Dissociation of thermoregulation in cats with cytotoxic pontine lesions
(brainstem/neurotoxic lesions)

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ABSTRACT

Neurons of the pontine tegmentum of the cat were lesioned by microinjection of ibotenic acid into the brainstem. The threshold ambient temperatures for heat-gain (shivering) and for heat-loss (panting) responses, together with brainstem and skin temperatures, were measured in intact animals and after the neurotoxic lesioning. After the lesioning the shivering threshold was altered but the panting threshold did not change. The results indicate that certain neurons involved in the shivering response reside in the pontine tegmentum. Neurons involved in the panting response, however, may lie outside the lesioned areas.

The investigation of extrahypothalamic thermoregulatory regions of the nervous system that started with Keller (1) has continued for nearly 60 years (2–16). Our previous studies on extrahypothalamic thermoregulatory areas have shown that damage to the pontine tegmentum alters the thermoregulatory responses of cats (2–6) and rats (7). Electrolytic lesions of the dorsolateral pontine tegmentum increase sensitivity to both low and high ambient temperatures (Tb) (4, 6). The thresholds for shivering (heat-gain) and for panting (heat-loss) are both altered, because these lesioned animals start to shiver at a higher Ta, and to pant at a lower Ta, than intact animals. These lesions also eliminate the atonia normally associated with paradoxical sleep (17, 18). Inactivating a shivering-inhibitory area located in the ventromedial pontine tegmentum by injection of a local anesthetic releases shivering in previously nonshivering animals. In mildly shivering cats an increase in intensity of shivering is observed as a result of the inactivation (2). Lesions of the lateral pontine tegmentum cause a cessation of shivering by destruction of the areas and pathways mediating the heat-gain response (3).

All previous studies have been based on ablation or on inactivation by local anesthetics or electrolytic techniques that affect both neurons and nerve fibers. In discussing the functional significance of the pontine tegmental areas in thermoregulation, it is important to understand the anatomical and physiological properties of these areas. As the first step I tried to determine whether the alteration of thermoregulatory responses caused by the pontine tegmental lesions is due to the destruction of neurons located in the pontine areas or to the interruption of the fibers passing through these areas. To do this I employed the technique of topical microinjection of a cytotoxin that destroys cells without disrupting nerve fibers. Part of this work has been published in preliminary form (5).

MATERIALS AND METHODS

Experiments were performed in two steps on seven adult cats weighing 2.7–4 kg. First, control data were obtained from intact cats with chronically implanted wires for recordings. Then, after the placement of pontine lesions, data were obtained again from the same cats and compared with those from the intact cats. Each cat served as its own control.

Surgical Procedures. Before surgery, cats were pretreated with atropine sulfate (5 µg/kg, i.m.) and given a prophylactic injection of benzathine penicillin (150,000 units, i.m.). Under halothane inhalant anesthesia the animals were placed in a stereotaxic instrument, and stainless steel wires were implanted for chronic recording of the electromyogram (EMG) of the dorsal cervical muscles. Stainless steel screws were placed in the skull for recording of the electroencephalogram (EEG) and the electrooculogram (EOG). (Data from the EEG and EOG will be reported elsewhere.) Thermocouples (type T copper–constantan) were implanted 1–2 mm into the cerebral cortex of the occipital lobe to record superficial brain temperature (Tb) and under the skin of the neck to record subcutaneous temperature (Ts) as an index of vasomotor activity. Although the neck skin is not a highly vascular area when compared with the tail or ears, this site was chosen because unanesthetized cats will not tolerate chronic implantation in other parts of the body. Lead wires from all electrodes were soldered to a single connector and embedded in dental acrylic affixed to the skull.

