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TEMPERATURE-SENSITIVE NEURONES IN THE DOG'S HYPOTHALAMUS

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It has been known for many years that the hypothalamus plays an important role in the homeostatic system of temperature regulation. Not only does ablation of the pre-optic and posterior regions cause serious disturbances in the maintenance of body temperature (Clark, Magoun & Ranson, 1939; Blair & Keller, 1946) but the pre-optic area has been shown to possess a marked sensitivity to temperature changes (Barbour, 1912; Magoun, Harrison, Brobeck & Ranson, 1938; Hemingway, Rasmussen, Wikoff & Rasmussen, 1940). Heating or cooling this region in a conscious animal provokes the appropriate thermoregulatory responses of vasodilatation and panting or vasoconstriction and shivering (Ström, 1950; Freeman & Davis, 1959; Hammel, Hardy & Fusco, 1960). This evidence indicates that the pre-optic area contains neurones which play an important part in the regulation of body temperature owing to their responsiveness to temperature change. Likewise skin receptors, besides supplying sensory information about temperature, have also been shown to provide a thermal drive in the regulation of body temperature in man (Kerslake & Cooper, 1950; Brebner & Kerslake, 1961) and in animals (Bligh, 1957; Bleakley & Findlay, 1955).

For an understanding of thermal homeostasis as a regulatory process, it is necessary that the identity and the characteristics of the sensory inputs to the system should be known. Recordings from fibres in cat and human cutaneous nerves have established that there are skin thermoreceptors which respond to both heating and cooling (Dodt & Zotterman, 1952; Hensel, Iggo & Witt, 1960; Hensel & Boman, 1960). Information about the hypothalamic temperature receptors has recently come from the work of Nakayama, Hammel, Hardy & Eisenman (1963). Using anaesthetized cats and locally heating and cooling the brain they found, with single unit recordings from micro-electrodes, that the thermoreceptors were only present in the pre-optic region, none being detected

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in the supra-optic nucleus, the posterior hypothalamus or thalamus. About 20 % of the neurones studied showed a marked thermal sensitivity and their discharge rate was found to increase with local temperature changes. The remainder of the units generally showed an insensitivity to temperature in the range 32–42° C; no cells were found which were cold-sensitive, i.e. increased their firing rate when cooled.

The present work was aimed at extending these observations to the dog—a species which has been used extensively for the study of temperature regulation. Extracellular recordings have been made from single hypothalamic neurones in the pre-optic region during imposed changes in the local temperature and are presented in this paper. For the sake of brevity, neurones which increased their rate of firing with increasing temperature will be referred to as 'warm cells'; those which did the reverse will be called 'cold cells'.

METHODS

Dogs weighing between 10 and 12 kg were used and anaesthetized with a solution containing 20 g of urethane and 2 g of α-chloralose per 100 ml. This was given intravenously for the initial preparation and small supplementary doses were given through a catheter as required during recording; the aim was to maintain as light a depth of anaesthesia as possible.

Micro-electrodes were of tungsten (Hubel, 1957) or stainless steel (Green, 1958) with a tip size of 1–2μ and a resistance at 1 kc of 1–5 MΩ. After a cathode follower-stage and conventional amplification (pass band 50–6000 c/s), the action potentials were displayed on an oscilloscope and recorded by the direct method on magnetic tape. Auditory monitoring was also used. For counting firing frequency the tape was played back to a recording galvanometer (Visicorder) or, in later experiments, to a frequency meter and printer (General Radio, types 1151 and 1137).

Hypothalamic temperature could be changed by the water perfusion technique of Hammel *et al.* (1960). With co-ordinates from the dog stereotaxic atlas (Lim, Liu & Moffitt, 1960), four thermode tubes (1 mm *o.d.*) of thin-walled stainless steel were inserted to the base of the brain in rostral planes 21, 24, 27 and 30 mm. To avoid the superior sagittal sinus, the tubes were placed 4 mm to the right of the mid line. Also on the right a fifth tube was placed 9 mm laterally in the rostral plane 25·5 mm. This enabled a thermocouple to be inserted to the base of the brain to record temperature at a lateral distance of 5 mm from the row of thermodes.

