Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs

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Hammel, H. T., J. D. Hardy and M. M. Fusco. Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. Am. J. Physiol. 198(3): 481-486. 1960.—A method is described for local heating or cooling of regions of the hypothalamus of the intact unanesthetized dog. Needle thermodes carrying warm or cold water are inserted through metal guides fixed permanently in the skull and placed symmetrically across the mid-line thus permitting the controlled raising or lowering of the hypothalamic temperature. Insertion of the thermodes is painless and causes no hyperpyrexia or other reaction. The thermodes are withdrawn at the end of an experimental period and the animal can be used repeatedly without ill effects. The following observations were made: a) mild cooling causes vasoconstriction and shivering. There is a marked rise in internal temperature and in temperature of skin over trunk as the result of increased heat production. There is also a large drop in skin temperature of extremities as the result of vasoconstriction. b) With continued cooling, shivering weakens and finally ceases, vasoconstriction continues but rise in body temperature slows. c) Rapidly alternating heating and cooling permits the simultaneous stimulation of panting and shivering responses. These observations suggest that in the hypothalamus there are areas sensitive to cooling, which have thermoregulatory responses. As a result of continued cooling of these regions, inhibitory responses are also evoked which are probably associated with warmth stimulation due to elevated skin and internal body temperature outside of the hypothalamus.

The effects of direct thermal stimulation of the brain stem upon the rectal temperature were first observed by Barbour (1) in 1912. In the anesthetized rabbit following a heat puncture, Barbour found that heating in the region of the corpus striatum would lower the rectal temperature by as much as 1.4°C. Likewise, he found that cooling the same region could produce a chill which would increase the rectal temperature by as much as 1°C. By noting changes in the ear temperature, he also concluded that peripheral vasoconstriction and vasodilatation are influenced by centrally applied cooling and warming. Although these classical observations pertain to an anesthetized animal and were obtained by circulating water through a large thermode at temperatures as high as 51°C and as low as 4°C, they have served as a basis for the hypothesis that there are thermal sensitive cells in the brain stem that participate in normal temperature regulation.

Repeatead observations of the effects of warming parts of the brain stem have served to localize the thermally sensitive cells in the anterior hypothalamus (2) and to define more clearly the motor responses that can be elicited by local hypothalamic warming in the anesthetized animal (3, 4) and in the unanesthetized animal (5-7). Attempts to elicit well defined shivering or even evidence for vasoconstriction in response to local hypothalamic cooling in the unanesthetized animal have not been as successful. Strom was unable to evoke cutaneous vasoconstriction in the anesthetized cat, dog or rabbit (8) in response to local hypothalamic cooling nor in the unanesthetized dog (6), except when the animals had been caused to vasodilate previously by local hypothalamic warming. More recently, Heussel and Kruger (9) were able to produce additional vasoconstriction by cooling the anterior hypothalamus of an animal in a neutral environment. Shivering was not elicited.

Recent experiments by Fusco (7) in our laboratory provide indirect, though convincing evidence that somewhere in the core of the dog there reside thermal receptors that respond to core cooling of no more than 1°C below the normal body temperature in a neutral environment. An unanesthetized dog was rendered hypothermic in a neutral environment by local heating of the anterior hypothalamus. If by this means the dog were caused to pant enough to drop the rectal temperature to between 36.5°C and 37.0°C, then upon cessation of the local hypothalamic heating the dog would start to shiver. Since the environmental temperature was neutral and the skin temperature was high following the stimulating phase, no thermal stimulus could be attributed to
the skin receptors. The only apparent thermal stimulus for driving shivering in this dog, must have been from the core.

The present experiments on local hypothalamic cooling in the unanesthetized dog are an attempt to show that at least some of the thermal receptors activated in moderate core hypothermia are in the hypothalamus.

