Thermal stimulation of electrical activity of single units of the preoptic region

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NAKAYAMA, TERUO, H. T. HAMMEL, J. D. HARDY, AND J. S. EISENMAN. Thermal stimulation of electrical activity of single units of the preoptic region. Am. J. Physiol. 204(6): I 122-1126. 1963.-Single unit activity has been recorded in the hypothalamus and preoptic region of cats anesthetized with urethan during local hypothalamic heating and cooling. Heating was accomplished by radio frequency current (3.7 megacycles/sec) and heating and cooling by circulating warm and cold water through small steel thermodes placed 4-6 mm on each side of the midline. Local tissue temperature was recorded by thermocouples and by thermistors. It was observed from a study of about 1,000 neurons that a) about one neuron in five increased its rate of spontaneous discharge with increase in local temperature from 32 to 41 C (Q10 = 5-15); b) 80% of the units did not respond to temperature change by changing their spontaneous discharge rate in the 34-y C temperature range; c) thermally sensitive neurons were found only in the midline region immediately rostroventral to the anterior commissure; d) no units were found for which the discharge frequency increased by decreasing the local temperature.

THAT THE NEURONS in the base of the brain have important functions in the regulation of body temperature has been generally accepted since the work of Ott (1) and Richet (2) in 1884. During subsequent years the responses of animals to heating and cooling of the hypothalamus were studied by many authors (3-5). This work has shown that by heating the midline area of the preoptic region just ventral and dorsal to the anterior commissure shivering is inhibited (6, 7) and panting and vasodilation are evoked. Cooling of the same area inhibits panting and evokes vasoconstriction (8) and shivering (5, 9). As these are appropriate responses for regulation of body temperature it has been generally inferred that this region of the hypothalamus contains at least one and perhaps the major thermostat of the temperature regulatory system. C. von Euler (10) in 1950 made a preliminary report of slow d-c potential changes in the hypothalamus with temperature changes. Although nothing certain can be stated about the nature of these potential changes and further studies of this phenomenon have not been reported, they might be involved in the temperature regulatory process. A study of the behavior of individual units could contribute to the problem. This report contains the results of experiments designed to explore the preoptic region with microelectrodes and to determine the frequency of impulse activity of the neurons in this area in response to local temperature changes.

METHOD

Anesthetized cats were used throughout the present experiments although it was recognized that anesthesia has a marked depressant action on the general temperature regulation of the animal. Urethan was chosen as the anesthetic because of its small depressing action on respiration; it was injected intraperitoneally (1.2 g/kg body wt.). After a cannula was inserted into the trachea and the skull was exposed, the animal was placed in the stereotaxic apparatus. With moderate doses of urethan used, the respiration of the cat was generally regular and uniform in frequency and amplitude. In some cases, however, experiments could not be started for an hour or more after the injection until the so-called "urethan polypnea" had disappeared. During the early stages of this study, the onset of polypnea was taken as the sign of effective heating. This procedure soon proved unsuitable because most neurons, even thalamic units, increased their discharge frequency at temperatures higher than 41-42 C, possibly a general nonspecific response. In later experiments attention was focused on the change in the unit response in a range of temperature between 34 and 41 C, irrespective of the respiration rate of the animal.

Four stainless tubes, $\frac{3}{16}$ in. long and 0.041 in. diameter, were implanted in the skull 9-16 mm rostral from the stereotaxic zero point, two on each side and 4-6 mm from the midline (see upper Fig 1). The brain was exposed on the midline between the tubes directly over

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the region to be subjected to microelectrode exploration. Through these guide tubes, four thermodes made of 20-gauge thin wall stainless tubing, 1 1/2 in. long, were inserted through the brain tissue into the hypothalamus up to 1 mm from the base of the skull. The thermodes were insulated except for 4 mm at the tip. Two water chambers were mounted on the skull (see lower Fig. 1) so that water at the desired temperature could be circulated between a constant temperature bath and one of the chambers. With a partial vacuum in one of the chambers and the appropriate valves opened, the warm or cold water flowed through the fine polyethylene tubes to the bottom of the thermodes and returned to the lower chamber, and from there to the constant temperature baths. In the first experiments diathermic heating was used in which low voltage radio frequency current of 3 megacycles/sec was applied to two central additional thermodes.