The micro-electrodes explored the hypothalamus on the left side between the levels of the base of the anterior commissure and the optic chiasma. In the cat most of the thermally-sensitive units lie within 2 mm of the mid line (Nakayama *et al.* 1963) and in order to reach this area, but avoid the sagittal sinus, the electrodes were inserted at an angle of 15° to the vertical. The lateral positioning of the electrode carrier was such that the tip of the electrode reached the mid line just above the chiasma. The recording tip was thus at a distance of 4–6 mm from the line of thermode tubes and the temperature at the tip would be comparable with that at the thermocouple 5 mm on the other side of the thermodes. The thermocouple e.m.f. (against an ice bath) was suitably backed off, amplified and displayed on a recording potentiometer so that the chart span was 5° C.

The animal's rectal temperature was measured with a thermistor and kept in the range 38–39° C by a heating pad. Usually at the end of the initial preparation (about 2 hr) the

rectal temperature had fallen to 37 or 36° C and after it had been raised to 38–39° C a steady state was apparently reached and little or no external heating was needed. Air temperature was 23–25° C.

RESULTS

The findings in this paper are based on recordings from a total of eighty-eight neurones which were held under observation for sufficiently long to determine whether they showed any temperature sensitivity. This procedure took about 8 min to allow for recording at the initial temperature for some 4 min and the alteration of local temperature, which was raised and lowered about this level by 1–2° C. If the firing rate did not show any

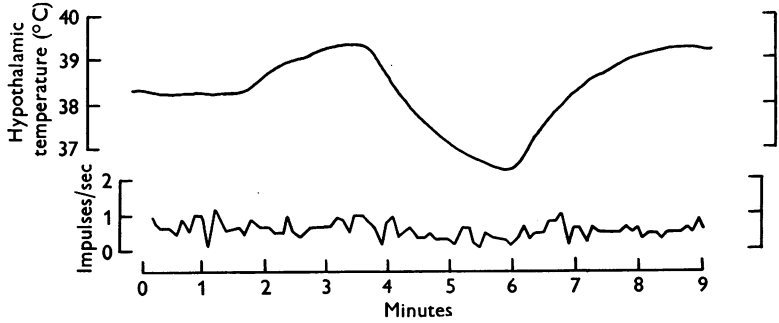

Fig. 1. Frequency response of a unit showing no response to temperature changes.

obvious relation to the imposed temperature changes the recording was discontinued. Otherwise the neurone was followed for as long as possible; the maximum duration was about 1 hr. During this time the hypothalamus was subjected to several heating and cooling cycles and, if time permitted, to various rates of change of temperature. This was achieved by stopping the perfusion of the thermodes at the peak of heating or cooling and allowing the temperature to drift back to its normal level.

There was a clear distinction between units which responded to temperature changes and those which did not; this was easily determined from the audio monitor without the necessity of a frequency plot.

Most of the neurones encountered were firing spontaneously and in a fairly steady fashion at rates ranging from 0·7 to 30 impulses/sec. The spike amplitude varied from 100 to 500 μV and the noise level was usually about 15 μV. Nearly all the cells had potentials which were positive-going.

Of the eighty-eight cells which have been recorded fifty-three or 60% showed no temperature sensitivity—at least within the physiological range which has been used in these experiments. An example of this type of response is shown in Fig. 1. In this experiment a slowly firing

cell was heated by 1° C, cooled through 2·9° C and finally heated through 2·9° C, but there was no evident change in the impulse frequency.

The remaining thirty-five cells have been classified as either 'warm' or 'cold' neurones, there being twenty-eight of the former and seven of the latter.

The warm cells generally followed the imposed swings of temperature quite smoothly. There was no overshoot nor during the several minutes at increased or reduced temperature did the firing rate show any evidence

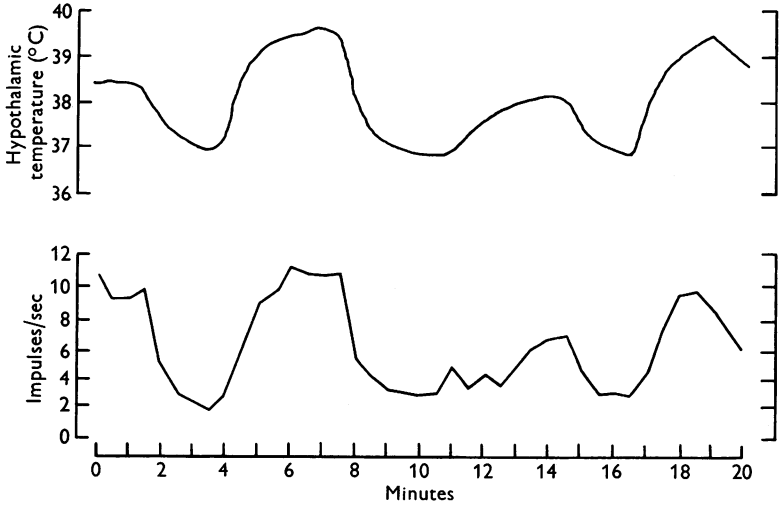

Fig. 2. Hypothalamic temperature and firing rate of a typical 'warm' cell.