METHODS

Female mongrel dogs were prepared for implanting thermodes by a method described by Lilly (10). Two rows of sleeve guides (6 guides in each row) were driven through the skull. Each guide was placed on a mandrel which was inserted in a director attached to the vertical rack of a stereotaxic instrument. While the dog was under general anesthesia, the guide was positioned with suitable coordinates and then driven through the skin, connective tissue, muscle and skull. After each guide (18 gauge thin wall stainless tubing 8–10 mm long) was driven through the skull, the mandrel was withdrawn and the skin was pulled over the upper end of the guide which remained 2–3 mm above the top of the skull. The two rows of guides were placed 4 mm on each side of the mid-line and the guides were spaced 3 mm apart in each row. The stereotaxic coordinates for the pair of guides in the number 3 position (fig. 1) were chosen so that this pair would straddle the anterior hypothalamus.

On the day of an experiment (from 1 week to 6 months following guide implantation) three pairs of thermodes were passed through the skin, sleeve guides, dura and brain substance to the base of the skull and then retracted 1 mm. A local anesthesia (1 ml of 1% procaine hydrochloride) was administered subcutaneously over the region of the guides in order to press the thermodes through the skin above the guides without pain. The animals showed no overt reaction to this procedure, and no hyperpyrexia was produced by the implantation of the needle thermodes. Thus, the needles must not have penetrated the areas of the corpus striatum described by Ott (11), Barbour (1) and others (12, 13) as producing this reaction. The thermodes were made of 20-gauge thin wall stainless steel tubing 51–55 mm long and sealed at the bottom end with a 1-mm rounded plug of the same metal. In order to serve as electrodes for electrical stimulation and radio frequency heating as well, the thermodes were enameled along the entire length except of 3 mm next to the tip. Four polyethylene catheters (PE 10) leading from the upper chamber of a small water circulator (fig. 1) were next inserted into two pairs of the implanted thermodes and passed to within 2 mm of the bottom. The larger polyvinyl catheters from the lower chamber were then pressed over the upper end of each thermode which were thereby sealed.

As shown in figure 1, the water chambers were mounted on a skull cap which rested on the head and was held in position with strips of adhesive tape passing under the chin. Water flowed through the upper chamber under pressure and at a high rate from a constant temperature bath. The water temperature in the chamber was measured with the thermocouple as indicated in figure 1. Water flowed from the upper chamber through the PE 10 catheter to the bottom of the thermode, and then outside the catheter inside the thermode and thence through the polyvinyl catheter to the lower chamber which was connected to a vacuum line. The total flow through the four thermodes was about 30 ml/ min. Although it is not possible to place thermocouples in the thermal field of the four thermodes and expect the measured temperature to represent the thermal stimulus to which the animal responds, the hypothalamic temperature was measured by thermocouples inserted to the bottom of the third pair of implanted thermodes which were 3 mm rostral or caudal to the two pair of active thermodes.

To obtain some idea of the temperature distribution in the brain, a mock-up was constructed with four thermodes implanted in gelatin with the same spacing as in the brain. Thermocouples were arranged in the thermal field in order to characterize roughly the temperature pattern in the field. With water circulating through the thermodes at 5.3°C above the ambient gelatin temperature, a thermocouple centered in the array of four thermodes measured 3.4°C above ambient temperature, a pair of thermocouples placed 1.5 mm behind the back pair of thermodes each measured 3.2°C above ambient temperature, a pair of thermocouples placed 3 mm ahead of the front pair of thermodes measured 2.3°C and 2.4°C above ambient temperature and a thermocouple placed 6 mm ahead of one of the front pair of thermodes measured 1.4°C above ambient. All of the above thermocouples were at the level of the end of the four thermodes. At a level of about 7 mm above the end of the thermodes, the temperatures were found to be about 0.4°C higher than at the lower level. Thus, with the four-thermode arrangement it was possible to change the temperature
of a fairly large volume of tissue in the hypothalamic region (approximately 250 mm²). The temperature of the hypothalamus is not uniform as shown by Fusco et al. (14) even without external heating or cooling, and the temperature within the region would be still less uniform in the experiments reported here.

