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THERMOREGULATORY NORADRENERGIC AND SEROTONERGIC PATHWAYS TO HYPOTHALAMIC UNITS

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SUMMARY

1. In guinea-pigs hypothalamic single units were extracellularly tested for their response to thermal stimulation of the skin and to electrical stimulation of two different pontine areas, the nucleus raphé magnus and the dorsomedial reticular formation. Furthermore, thermoregulatory control actions were measured during the stimulations.

2. Electrical stimulation of those reticular formation areas containing noradrenaline cells caused an increase of oxygen uptake, electrical muscle activity and body temperature, while stimulation of the nucleus raphé magnus, known to contain serotonin cells, brought about inhibition or had no effect.

3. The recorded units could be subdivided into three groups.

Cell type a. Neurones on the boundary of preoptic and anterior hypothalamic regions which increased their firing rate when the skin was cooled and decreased it when the nucleus raphé magnus was stimulated.

Cell type b. Neurones in the anterior hypothalamus which did not respond to brain-stem stimulation.

Cell type c. More posterior neurones which increased their firing rate when the skin was warmed or when the nucleus raphé magnus was stimulated and decreased their firing rate when the reticular formation was stimulated.

4. Cell type *a* seems to represent interneurones which are connected to the ascending serotonergic thermoregulatory pathway. As for cell type *c*, it is inferred that it could represent interneurones which control the threshold for shivering and non-shivering thermogenesis.

INTRODUCTION

Neurones sensitive to local temperature have been identified in the anterior hypothalamus (Nakayama, Eisenman & Hardy, 1961) and preoptic region (Nakayama, Hammel, Hardy & Eisenman, 1963) and neurones responsive to thermal stimulation of the skin in the preoptic region (Wit & Wang, 1968) and anterior (Hellon, 1970) and posterior (Nutik, 1973) hypothalamus. The present paper investigates the responsivity of the latter neurones to stimulation of the nucleus raphé magnus and the dorsomedial reticular formation of the pons. On the basis of previous

The authors' names are in alphabetical order.

studies (Szelényi, Zeisberger & Brück, 1976) in which electrical pons stimulation and intrahypothalamic microinjections of noradrenaline caused excitatory thermoregulatory effects, a catecholaminergic pathway ascending from the pontine reticular formation to the hypothalamus has been postulated (Zeisberger, Szelényi & Brück, 1977). The aim of the present study was to substantiate this pathway by evaluating hypothalamic single-unit activity and thermophysiological reactions following selective stimulation of either the nucleus raphé magnus or the dorsomedial reticular formation. Thermoregulatory effects of nucleus raphé magnus stimulation have not yet been described. There is however evidence from electrophysiological studies that there is a connexion between cutaneous thermoreceptors and the hypothalamus via the nucleus raphé magnus (Dickenson, 1978). The neurones ascending from the nucleus raphé magnus may act on hypothalamic and preoptic neurones via serotonergic pathways since microinjections there of serotonin (Komiskey & Rudy, 1977; Zeisberger & Wissel, 1978) produce thermoregulatory responses. On the other hand, cells in the nucleus raphé magnus contain serotonin (Dahlström & Fuxe, 1964), whereas cells in the dorsomedial pontine reticular formation contain noradrenaline (Ungerstedt, 1971). The present study describes direct evidence of pathways from these brain-stem centres to the hypothalamus.

METHODS

Thirty-two guinea-pigs, 6–10 weeks of age and weighing 309–470 g, were used in these experiments. In the electrophysiological single-unit experiments the animals were anaesthetized with urethane (0·8 to 1·2 g/kg I.P.) and placed in a stereotaxic apparatus. The heads were fixed according to the co-ordinate system of Tindal (1965). The co-ordinates were scaled up according to the size of the animal. The activity of single units was extracellularly recorded with stainless-steel electrodes (F. Haer, U.S.A.; impedance values 4–5 MΩ at 1000 Hz and 10^{-9} A). For the stimulations four-pole electrodes were implanted in order to excite two different tracts in the pons by the bipolar technique. The stimulating electrodes (Fig. 1) were made of stainless-steel wires 100 μm in diameter and insulated with multiple layers of Epoxylite (Epoxylite Corp., U.S.A.). Each pole was sloped at the tip by etching. The distance between two corresponding tips was 100 μm. To stimulate alternately the nucleus raphé magnus or the reticular formation, the distances between paired tips were selected to correspond to the age of the animal, at 500, 600, 700 and 1000 μm, respectively. For the stimulations square-wave impulses from a stimulator (H. Sachs Elektronik, W. Germany) were applied at frequencies of 1, 3, 5, 10 or 20 Hz. The pulse duration was preferentially 1 ms, and the intensity of stimulation was not greater than 1 μA and 30 mV, to avoid interspersing signals. Each single unit was tested for its response to electrical stimulation of the nucleus raphé magnus, the reticular formation or both. Unit activity was amplified by a type DAM 5-A amplifier (WPI, U.S.A.), and displayed on a storage oscilloscope (Tektronix, U.S.A.). Abdominal skin and paws were warmed or cooled by the water-perfused support plate of the stereotaxic apparatus. The water was taken from three sources, the temperature of which could be kept constant. The water temperature of two of them could be changed between +18 and +58 °C, the temperature of the third one between −5 and +25 °C.

