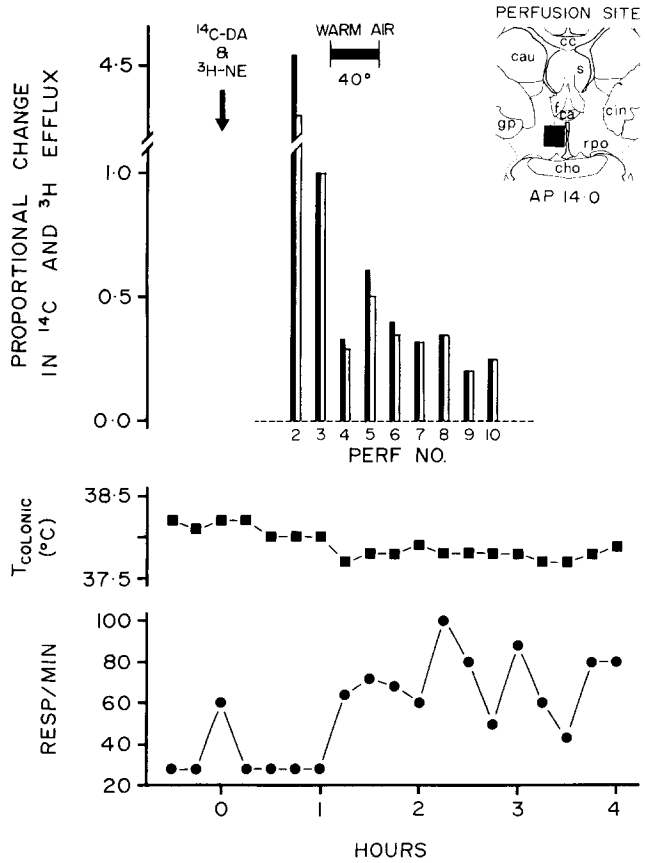

FIG. 13. Proportional efflux (Top) of ^{14}C-DA (solid vertical bars) and ^{3}H-NE (open vertical bars) in successive push-pull perfusates collected within preoptic site (histological inset—■) of the cat. At the arrow, 0.75 μCi ^{14}C-DA and 7.5 μCi ^{3}H-NE were microinjected into this site. Ambient temperature raised to 40°C (solid horizontal bar) by warm air during 30 min interval. Colonic temperature (Middle) is °C and respiratory rate (Bottom) is in resp/min. Anatomical abbreviations as in Fig. 1.

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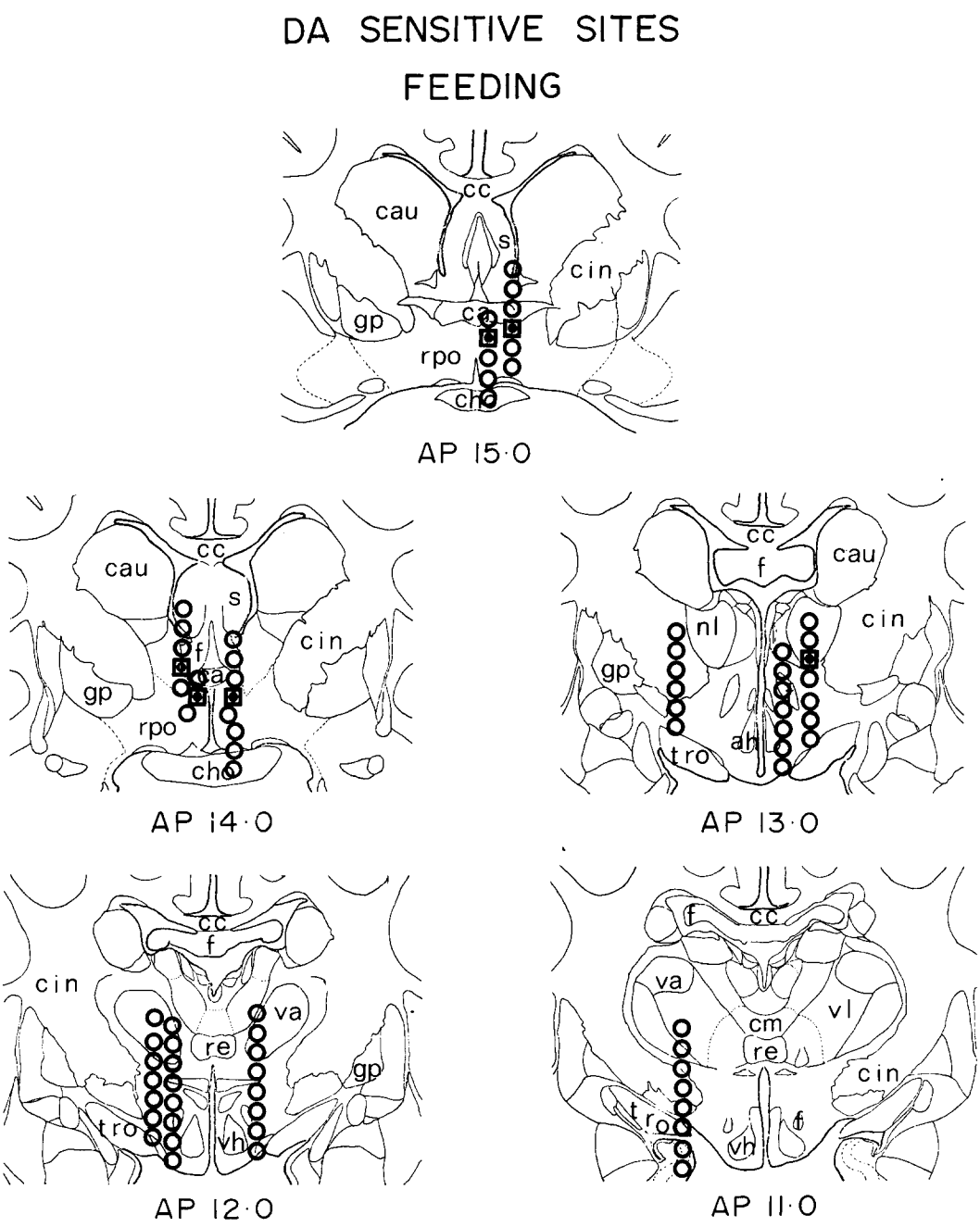