of adaptation. Figure 2 illustrates the responses of a representative warm cell. After rapid cooling, warming, and cooling again, the temperature was allowed to increase passively to about its initial level and then a further cycle of rapid cooling and warming was performed. Throughout the 20 min of observation, the firing rate followed these temperature changes quite faithfully, particularly in regard to rate of change. It can be seen that there was some variation in response to the absolute levels of temperature. The initial frequency of 9–10/sec at 38·4° C is similar to the frequency in the final phase of heating where the temperature was 39·5° C. Many of the warm cells showed this variability to a greater or lesser extent and, while it may have been partly due to injury caused by the electrode, it was also seen in cells which were followed for up to 1 hr and which were presumably not injured.

About half the cold cells which have been studied responded smoothly to the temperature changes; that is to say their firing rate was inversely

related to temperature. The results shown in Fig. 3 illustrate this type of response. The other cold cells changed their activity more abruptly, particularly with cooling, and showed some evidence of adaptation. Data from one of these neurones is shown in Fig. 4. On heating through 1·3° C the firing rate was reduced from 8/10 sec to about 2/10 sec. Immediately

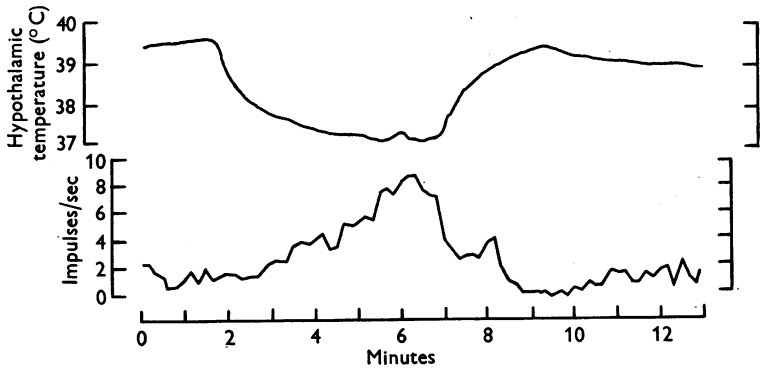

Fig. 3. Hypothalamic temperature and firing rate of a 'cold' unit.

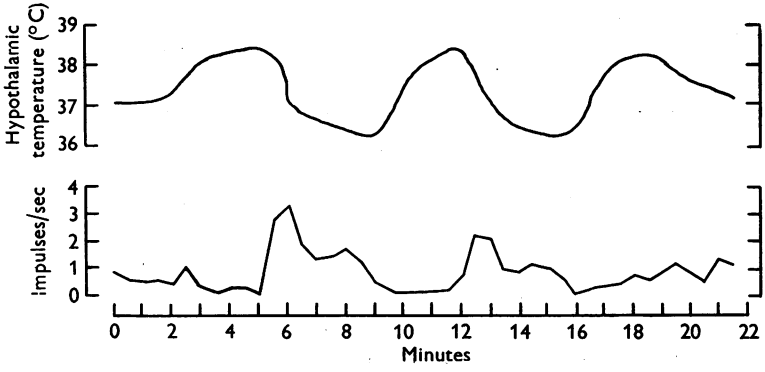

Fig. 4. Responses of a 'cold' unit showing phasic effect of cooling.

on cooling there was a dramatic outburst of activity to a rate of 30/10 sec, but this gradually subsided during the remainder of the cooling. It can be seen that this pattern of response was repeated in the two following temperature cycles, except that the final slower cooling was not accompanied by an outburst or indeed by any obvious change in firing rate. To what extent the sudden outburst or phasic response was related to the rate of change of temperature cannot be decided since it was only possible to subject this one neurone to rapid and slow cooling.

Only once during the experiments was it possible to detect any overt

responses in the form of panting or shivering when the hypothalamic temperature was changed in the anaesthetized dog. In this instance the respiratory rate increased when heating was applied, but there was no indication that the anaesthetic level was unusually light.