For chronic implantation of the stimulating electrodes and hypothalamic thermocouples used in the thermophysiological experiments, the guinea-pigs were anaesthetized with Nembutal (40 mg/kg I.P.). Shortly before the experiment, while the animal was kept under Halothane inhalation anaesthesia, additional electrodes were inserted into thigh and masseter muscles and thermocouples into the interscapular brown adipose tissue, the colon and under the skin of the back. The subsequent measurements were made with the animals in a fully awake state. Owing to the small electrode tips (100 μm), the stimulating currents were lower (25 μA) than those used in a previous study (Szelényi *et al.* 1976). The guinea-pigs were placed in a metabolic chamber in which the air and wall temperatures could be kept constant in a range between 15 and 35 °C. Oxygen consumption, the four body temperatures and the integrated electrical activity in the two muscle groups were continuously recorded on two polygraphs.

To mark the electrode locations by deposition of iron at recording and stimulation sites, current (10–20 μA, 0·8–1·8 V) was passed through the electrodes for 10 sec. The marker current was monitored by connecting a standard micro-ampere meter in the ground leg of the set-up. The positions of the deposition sites were marked with the Prussian Blue technique.

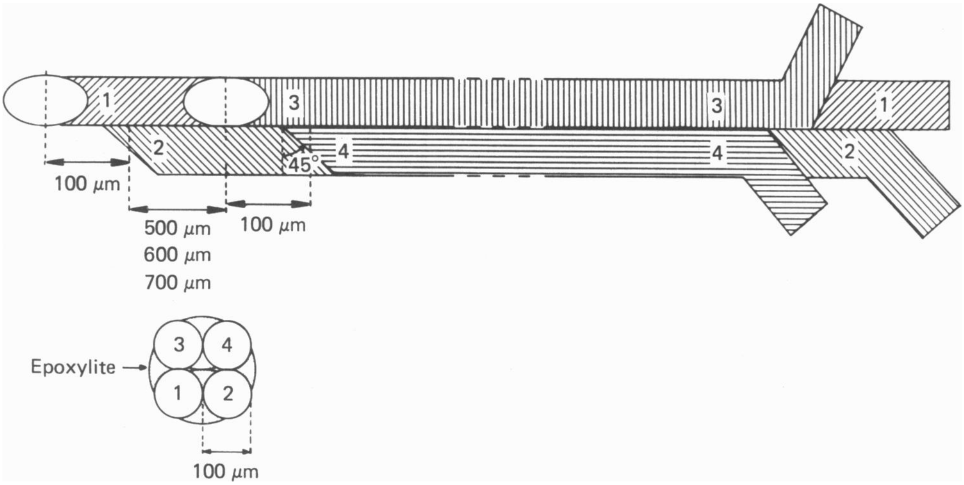

Fig. 1. Technical drawing of the electrical four-pole stimulating electrode.

RESULTS

Thermophysiological studies

Electrical stimulation was applied to the dorsomedial reticular formation (Figs. 2, 3) in eight experiments on three guinea-pigs. The stimulations caused an increase of oxygen uptake, electrical muscle activity and body temperatures. An example of such a reaction is shown in Fig. 4.

Similar stimulation was applied to or around the nucleus raphé magnus (Fig. 3) in six experiments on two guinea-pigs. The stimulations were found either to have no effect (four experiments) or to cause inhibition (two experiments) when a thermal response had previously been evoked by slight cooling.

Extracellular reactions of single-unit activity

Extracellular recordings of hypothalamic single units were made during thermal skin stimulations and electrical stimulations of the reticular formation and the nucleus raphé magnus.