FIG. 14. Composite anatomical mapping of sites within coronal planes AP 11.0 through AP 15.0 at which dopamine (DA) was microinjected. An enclosed circle (⊡) indicates site at which DA caused the cat to feed spontaneously; an open circle (○) denotes no effect on ingestive behavior. Anatomical abbreviations as in Fig. 1.

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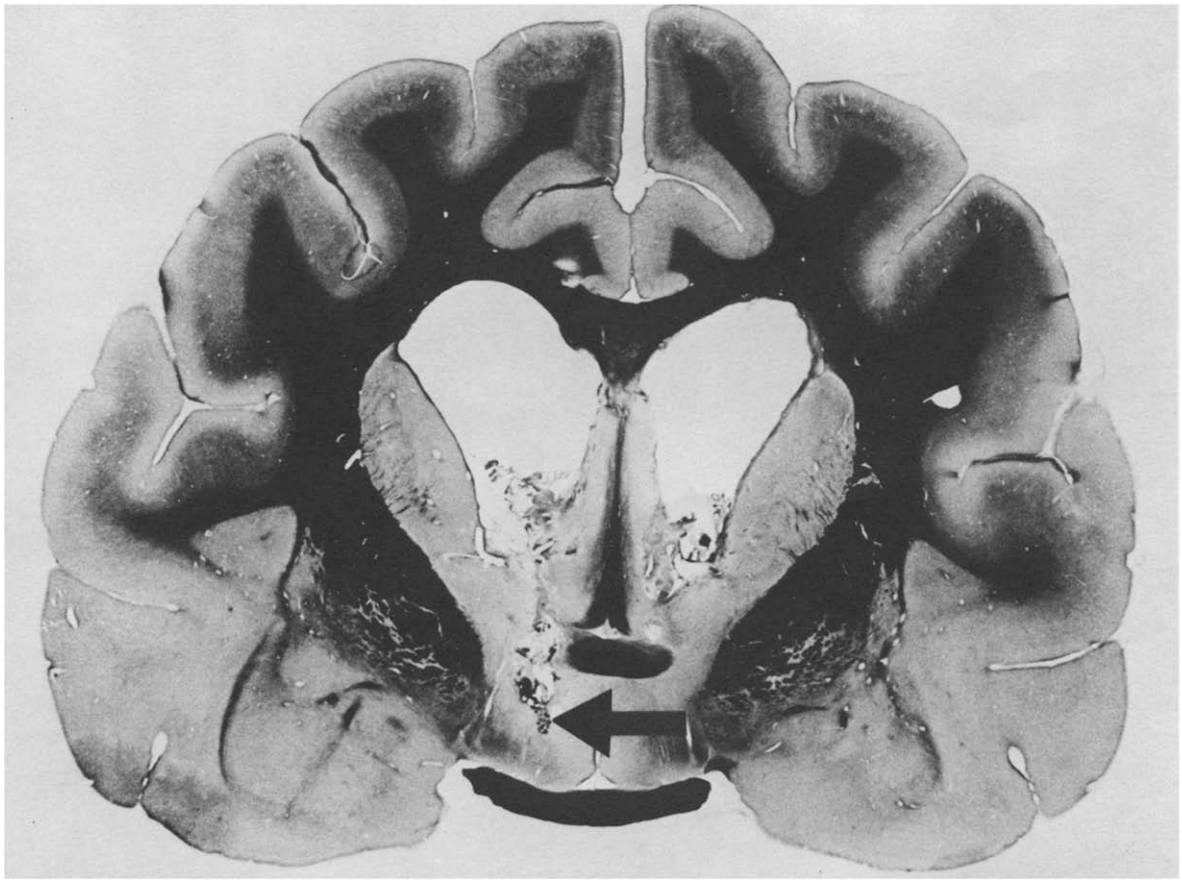

FIG. 15. Representative histological section illustrating microinjection site (arrow) in preoptic area of the cat at coronal plane AP 15.0. Section was cut at 100 microns and stained with hematoxylin according to the method of Wolf [44].

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