On several occasions when a sensitive cell has been detected, attempts were made to drive it by heating or cooling the dog's snout with wet sponges or infra-red radiation. There were no responses in the hypothalamus to this peripheral stimulation.

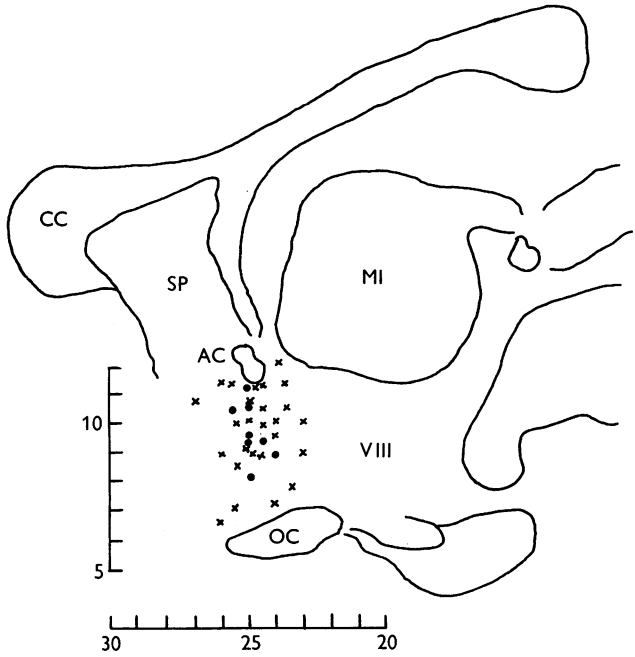

Fig. 5. Position of warm (×) and cold (●) cells taken from stereotaxic co-ordinates and projected on a para-sagittal section of the dog's brain. Vertical and horizontal scales show distance (mm) dorsal and rostral to the zero planes. CC, corpus callosum; SP, septum pellucidum; MI, massa intermedia; AC, anterior commissure; V III, third ventricle; OC, optic chiasma.

No histology has been done to show the sites of the sensitive neurones. However, if the dorso-ventral and rostro-caudal co-ordinates for each cell are projected on a parasagittal section of the dog's brain (traced from Singer, 1962), the positions shown in Fig. 5 are obtained. The cells appear to be scattered fairly uniformly between the anterior commissure and the chiasma and do not fall within any recognized nucleus of this region. In the present experiments the search for sensitive cells was made in the area from 23 to 27 mm anterior to the vertical inter-aural plane and all the insensitive units also lay within this area.

DISCUSSION

The first aspect of these results which should be considered is the question whether the records have been taken from the actual receptors in the anterior hypothalamus or from interneurones which are modulated by the receptors. While there can be no unequivocal answer on this aspect we believe it is justifiable for the moment to refer to these temperature-

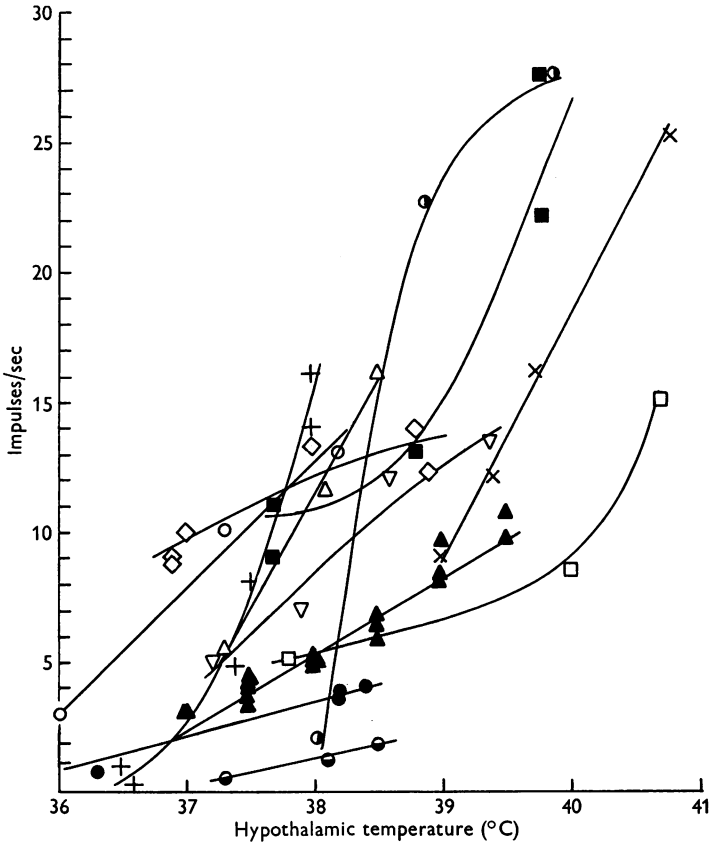

Fig. 6. Steady-state firing rates and hypothalamic temperatures of 'warm' cells. Each symbol applies to one neurone.

