主論文

RESPONSE OF GLUCOSE RESPONSIVE VENTROMEDIAL HYPOTHALAMIC NEURONS TO SCROTAL AND PREOPTIC THERMAL STIMULATION IN RATS

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Almost all ventromedial hypothalamic (VMH) neurons facilitated by electro-osmotic application of glucose responded to scrotal thermal stimulation. On the other hand, only 50 % neurons which did not respond to glucose responded to the thermal stimulation. The VMH neurons facilitated or inhibited by scrotal warming were mostly facilitated or inhibited by preoptic warming, respectively. These suggest that thermal signals from scrotal skin and preoptic area were conveyed to VMH neurons, especially to glucose responsive ones, and could influence feeding control.

The warm-blooded organism eats more in a cold environment than it does in a warm one [9]. The ambient temperature thus not only produce thermoregulatory responses but also influence the amount of food intake. This suggests that peripheral thermal signals may act on feeding control. We investigated whether the peripheral and central thermal stimulation could act on feeding related neurons.

In the lateral hypothalamus (LH) and the ventromedial hypothalamus (VMH), are found glucose responsive neurons [8]. The LH glucose responsive neurons are inhibited and the VMH ones are facilitated by electro-osmotic application of glucose. These neurons are considered to be involved in feeding control. Our previous papers [6,10] showed that the glucose responsive neurons in LH and VMH responded also to thermal stimulation of preoptic area (PO). The PO thermosensitive neurons are known to respond to changes in both brain and peripheral temperature. Further, we reported that almost all LH glucose responsive neurons respond to

peripheral thermal stimulation [2]. The scrotal thermal stimulation was often used as a peripheral stimulation because it can produce thermoregulatory responses [3] and change unit discharges of PO thermosensitive neurons [5] in rats.

In this study, the effects of scrotal thermal stimulation on VMH neurons were observed in rats. The neuronal responses to the peripheral thermal stimulation were compared with those to the central thermal stimulation in the same VMH neurons.

Male Wistar rats weighing 300-420 g were anesthetized with 0.8 g/kg urethane and 60 mg/kg α-chloralose injected intraperitoneally. The methods of PO and scrotal thermal stimulations, unit recording and chemical applications to neurons were previously described [2]. Main experimental procedures were as follows. Single unit activity was recorded from VMH neurons using a multibarreled electrode. The recording barrel which contained 2 % Pontamine sky blue in 0.5 M sodium acetate was glued to a 3-barreled glass pipette. Each pipette was filled with 2 M monosodium-L-glutamate (pH 8.0), 0.5 M glucose in 0.9 % NaCl, or 0.9 % NaCl. The chemicals were applied to the neuron by means of iontophoresis and electroosmosis. Neurons responding to glutamate were subjected to further studies to see the responses to glucose and Na. The data of direct current effects were excluded by the criteria of Curtis et al. [1].

Following chemical stimulations and recording to determine chemosensitivity of neurons, scrotal temperature was changed between 25 and 42°C and PO temperature between 34 and 40°C. The neuronal responses to the thermal stimulation were observed. Following each experiment the rat was perfused with saline followed by 10 %

formalin. Frozen sections of the brain were cut at 100 μm to confirm the position of the electrode. The locations of 68 electrodes tips were identified in VMH.

The VMH neurons were classified according to their responses to glucose, Na and scrotal thermal stimulation, as shown in Table I. Fifteen out of 68 neurons were facilitated by glucose but had no response to Na. Fifty-two neurons had no responses to glucose. Only one neuron was facilitated by both glucose and Na. The facilitatory effect might be attributed to the action of Na of the solvent, so that we did not include the neuron facilitated by both glucose and Na into glucose responsive neurons. Out of 15 neurons facilitated by glucose, 14 (93 %) neurons responded to scrotal thermal stimulation. We regarded a neuron as thermally responsive when increase or decrease of neuronal discharges occurred with visual obviousness in response to gradual warming or cooling repeatedly. Of these neurons, 9 were facilitated and 5 were inhibited by scrotal warming. Twenty-six (50 %) out of 52 neurons which had no response to glucose were influenced by scrotal thermal stimulation. Of these neurons, 18 were inhibited and 8 were facilitated by scrotal warming.