In addition to two thermocouples in the hypothalamus and one in the circulating water, eight thermocouples were attached to the skin surface with beeswax. The thermal potentials of the skin thermocouples could be measured individually or averaged by connecting the thermocouples in parallel (all skin thermocouples had the same internal resistance). Another thermocouple was attached to the skin surface of the ear and a thermocouple was inserted 10 cm into the rectum. The skin thermocouples, the circulating water thermocouple, and ambient temperature thermocouple were recorded directly on a Leeds and Northrup Speedomax multiple channel recording potentiometer (0 mv full scale). The hypothalamic and rectal thermocouples were connected to an L and N linear amplifier (× 10) and then to the Speedomax.

Preparation of the dog, implanting the thermodes, mounting the thermal stimulator and attaching the thermocouples required about an hour. During the experimental period, lasting 3 or 4 hours, the animal rested on a table in the laboratory with no restraint other than the attached thermocouples and rubber tubing. To provide a quiet environment a screen with a one-way mirror was placed between the dog and the investigator. A 16-mm movie camera was mounted near the animal for photographic recording of the shivering and panting responses of the animal. In addition, notes of shivering, panting and respiration rate were recorded on the temperature record. Following each experimental period, the animal was allowed between consecutive periods. After 8 months the animal shows no behavioral changes. To maintain its familiar association with the investigators it is brought frequently to the laboratory and gently handled without being stimulated.

Although the dog used in this study is being saved for total calorimetric measurements during thermal stimulation, two less tractable preparations that gave similar responses to thermal stimulation have been sacrificed and gross sections made of the brains. In both these animals the anatomical examination of the thermode tracts confirmed functional evidence (based on a panting response elicited by r-f diathermy) that the pair of thermodes in positions 2 and 3 were astraddle the preoptic region of the anterior hypothalamus.

**RESULTS**

The responses obtained during two experimental periods are shown in figures 2, 3, 4 and 5. In the record shown in figure 2, obtained on March 6, two pair of thermodes were implanted in positions 2 and 3. The first part of this record illustrates the functional probing that was made with r-f diathermy to determine the more responsive locations in the hypothalamus. On previous occasions it was found that radio frequency energy (3.7 megacycle) was most effective in causing panting when applied between the electrodes in position 3, less effective in position 2 and only slightly or not effective in positions 1, 4, 5 and 6. Electrode temperatures no higher than 49°C were used. In this record two transmitters were employed, one delivering energy to the pair of electrodes in position 2, and the other to electrodes in position 3. Vigorous panting was obtained for about 5 minutes during the first part of the heating causing a rapid fall in rectal temperature. Panting ceased and the rectal temperature stopped falling, however, even though the heating continued and was increased somewhat. The latter part of the record shows the effect upon the rectal temperature of alternately flowing warm (42°C) and cool (33°C) water through the two pairs of thermodes in positions 2 and 3. During the cooling phase, vigorous shivering was elicited and produced a rapid rise in rectal temperature. The onset of shivering was delayed a minute or two following the change in thermode temperature. Also, shivering would sometimes persist after warm water was perfusing the thermode. On occasion, upon changing from a cooling to a warming phase the dog would even shiver and pant at the same time. Then it would stop shivering and pant vigorously during the warm phase of the stimulation. On each
occasion of warm stimulation, panting produced a rapid decline of the rectal temperature.

The consecutive records shown in figures 3, 4 and 5 of an experimental period on March 27 are presented, since skin temperatures and the hypothalamic temperature 3 mm behind the stimulating thermodes were obtained in addition to rectal and thermode temperatures. In this experiment the two pairs of stimulating thermodes were in positions 3 and 4 and the hypothalamic thermocouple was in position 5. During the heating phase (fig. 3) the thermode temperature was maintained at about 42°C for an hour. No panting was elicited although the respiration rate was elevated above the post heating rate, approximately 55/min. and 25/min., respectively. The average skin temperature was maintained at a high level, 36.4°C, and the rectal temperature fell only 0.5°C during the hour of heating.