Out of the total of forty-one single units in twenty-nine guinea-pigs, twenty single units located in the hypothalamic regions could be classified according to their sites, thermoresponsivity and stimulation response. Twenty-one units were discarded from evaluation as they could not be held long enough to classify them according to all three criteria.

Three functional groups (a, b, c) could be distinguished (Table 1):

(a) Three units around the boundary between the preoptic area, PO, and the anterior hypothalamus, AH (Fig. 2) responded to cooling of various skin areas

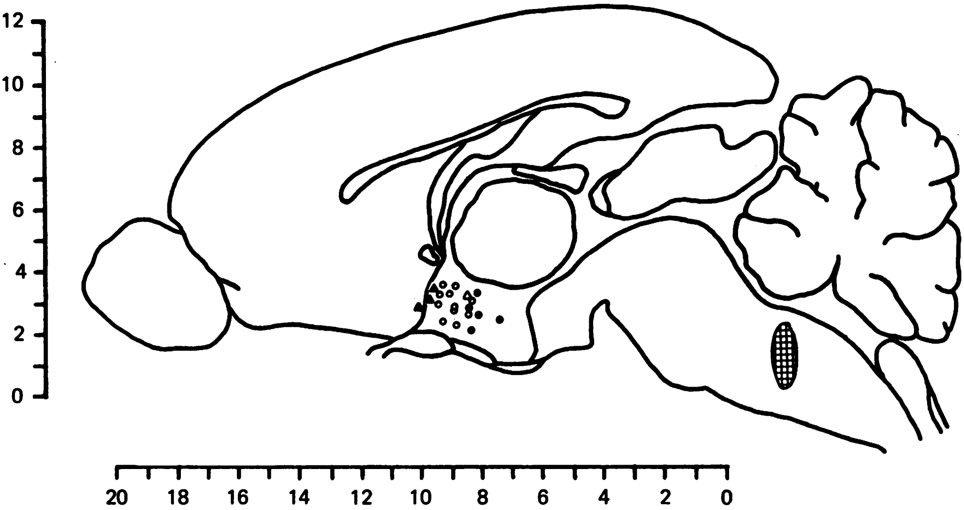

Fig. 2. Sagittal view of recording and stimulation sites. Locations of the recording sites in the hypothalamus are as follows. ▲, cold-responsive neurones of cell type *a* (Table 1); ○, non-thermoresponsive neurones of cell type *b*; △, warm-responsive neurone of cell type *b*; ●, warm-responsive neurones excited by stimulation of the nucleus raphé magnus and inhibited by stimulation of the reticular formation (cell type *c*); ⊕, area of the stimulation sites in the pons. The horizontal and vertical scales (mm) represent the stereotaxic co-ordinates according to the atlas of Tindal (1965). The zero marks of both the horizontal and vertical scales correspond to the interaural line.

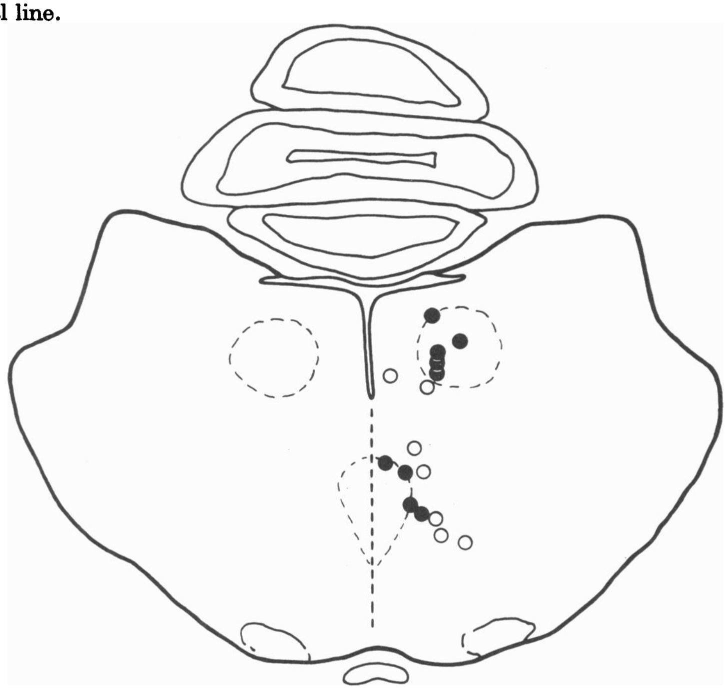

Fig. 3. Positions of the pontine stimulation sites in a frontal view. ●, sites located in the dorsomedial reticular formation and within and around the nucleus raphé magnus, stimulations of which caused responses of hypothalamic units; ○, sites where stimulations did not cause responses of hypothalamic units. Dashed lines indicate areas stimulated in the present 'thermophysiological studies'. They represent noradrenaline-containing parts (Ungerstedt, 1971) of the dorsomedial reticular formation (upper fields), and areas within and around the nucleus raphé magnus (lower field).