The cytotoxic lesions were made bilaterally by microinjection of a cytotoxin (ibotenic acid, 0.5 µl per injection site, 10 µg/µl) into the pontine tegmentum of the cats by means of a sterilized 5-µl Hamilton syringe with a 30-gauge needle. The syringe was mounted on a stereotaxic carrier inclined at 45° angle. The needle was lowered and inserted into the pontine tegmentum through the cerebellum. Four lesions were made, two on each side at P, 2.5 and 3.5; L, 2.5; and H, −3.0 (the horizontal zero plane is 10 mm above the interaural line). All surgical procedures were performed under aseptic conditions. Immediately following each surgery a non-narcotic analgesic (Nubaine) was injected (1 mg/kg, s.c.). Postoperative recovery was uneventful, with cats showing normal appetite and home cage behavior.

Recording Procedures. Control recordings and observations began after a recovery period of 1 week. On each experimental day a cat was placed in the temperature-controlled chamber (see ref. 6 for details) at a predetermined Ta between 5°C and 44°C, in random order and with an interval of 4–7 days between each session. EEG, EOG, and EMG were continuously recorded on a computer using a Codas data acquisition system. Both Tb and Ts continuously recorded on a computer using an Omega data acquisition system. Respiratory movement was recorded on a polygraph using an open mercury strain gauge. Shivering threshold was determined by observation of the first appearance of regular bursts of shivering activity recorded above the baseline EMG. Panting threshold was regarded as respiratory move-

Abbreviations: Ta, ambient temperature; Tb, brain temperature; Tg, subcutaneous temperature; EEG, electroencephalogram; EMG, electromyogram; EOG, electrooculogram.
ments at or above 90/min, usually coupled with narest movement. Body posture at various $T_a$ values was rated on a scale extending from tightly curled (cold) to completely stretched (hot). Records were taken within the first 30 min at each $T_a$. The thresholds for shivering and for panting, and body posture, were determined for each cat from at least two recording sessions at each $T_a$. In addition, in five of the seven cats $T_a$ and $T_b$ were recorded (LA15, LA16, LA17, LA20, LA21). During the intervals between tests the cats were housed in the animal quarters, where the $T_a$ was kept constant at 20–22°C and there was a 12-hr light/dark routine. Together with random placements in various $T_a$ conditions, this procedure minimized any adaptation to a thermal zone different from 20°C.

One week after placement of the lesions, the above protocol was used for further recordings. These experiments were performed on unrestrained, unencumbered cats, studied over a period of several months.

After completion of all studies, the animals were anesthetized with sodium pentobarbital and perfused through the heart with normal saline followed by 10% formalin for later histological analysis of the lesioned sites (see Fig. 1).

**RESULTS**

At room temperature (20°C) $T_a$, $T_b$, respiratory rate, and body posture of the lesioned cats were the same as for intact animals. Of seven cats lesioned by the cytotoxin, six showed no change in the threshold for heat-loss (panting) response. All seven, however, showed a change in the threshold for heat-gain (shivering) response (Fig. 1).

During a 30-min exposure to a $T_a$ of 15°C, the intact animals did not shiver (Table 1, Fig. 2) but exhibited a slight decrease in $T_b$ (Fig. 3). At a $T_a$ of 7–10°C, intact cats showed piloerection and slight shivering localized to the neck and face. Body posture was mildly curled. At a $T_a$ of 5°C the intensity of shivering increased (Table 1, Fig. 2) and was observed over the entire body; at the same time the body posture was more curled. $T_b$ of the intact cats did not change within the first 30 min of exposure to cold (Table 2).