sensitive cells as receptors, particularly since the pre-optic region is the area of the brain stem which, when heated or cooled, evokes all of the physiological responses of temperature regulation in the conscious animal. It is conceivable that the cold cells are not true receptors, but simply neurones which receive inhibitory synaptic connexions from the warm cells. Although this cannot be ruled out, it is perhaps unlikely in view

of the different time courses of the smooth responses of the warm cells and the abrupt changes of the cold cells. In addition the phasic type of discharge shown in Fig. 4 is very similar to the responses of peripheral cold receptors in the tongue (Hensel & Zotterman, 1951) and the skin (Hensel *et al.* 1960; Hensel & Boman, 1960).

The presence of warm cells in the pre-optic region of the dog confirms the results of Nakayama *et al.* (1963) from the cat. They were unable to locate any cold neurones and it seems possible that this represents a species difference. It is probably not due to a sampling error, since a considerably larger total of cells was studied in the cat than in the present experiments. Caution should therefore be exercised in extrapolating from one species to another.

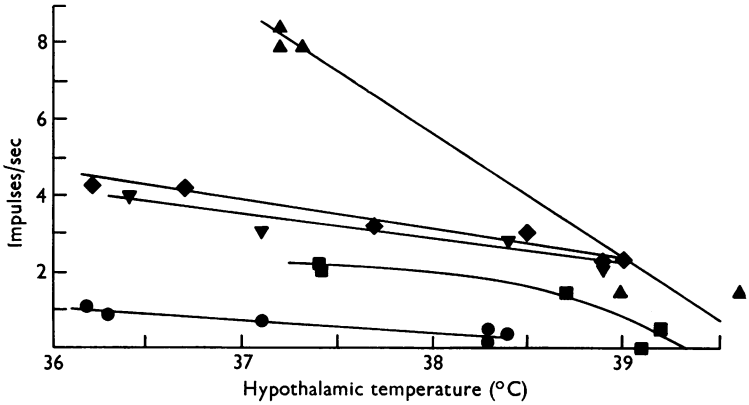

Fig. 7. Steady-state firing rates and hypothalamic temperatures of 'cold' cells. Each symbol applies to one neurone.

The properties of the sensitive cells have so far been presented only against a time scale and it is of interest to show their static discharge rates plotted against hypothalamic temperature. To do this, values of frequency and temperature were selected from the original records when both variables had been constant for several minutes. The resulting curves are shown in Figs. 6 and 7, where considerable variations in sensitivity (slope) can be seen for both the warm and cold cells. Most of the curves are linear but some do show sudden inflexions. The sensitivities of the warm cells varied from 1 impulse/sec.° C to 21 impulses/sec.° C with a mean value of 7. The cold cells shown in Fig. 7 were less responsive, having a mean sensitivity of -1 impulse/sec.° C (range $-0{\cdot}4$ to $-3{\cdot}5$). However, these figures refer to the stable sensitivities of the cold units and, when the dynamic or phasic response is considered, very much higher sensitivities are evident. For example, in Fig. 4 the change in activity with the cooling

at 5 min corresponds to a sensitivity of about −50 impulses/sec. ° C. It is of interest to compare these figures with those which have been found from other temperature receptors. In a specific cold receptor in human skin, Hensel & Boman (1960) recorded a dynamic sensitivity of −45 impulses/sec. ° C but at steady temperatures the sensitivity was reduced to −0·65 impulses/sec. ° C. The similarity of these with the sensitivities we have observed in the dog's hypothalamus will be apparent. There is also good agreement between our results and those which have been recorded from C fibres in the cat's skin (Hensel *et al.* 1960). The mean sensitivity of the warm cells observed in the cat's hypothalamus by Nakayama *et al.* (1963) was 4·2 impulses/sec. ° C, which again is of the same order as the present mean of 7 impulses/sec. ° C.

Although no histological marking of the sites of sensitive cells was done, the approximate co-ordinates shown in Fig. 5 agree well with the marked sites of Nakayama *et al.* (1963) and with the position of the area in which von Euler (1950) was able to record slow temperature potentials. In the present experiments the electrodes were so angled that while recording between the anterior commissure and the optic chiasma the tip was within 2 mm of the mid line.