(abdominal skin, paws) by increasing the firing rate (Fig. 5). They are referred to as 'cold-responsive'. The impulse frequency of two units decreased during stimulation of the nucleus raphé magnus (Fig. 5) but did not change during stimulation of the reticular formation.

(b) Twelve units located in the AH (Fig. 2) did not respond to stimulation of the reticular formation or the nucleus raphé magnus. Only one of these units was thermoresponsive (Table 1).

(c) Five units in the transition area between the AH and the posterior hypothalamus, PH, and in the rostral PH (Fig. 2) were warm-responsive. Their firing rate increased in response to electrical stimulation of the nucleus raphé magnus and decreased to stimulation of the reticular formation (Fig. 6). Three units of cell type c could be studied over a wide range of plate temperatures. Fig. 7 shows the temperature response characteristic of such a unit as an example.

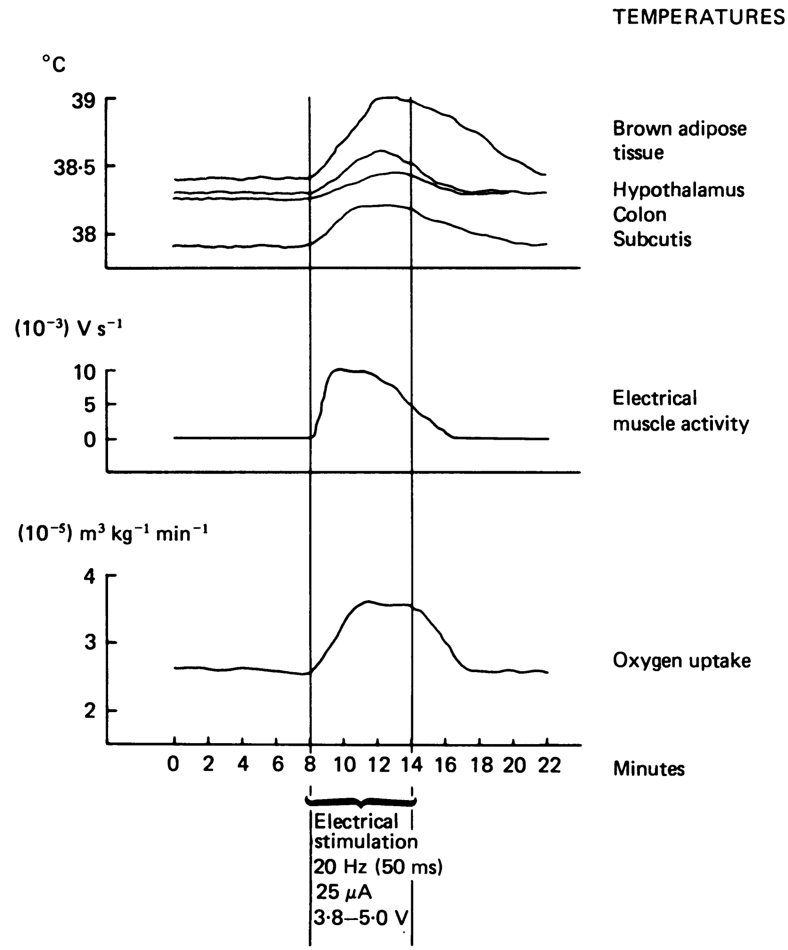

Fig. 4. Thermoregulatory responses to electrical stimulation of the pontine reticular formation in a female guinea-pig weighing 366 g. Ambient temperature: 22 °C. Electrical stimulation parameters: Square-wave pulses of 2 ms duration at a frequency of 20 Hz (corresponding to intervals of 50 ms), 5 sec on/off. Increase in the temperature of the brown adipose tissue in connexion with rising oxygen uptake indicates non-shivering thermogenesis in addition to shivering (increased electrical muscle activity).