Tungsten, steel, or platinum electrodes, the exposed tips of which were about 1 μ, were used for recording single unit activity. Hypothalamic and rectal temperatures were monitored throughout the experiment. A heating pad was placed beneath the animal to keep the rectal temperature in a normal range (38–39 C). The room temperature was 25 C.

With diathermic heating, the temperature at the tip of the microelectrode was estimated indirectly from the reading of a thermocouple inserted to the bottom of the thermode. To relate the thermode temperature to the tissue temperature at the site of the tip of the recording electrode, the microelectrode was replaced at the end of an experiment by a thermocouple and the thermode temperature raised in steps. Generally, the difference in temperature between the position of the microelectrode (midway between the thermodes) and the thermode increased from 0 to 5 C as the thermode temperature increased from 37 to 45 C (see lower Fig. 2). The time course of temperature elevation at the tip of the microelectrode was approximately the same as that of the midline tissues (see top Fig. 2). Hypothalamic temperature during heating or cooling by circulating water was registered by thermistor and recorded simultaneously with the neuron discharge on an oscilloscope. The thermistor was placed in a position between the thermodes similar to that of the microelectrode and thus its temperature was taken as representative of the temperature at the tip of the microelectrode. The time constant of the thermistor was 0.8 sec as measured by plunging it into water. When desirable, the respiration was registered by a thermopile placed in the tracheal cannula and recorded on one beam of the oscilloscope.

In some experiments, the area containing a heat-sensitive neuron was marked for histological localization. The Prussian blue marking technique was used. A current of 10–30 μA was passed through the steel recording electrode, causing the electrolytic deposition of iron from the tip. The cats were sacrificed by perfusion with saline-acacia solution, followed by formalin-acacia. Dur-
ing this perfusion, 10-20 ml of saturated potassium ferrocyanide was injected through the perfusion cannula. Frozen sections 100 μ thick were made, stained with neutral red, and permanently mounted for study.

RESULTS

Temperature response of sensitive units. Continuous impulse activity from the hypothalamus and preoptic area was characteristic of the units recorded in the absence of intentional stimulation. This “spontaneous” activity ranged from 2 to 30 impulses/sec with amplitudes as recorded by our electrodes in the range 100 to 200 μV. The discharge frequency was roughly stable for periods up to 30 min but some units showed rhythmic changes of discharge interval. Units which were unstable or changed their frequency with the slight movements of the microelectrode could not be studied for temperature sensitivity. As illustrated in Fig. 3, some units increased or decreased the amplitude of the impulses with change in temperature although the frequency of this discharge remained constant. After cessation of heating, the amplitude returned gradually to the normal height. It is suggested that this effect is the result of tissue movement with respect to the electrode, possibly related to vaso-

**Fig. 4.** Changes in frequency of discharge of neuron in preoptic region and respiration rate with 3 mc heating. Effect of local rf heating of anterior hypothalamus on: A: electric activity of single neuron; B: respiration.

**Fig. 5.** Discharge rates of a neuron in preoptic region at various maintained temperature levels showing little adaptation.

**Fig. 6.** Impulse frequency versus hypothalamic temperature for thermally responsive units of the preoptic region.
was frequently accompanied by the "doubling" phenomenon, i.e., a small notch appeared on the top of the record of the inspiratory phase and deepened little by little and finally divided the cycle of respiration into two. The hypothalamic temperature at which the polypnea was observed varied in different experiments but in most cases temperatures of 39.5 or over evoked polypnea to some degree. However, hypothalamic temperatures 42-44 C did not elicit polypnea in some preparations even under apparently the usual experimental conditions.

In the experiment illustrated in Fig. 5 the hypothalamic temperature was changed slowly from 40 C to 37 C to 33.8 C and returned to 37 C. The four records were taken 1 min after the hypothalamic temperature had stabilized at the above temperatures. Discharge rates remained roughly constant for a given temperature indicating little adaptation during the several seconds of observation.

With local heating, the impulse frequency of heat-sensitive units increased with the temperature in a roughly linear fashion. Figure 6 shows the discharge rate of ten neurons measured after the hypothalamic temperature had reached a steady state. As with the thermally insensitive neurons, the discharge rate at 38 C was highly variable between 2 and 40 impulses/sec from unit to unit of the thermally responsive neurons. Based on the activity change in the 34-40 C range, the Q10 of the thermally responsive units was between 5 and 15. About one unit in five of those studied was thermally sensitive.