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**Table 1. Effects of 30 min of exposure of intact and lesioned cats to various $T_a$ conditions**

<table>
<thead>
<tr>
<th>$T_a$, °C</th>
<th>Shiv</th>
<th>Pilo</th>
<th>P</th>
<th>RR</th>
<th>Shiv</th>
<th>Pilo</th>
<th>P</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>++</td>
<td>+</td>
<td>C</td>
<td>47±5</td>
<td>+++</td>
<td>++</td>
<td>C</td>
<td>45±5</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>SC</td>
<td>40±5</td>
<td>++</td>
<td>++</td>
<td>C</td>
<td>35±5</td>
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<tr>
<td>15</td>
<td>–</td>
<td>N</td>
<td>25±5</td>
<td>+</td>
<td>+</td>
<td>SC</td>
<td>25±5</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>–</td>
<td>N</td>
<td>25±5</td>
<td>–</td>
<td>–</td>
<td>N</td>
<td>25±5</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>–</td>
<td>N</td>
<td>25±5</td>
<td>–</td>
<td>–</td>
<td>N</td>
<td>25±5</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>–</td>
<td>N</td>
<td>40±10</td>
<td>–</td>
<td>–</td>
<td>N</td>
<td>40±10</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>–</td>
<td>SS</td>
<td>40±10</td>
<td>–</td>
<td>–</td>
<td>SS</td>
<td>40±10</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>–</td>
<td>CS</td>
<td>105±15</td>
<td>–</td>
<td>–</td>
<td>CS</td>
<td>105±15</td>
<td></td>
</tr>
</tbody>
</table>

Shiv, shivering; Pilo, piloerection; P, posture; RR, respiratory rate (min⁻¹); SS, slightly stretched; CS, completely stretched; SC, slightly curled; TC, tightly curled; N, normal; –, no shivering, no piloerection; +, slight shivering, slight piloerection; ++, moderate shivering, moderate piloerection; ++++, marked shivering, marked piloerection. At $T_a$ of 44°C, exposure was continued until the onset of panting (50–90 min of exposure), at which time the data of this table were recorded. Each value represents 12 repeated observations in six cats.

After the cytotoxic lesioning the thresholds for heat-gain responses (shivering, piloerection, and curled body posture) were altered in that the animals were more sensitive to cold. They started to shiver, piloerect, and curl their bodies at a higher $T_a$ than intact animals (Table 1). The intensity of shivering increased and the shivering was observed over the entire body at a $T_a$ of 10°C (Fig. 2). As in intact animals, however, the $T_a$ of the lesioned cats did not show any change at low $T_a$ (Table 2). $T_a$ of the lesioned cats unexpectedly increased at low $T_a$ (Table 2, Fig. 3). Although $T_b$ recorded in these experiments may not be representative of mean $T_b$, it did serve as an indicator of an obvious difference in lesioned animals.

At high $T_a$ of 35°C and 40°C, in spite of an increment in $T_b$ (0.3°C and 0.6°C, respectively), six of seven intact cats did not pant during a 30-min exposure. After exposure for 50–85 min to a $T_a$ of 44°C these six animals finally started to pant.

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**FIG. 1.** Cross sections drawn from histological sections of brains with pontine tegmental lesions caused by injection of ibotenic acid. The most rostral area is at the top, BC, brachium conjunctivum; IC, inferior colliculus; M5, motor nucleus of trigeminal nerve; SO, superior olive. Below the drawings is the change in threshold $T_b$ of shivering (Shiv) or panting (Pant) for each cat after the cytotoxic lesioning. Some of these drawings appeared in ref. 5 and are reproduced here with permission of the copyright holder (Elsevier Science Publishers).
with a respiratory rate of >90/min. \( T_b \) at this point was elevated by 0.8°C, and \( T_e \) was elevated by 1.6°C. The body posture was stretched, but there were no signs of extreme discomfort such as restlessness and assumption of an extended, recumbent posture. In these cats the threshold for heat-loss responses (panting, stretched body) did not change after lesioning (Fig. 1). These animals did not pant at \( T_a \) conditions of 35°C and 40°C, where their \( T_b \) was elevated by 0.2°C and 0.6°C, respectively. The respiratory rate of both intact and lesioned cats at \( T_a \) of 35°C and 40°C increased slightly (30–50/min) but stayed below 90/min during the first 30 min of exposure. When exposed to a \( T_a \) of 44°C for 60–90 min, the lesioned cats started to pant with a respiratory rate of >90/min. \( T_a \) at this point was elevated by 0.8°C and \( T_e \) was elevated 1.2°C. Body posture was completely stretched, but the cats did not show any signs of thermal discomfort, as electrolytically lesioned cats do (6). In one cat (GM46), however, the threshold for panting was 37°C before the lesioning and decreased to 33°C after the placement of the lesions.