TABLE 1. Survey of the single units categorized according to thermal response and electrical stimulations (NRM: nucleus raphé magnus)

Group	Thermal response	Number of single units	Response to stimulation of brain stem: Reticular formation	Response to stimulation of brain stem: NRM	Site
a	Cold responsive	2	→	↓	PO/AH'
		1	→	→	PO/AH
b	Non thermoresponsive	11	→	→	AH
	Warm responsive	1	→	→	AH
c		5	↓	↑	AH, PH
	Questionable	21			

↑ Excited; ↓ inhibited; → no change in firing rate; PO/AH, Preoptic/Anterior hypothalmus; AH, Anterior hypothalamus; PH, Posterior hypothalamus.

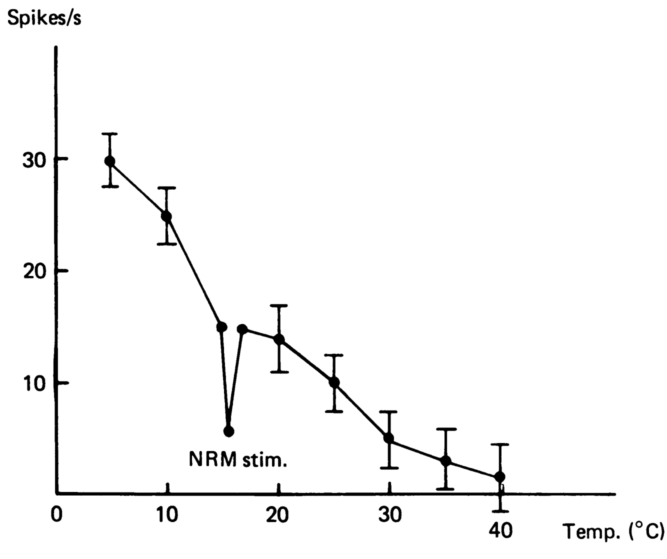

Fig. 5. Relation between unit activity and temperature of the support plate for a cold-responsive neurone of type a with the response to electrical stimulation of the nucleus raphé magnus (NRM) (no effect during electrical stimulation of the reticular formation at plate temperatures of 10, 15, 22, 31 and 40 °C).

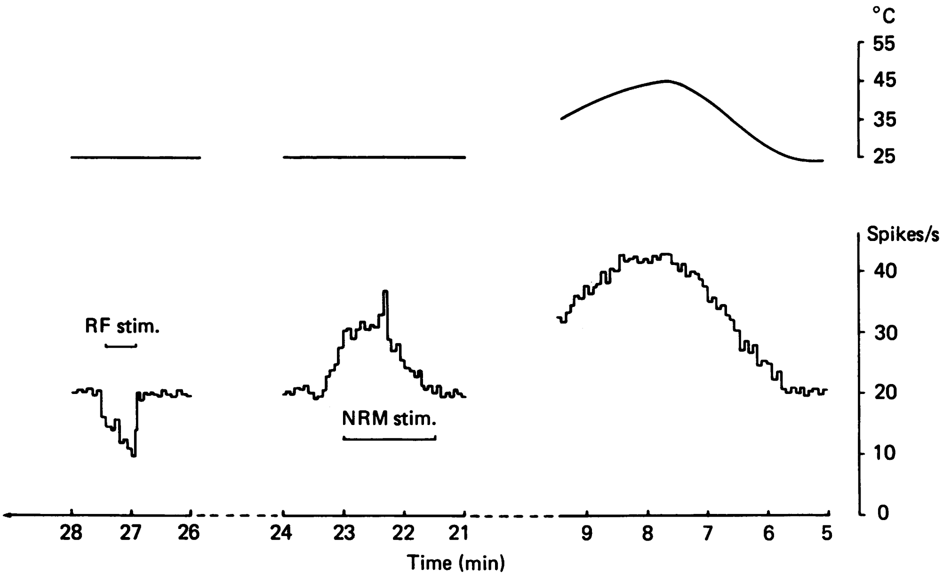

Fig. 6. Response of a neurone (type *c*) in the transition area between the AH and PH to changes in the temperature of the support plate and to electrical stimulations of the nucleus raphé magnus (NRM) and reticular formation (RF).