Finally, it remains to discuss the possible role of these sensitive cells in the temperature regulatory system of the dog. It would be easy to propose a simple model in which, for example, the warm cells had excitatory and inhibitory connexions with neurones governing heat-dissipating and heat-conserving mechanisms, respectively. Similarly, the reverse of these arrangements could be envisaged for the cold cells, with temperature regulation being achieved as the result of a balance between the total outputs of the two types of pre-optic sensor. Further, the possible importance of the temperature-insensitive cells should not be overlooked. Should the temperature regulator of the dog possess a 'set point' it would be expected that a temperature-*insensitive* input into the system would be required for the reference of set point. The temperature-*sensitive* elements would provide the temperature information for comparison with the signals from the insensitive cells. While simple systems such as these might be adequate to account for the responses observed in a conscious animal when its hypothalamus is artificially heated or cooled, they fail in several ways to account for other known facts concerning the thermoregulatory system.

First of all they do not describe how the temperature receptors in the skin come to play their undoubtedly important part in temperature regulation (Fusco, Hardy & Hammel, 1961; Belding & Hertig, 1962). It has recently been demonstrated that a dog may shiver in the cold with a hypothalamic temperature which is the same or even higher than the

temperature at which it pants in the heat (Hammel, Jackson, Stolwijk, Hardy & Strømme, 1963). The drive for regulation in this case is presumably arising from skin receptors, but how these impulses integrate with the hypothalamic receptors is not clear. In our experiments it did not prove possible to change hypothalamic firing rate by heating or cooling the dog's snout (an area which Murgatroyd, Keller & Hardy (1958) have shown to be highly sensitive to temperature changes). This negative finding suggests that the skin impulses do not modulate the firing of the anterior hypothalamic receptors directly and presumably integration occurs at a lower level (Randall, 1963). It is uncertain whether the anaesthetic was a complicating factor in these efforts to drive the hypothalamic cells from the periphery. However, in cats and rabbits under urethane or chloralose, units in the lateral or posterior hypothalamus (Cross & Silver, 1963) or supra-optic nucleus (Brooks, Ishikawa & Koizumi, 1963) can still be affected by peripheral stimuli. On the other hand, Stuart, Porter, Adey & Kamikawa (1964) were only able to record in the cat's hypothalamus in the absence of urethane or barbiturates.

The second failure of a simple model of temperature regulation involving a single temperature-sensitive site lies in the fairly large changes which have been observed in hypothalamic temperature when an animal falls asleep. Hammel *et al.* (1963) have shown that with the onset of sleep or dozing there is an increased rate of heat loss accompanied by a *falling* hypothalamic temperature. Presumably the pre-optic receptors respond to this falling temperature, but their output is in some way inhibited and a reduction of body temperature follows. The 'adjustable set-point' hypothesis of Hammel *et al.* provides a possible explanation for this phenomenon.

The recent work of Andersson, Ekman, Gale & Sundsten (1963) indicates that, besides their role in the 'physical' regulation of body temperature, these hypothalamic receptors may also have a part to play in the hormonal control of heat production through thyroid secretion. It was found that pre-optic cooling in conscious goats caused a marked increase in blood levels of protein-bound iodine with a time course similar to that following administration of thyrotrophic hormone. The response to cooling was abolished by a lesion placed in the median eminence, and so appears to be activated through the pituitary.

SUMMARY

1. Micro-electrodes have been used in dogs to explore the anterior hypothalamus for neurones which were sensitive to small temperature changes. Thermode tubes were implanted lateral to the region being explored and enabled local temperature to be raised or lowered.

2. Searches were made within 2 mm of the mid line between the base of the anterior commissure and the optic chiasma.

3. Raising or lowering the hypothalamic temperature by 1–2° C had no effect on the firing rate of about 60 % of the units.

4. 80 % of the remaining neurones showed a sensitivity to temperature changes by increased firing rate on heating and decreased with cooling. Firing rate followed different rates of change of temperature quite smoothly and with a mean sensitivity of 7 impulses/sec. ° C. There was no evidence of adaptation.

5. 20 % of the sensitive cells increased their firing rate with cooling. Some of the units followed the temperature swings smoothly but others showed an abrupt outburst or phasic response with cooling.

6. It is suggested that the sensitive cells are receptors which play an important role in temperature regulation.

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