Unlike intact animals, when they were observed during paradoxical sleep, these lesioned cats had excessive proximal movements and showed muscle tonicity, as do electrolytically lesioned cats.

Histological examination showed that although the location of lesions varied in different cats, in every case the lesions involved brainstem mesencephalic and pontine tegmental regions (Fig. 1). This suggests that certain neurons involved in the heat-gain response are probably located both medially and rostrocaudally throughout the reticular formation.

**DISCUSSION**

In spite of extensive work on central control of thermoregulation, there still are many open questions in this field. For a long time the hypothalamus was believed to be the only area involved in thermoregulation, and therefore an extensive literature has developed about experiments in this area. Although now it is known that many areas of the neuraxis are involved in integrating thermal information, only limited information is available about the properties of these areas and their interaction with the hypothalamic regions and with each other. It is clear that the activity of many cells in preoptic–anterior hypothalamus is affected by thermal changes in the spinal cord or midbrain (12) or by pontine electrical stimulation (11). Also it is known that changes in the temperature of the skin affect the activity of hypothalamic neurons (15), and that neurons of the nucleus raphe magnus and the subcoeruleus region are relay stations for thermal afferent projections from skin to the hypothalamus (13). Moreover, studies on intact, unanesthetized animals have shown that the pontine thermoregulatory regions have active functions in thermoregulation (6, 7). Further electrophysiological studies may reveal whether the function of pontine thermoregulatory areas is controlled directly through a peripheral input (14) or through a reciprocal interaction with thermoregulatory areas located in other parts of the neuraxis.

Previous work has shown that electrolytic lesioning of the pontine tegmentum alters the thresholds to both heat-gain and heat-loss responses (6). In the present study the cytotoxic lesioning of the pontine tegmentum affected only the threshold for heat-gain responses and did not change the threshold for heat-loss responses.

The alteration in the threshold for shivering in the lesioned animals was not due to a lowered brain temperature, because \( T_b \) of the lesioned cats was the same as that of intact cats. The change may have been due to inactivation of heat-gain inhibitory neurons of the pontine tegmentum, resulting in an imbalance of the central warmth-sensitive and cold-sensitive...
neurons. The skin temperature of the cytotoxic lesioned cats at room temperature was similar to that of the intact cats. At low $T_a$, the intact cats showed vasoconstriction ($T_w$ decreased in spite of piloerection and shivering), whereas $T_w$ of the lesioned cats increased. Although this may suggest that vasomotion, in particular vasoconstriction, was altered in the pontine lesioned cats, the underlying mechanisms are not known.

The threshold for panting did not change in the cytotoxically lesioned cats, and at high $T_a$ the $T_w$ of the lesioned cats increased as did the $T_b$ of intact animals. In addition, both intact and lesioned cats exhibited vasodilatation. The different results upon heat versus cold exposure may mean that the afferent inputs from peripheral receptors for heat and cold were differently affected by the cytotoxic lesions. The results of these experiments suggest that thermoregulatory mechanisms are specific. Heat-gain and heat-loss regulatory mechanisms can be dissociated. Change in the threshold for shivering in these experiments followed destruction of cells residing in the pons. By contrast, cell somata involved in the panting response must lie outside the lesioned areas, although their fibers pass through these regions. It seems that when damage to the mesencephalic and pontine tegmentum causes alteration in thermoregulation, the alteration in heat-gain responses is due at least in part to the destruction of neurons in these regions.

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