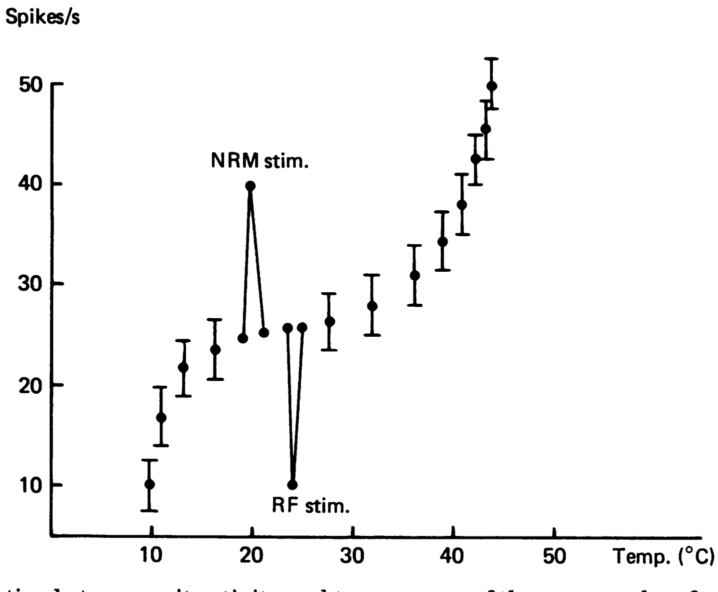

Fig. 7. Relation between unit activity and temperature of the support plate for a warm-responsive neurone of type *c* (Table 1) with the effects of electrical stimulations of the nucleus raphé magnus (NRM) and reticular formation (RF).

DISCUSSION

In the present study it was demonstrated that electrical stimulation of a circumscribed area of the dorsomedial reticular formation, first, evokes a typical thermoregulatory response (Fig. 4) in the unanaesthetized animal and, secondly, inhibits warm-responsive neurones (cell type *c*) located at the border of the anterior (AH) and posterior (PH) hypothalamus and within the PH. It is suggested that both the inhibitory effect on cell type *c* and the thermoregulatory effect are mediated through an ascending catecholaminergic pathway as described in a previous study (Szelényi *et al.* 1976). Evidence for this assumption is derived from the following previously obtained results. An accumulation of noradrenaline-containing cells has been demonstrated in the rat in the area of the reticular formation stimulated in the present study (Ungerstedt, 1971). Noradrenaline has been shown to act as an inhibitory transmitter on warm-responsive hypothalamic neurones (Hori & Nakayama, 1973; Jell, 1974). Microinjection of noradrenaline into an area located close to the sites of cell type *c* has previously been shown to produce thermoregulatory effects which greatly resemble those which followed stimulation of the reticular formation in the present study. In a previous study it was possible to inhibit those thermoregulatory effects by hypothalamic microinjections of phentolamine, an adrenergic blocking agent (Zeisberger & Brück, 1976). Morphological evidence for the proposed ascending catecholaminergic pathway was obtained from retrograde horseradish peroxidase tracing studies (Merker, Zeisberger, Bergheim-Hackmann & Brück, 1977). Thermal stimulation of the area containing cell type *c* did not cause thermoregulatory responses in a previous study in the guinea-pig; such effects could be produced however by thermal stimulation of the more rostrally situated preoptic area (Zeisberger & Brück, 1973). In addition the present study shows that cell type *c* reacts to cutaneous cold stimulation by a decrease in firing rate. It is suggested that this cell type represents interneurones, the action of which determines the threshold for shivering and non-shivering thermogenesis (cf. neuronal model in Brück, 1976).

In addition to the noradrenergic projection, cell type *c* seems, according to the present study, to receive a serotonergic projection from the nucleus raphé magnus which exerts a stimulatory effect on it. These results are in accordance with the function of serotonin as a predominantly activating transmitter on warm-responsive hypothalamic neurones (Hori & Nakayama, 1973; Jell, 1974).

The present study also confirms the thermal pathway ascending from skin thermoreceptors via the nucleus raphé magnus to the PO/AH region (Dickenson, 1978). As was shown by a recent study, the serotonin cells in the nucleus raphé magnus receive projections from cutaneous warm and cold receptors (Dickenson, 1977). The projection from the nucleus raphé magnus to the PO/AH region which was primarily demonstrated by an autoradiographic study in the cat (Bobillier, Seguin, Petitjean, Salvert, Touret & Jouvet, 1976) was electrophysiologically verified by our experiments (cell type *a*).

The existence of the different cell types a and c indicates two hypothalamic integrative networks. Cell type a in the rostral area (PO/AH) seems to be connected with the serotonergic pathway, and cell type c in the caudal area (AH/PH) with both the serotonergic and catecholaminergic pathways. This latter convergence is confined to cell type *c* and was not seen in the preoptic and rostral hypothalamus (cell types *a* and *b*). Cell type *b* does not seem to be directly connected with the thermoregulatory system.

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