Following a 25-minute period of no thermal stimulation, cool water, 32°C, was circulated through the thermodes (fig. 4). With a delay of no more than a minute, vigorous shivering was produced by this stimulus. This full scale shivering persisted for about 15 minutes, then weakened and became intermittent so that after about 50 minutes observable shivering ceased although the local hypothalamic cooling continued for another 15 or 20 minutes. During the entire period of cooling the rectal temperature rose rapidly. The rate of rise of rectal temperature was 1.3°C/hr; during the 15 or 20 minutes the animal was shivering vigorously, 0.8°C/hr; for 20 minutes of weak and intermittent shivering and 0.6°C/hr. for a period of 15 minutes after the dog had stopped observable shivering. At the end of cooling the rectal temperature reached a high of 39.0°C, the average of the eight skin temperatures fell 2.1°C.

FIG. 3. Effect of prolonged gentle heating of hypothalamus by circulation of water at 42°C through thermodes. Hypothalamic temperature (HYOTEMP) measured 3 mm caudal to heating thermodes. RESP. indicates respirations per minute.

FIG. 4. Changes caused by a period of moderate cooling of hypothalamus by circulation of water at 32°C through thermodes. TTH—thermode temperature; TR—rectal temperature; THY—temperature of hypothalamus 2 mm caudal to cooling thermodes; TS—av. skin temperature; TETR ear surface temperature; RESP—respirations per minute.
from 35.7°C and the ear temperature fell 3.8°C from 35.0°C. In table 1 are shown several skin temperatures before, during and after hypothalamic cooling. Three skin temperatures (hind paw, front paw and ear margin) measured when the dog had stopped shivering were 6-8°C below the precooling temperatures indicating strong vasoconstriction. On the other hand, at the time the dog had stopped shivering all the skin temperatures on the trunk were 0.5-1.0°C higher than the precooling. This elevation of skin temperature of the trunk was associated with the storage of body heat from shivering.

Immediately after the hypothalamic cooling ended, the skin temperatures of the extremities rose rapidly (within 6 min.) to 36°C or more due to a vasodilatation and a greatly increased flow of blood to the periphery. The resulting redistribution of heat from the core to the peripheral tissue produced an initial rapid rate of fall of rectal temperature (greater than 3°C/hr.) for the first few minutes following the period of hypothalamic cooling. Thereafter the rectal temperature continued to fall at a decreasing rate. High skin temperatures and a high respiration rate both contributed to the excess of heat loss over heat production during this period. However, no frank panting was observed at this time.

Another period of heating the hypothalamus followed a 40-minute period of no stimulation (fig. 5). Although there was evidence of vasodilation and the respiration rate was increased by the stimulus no panting occurred. The rectal temperature, however, fell at an increased rate during the heating.

**DISCUSSION**

The results reported above confirm and extend the findings of Barbour (1). However, to establish the hypothesis, that the hypothalamic temperature in some way combines with the skin temperature to produce and maintain the appropriate motor responses to a cold environment, requires as a major objective a demonstration that moderate hypothalamic hypothermia elicits vasoconstriction and shivering without thermal drives from the periphery and also in the presence of possible inhibitory drives due to elevated skin and visceral temperature. This finding rules out the theory of cold response in temperature regulation suggested in 1932 by Hensel (15) who proposed that all cold stimulation arises in the periphery and none from the hypothalamus. Hensel considered cold, physiologically, to occur only with the cooling of the exposed parts of the body.