Temperature response of insensitive units. Most of the units studied in the anterior and posterior hypothalamus changed their discharge frequency only slightly if at all with change in local tissue temperature. Several hun-
dreds of these units were observed in the search for neurons which had some response to temperature and Fig. 7 shows a plot of the discharge frequencies of 11 of these units. The level of impulse activity of these neurons at 38 C varied between 3 and 32 impulses/sec and warming the units between 34 C and 40 C caused on the average an increase of 1–2 impulses/sec. About 80% of the neurons studied in the preoptic region showed this small response to temperature. The high variability of the spontaneous discharge level at 38 C may be characteristic of the neurons or may possibly be related to some experimental artifact. In a single preparation and within a few minutes, neurons of very different discharge rates were observed, thus apparently excluding variability in anesthesia as a possible cause of the variable discharge level.

Response to cooling. No unit responded with an increase of the steady-state discharge frequency following local cooling out of the 1,000 neurons studied in the anterior and posterior hypothalamus. Occasionally, a neuron responded with a short transient increase in discharge frequency with rapid cooling but soon returned to its former discharge rate.

Under our experimental conditions, cooling the hypothalamus did not bring about shivering. Instead, local cooling of the anterior hypothalamus caused a transient restlessness and increase of respiration rate. In one experiment in which the hypothalamic temperature was lowered to 31.6 C, the respiration rate increased temporarily from 54 to 90 while the rectal temperature remained unchanged.

Localization of temperature sensitive units. Seventeen of the heat-sensitive units were marked for histological localization. Figure 8 is the frontal section of the cat brain, 15 mm rostral from the stereotaxic zero point. The large lesions lateral to the hypothalamus were made by the thermodes. (These lesions have no observable effect on the thermoregulatory response in the intact dog (5, 8).) Two points at which thermally sensitive units were observed are seen located ventral to the anterior commissure near the midline. In Fig. 9, points marked were projected onto the parasagittal plane and horizontal plane. Neurons responding to heating were found in the anterior hypothalamic and preoptic areas from Fr. 14 to Fr. 17 mm. The depth was from H-1 to H-4.0, very close to the lower border of the preoptic area. Fifty per cent of the marking spots were within 0.5 mm of the midline, although heat-sensitive units were found as far lateral as 2.5 mm. In the area more rostral than 17 mm from the zero points, no heat-sensitive units have been found. The responsive units were not confined to a single nuclear mass but were diffusely distributed in the lateral and anterior hypothalamic areas and the nucleus of the diagonal band of Broca.

Exploration in the posterior hypothalamus, 10.5–13 mm rostral from the zero points, revealed no thermally sensitive neurons among the 95 units studied in this area. The supraoptic nuclei have sometimes been mentioned as possible sites of temperature regulatory sensitivity so exploration for thermally sensitive neurons was made in this area. However, no thermally sensitive neurons were found.

DISCUSSION

The presence of thermally sensitive neurons in the preoptic region is further evidence of the importance of this area in the thermal regulation of the animal. Although it is not possible to state that the neurons reported on have a function in the temperature regulation of the normal cat, it is probably significant that those neurons which respond to temperature change are located only in the area which, when stimulated thermally (4), evokes appropriate thermoregulatory responses in the intact animal and which, when ablated, causes svcccr disturbance of the animal’s temperature (11). Some speculation as to a possible role of the neurons thus seems warranted. Plotting the means of the discharge rate for temperatures between 34 and 41 C for the neurons which are shown in Fig. 6 and Fig. 7 gives the curves in Fig. 10. From available evidence it cannot, of course, be supposed that these two curves have any significance for temperature regulation and speculation as to their relationship to such a function must be guarded. However, the form of the curves is suggestive of regulatory function (12). For example, it might be that the thermoregulatory functions of shivering and vasoconstriction are stimulated and panting is inhibited when the firing rate of the thermally responsive neurons has fallen somewhat below the average of the thermally unresponsive neurons. A reverse situation could be thought of for hypothalamic temperatures above about 38 C when the mean firing rate of the responsive neurons is greater than that of the unresponsive neurons.

REFERENCES

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