Our method of direct application of cooling surfaces to the hypothalamus has achieved a limited objective of showing that thermally sensitive cells reside in the hypothalamus which when cooled activate vasoconstriction and shivering. In the neutral environment maintained in this study, no drive for shivering could have originated from the skin. The fact that frank shivering diminished and disappeared after 50 minutes of continuing hypothalamic cooling does not negate this interpretation of the results. The increased production of heat by shivering slowly increased both the core temperature and the skin temperature over the trunk so that increasing inhibitory drives (warmth) were coming from the skin (except from the extremities) and possibly from the core outside the hypothalamus. It appears also that although the effects of warmth and cold stimulation of the hypothalamus are mutually inhibitory they are not completely so, as both shivering and panting can be simultaneously stimulated by alternating the warming and cooling sufficiently rapidly.

One troublesome fact must be mentioned. On one occasion, on March 20, the same dog as studied above appeared to be much less responsive to thermal stimulation and for no reason that could be found. Circulating 32°C water in thermodes in positions 3 and 4 produced no frank shivering, and only a moderate rise in rectal temperature and fall in the average of eight skin temperatures. Circulating 25°C water did elicit some shivering and a rising rectal temperature but the animal was

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**TABLE 1. Skin Temperatures Before, During and After Hypothalamic Cooling on March 27**

<table>
<thead>
<tr>
<th>Skin Surface</th>
<th>Before</th>
<th>During</th>
<th>Change</th>
<th>After</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(4:45)</td>
<td>(5:17)</td>
<td></td>
<td>(0:23)</td>
<td></td>
</tr>
<tr>
<td>Hind paw</td>
<td>36.6</td>
<td>28.8</td>
<td>-7.8</td>
<td>37.8</td>
<td>+0.0</td>
</tr>
<tr>
<td>Hip</td>
<td>35.7</td>
<td>37.2</td>
<td>1.5</td>
<td>36.1</td>
<td>-0.1</td>
</tr>
<tr>
<td>Kump</td>
<td>35.3</td>
<td>35.9</td>
<td>+0.6</td>
<td>33.7</td>
<td>-0.2</td>
</tr>
<tr>
<td>Flank</td>
<td>36.5</td>
<td>37.3</td>
<td>+0.8</td>
<td>35.9</td>
<td>-1.4</td>
</tr>
<tr>
<td>Chest</td>
<td>34.7</td>
<td>33.7</td>
<td>+1.0</td>
<td>33.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>Neck</td>
<td>38.2</td>
<td>32.1</td>
<td>-6.1</td>
<td>37.2</td>
<td>+0.1</td>
</tr>
<tr>
<td>Ear (margin)</td>
<td>36.3</td>
<td>29.5</td>
<td>-6.8</td>
<td>37.3</td>
<td>+0.0</td>
</tr>
<tr>
<td>Front paw</td>
<td>39.7</td>
<td>39.3</td>
<td>-0.4</td>
<td>37.3</td>
<td>+0.0</td>
</tr>
</tbody>
</table>

* Refer to fig. 4.
very restless and irritable. On the same occasion, the animal was only slightly responsive to r-f diathermy applied to electrodes in position 3. Only a trace of panting was noted although the respiration rate was two to four times the normal rate. On no other occasion, before or after, was this dog so unresponsive to thermal stimulation (see figs. 2 and 3, 4 and 5).

Another limitation of our method of direct thermal stimulation is that it is not suitable for sharp localization of the sensitive areas of the hypothalamus. The animal was most responsive to cooling with the thermodes in positions 2 and 3 or 3 and 4 and did not respond with the thermodes in position 4 or 5. However, there was considerable spread of the thermal stimulus so that it is not possible to separate the particular part of the hypothalamus responding by panting upon heating from the part responding by shivering upon cooling.

ADDENDUM

Our attention has been drawn to a study by Kundt, Brück and Hensel (16) on the behavior of the skin blood flow while cooling the anterior hypothalamus of unanesthetized cats. In one sentence of this study it was noted that regular shivering was induced when the stimulating thermode was at 25°C.

REFERENCES