The sample responses of neurons facilitated by glucose are shown in Fig. 1. Fig. 1 A shows the neuron whose firing rate was increased by scrotal warming and Fig. 1 B shows another neuron whose firing rate was decreased by scrotal warming but increased by scrotal cooling.

We observed the responses to PO thermal stimulation in 45 out of 68 VMH neurons. The results are summarized in Table II. The

neurons facilitated by scrotal warming were mostly facilitated by PO warming, and all neurons inhibited by scrotal warming were inhibited by PO warming. The typical examples of these responses are shown in Fig. 2. Almost all neurons which showed no response to scrotal thermal stimulation also did not respond to PO thermal stimulation.

The most important result in this study was that 14 out of 15 neurons facilitated by glucose also responded to scrotal thermal stimulation. On the other hand, in the VMH neurons which did not respond to glucose, only 50 % neurons responded to the thermal stimulation. These results indicate that the VMH neurons which respond to glucose also more certainly receive thermal signals from scrotal skin than those which do not respond to glucose. This is quite similar to LH neurons we reported earlier [2]. All but one LH neurons inhibited by glucose responded to scrotal thermal stimulation.

In the morphological study, the difference between glucose responsive neurons and glucose irresponsive neurons in VMH was clearly noticeable [7]. Using intracellular horseradish peroxidase staining of VMH tissue slices, it was found that glucose responsive neurons have multipolar dendrites predominantly, while in glucose irresponsive neurons, half of them have bipolar dendrites and half have multipolar dendrites. These morphological aspects may exlpain our results that glucose responsive neurons responded to scrotal thermal stimulation more than glucose irresponsive neurons.

The statistical significance of the difference between distribution of responses to scrotal thermal stimulation in glucose responsive and irresponsive neurons was assessed by χ^2-test. The

proportion of neurons responded to scrotal thermal stimulation in glucose responsive neurons (93 %) was significantly ($p<0.005$) higher than that in glucose irresponsive neurons (50 %). Furthermore, among thermally responsive neurons, the proportion of neurons facilitated by scrotal warming in glucose responsive neurons (9 out of 14 neurons) was significantly higher ($p<0.05$) than that in glucose irresponsive neurons (8 out of 26 neurons). In the case of LH neurons which we earlier reported [2], almost all LH glucose responsive neurons (inhibited by glucose) also responded to scrotal thermal stimulation, while in those neurons, the neurons inhibited by scrotal warming were found more frequently in contrast with VMH neurons. There is a strong connection between VMH and LH and the reciprocity of the function of VMH and LH is well known [4]. In this and our earlier studies, it appears that the responses to scrotal thermal stimulation in VMH and LH glucose responsive neurons show the reciprocity.

In most VMH neurons, the direction of responses to PO thermal stimulation was the same as that to scrotal thermal stimulation. In most of PO neurons, warm sensitive neurons were facilitated, cold sensitive neurons were inhibited by scrotal warming and thermally insensitive neurons were not affected by scrotal thermal stimulation [5]. It is clear from our result that many VMH neurons receive both central and peripheral thermal signals. It is possible that the peripheral thermal signals from scrotal skin are transmitted to VMH neurons via PO thermosensitive neurons.

We conclude that the thermal signals from scrotal skin and PO were conveyed to VMH neurons, especially to the glucose responsive

ones, and thus could influence the control of feeding.

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Fig. 1. Responses of two VMH neurons to iontophoretically applied glutamate (Glt), glucose (Glu), sodium (Na), and to the scrotal temperature (Tscr) at constant preoptic temperature (Tpo). The numbers under the chemicals denote the order of flowing currents in nA.

Fig. 2. Responses of two VMH neurons to Tscr and Tpo.

TABLE I

NUMBERS OF VMH NEURONS CLASSIFIED BY THEIR RESPONSES TO GLUCOSE, Na AND TO Tscr

W, facilitated by warming; C, inhibited by warming.

TABLE II

NUMBERS OF VMH NEURONS CLASSIFIED BY THEIR RESPONSES TO Tscr AND Tpo

W, facilitated by warming; C, inhibited by warming; N, not responsive to thermal stimulation.

Fig. 1

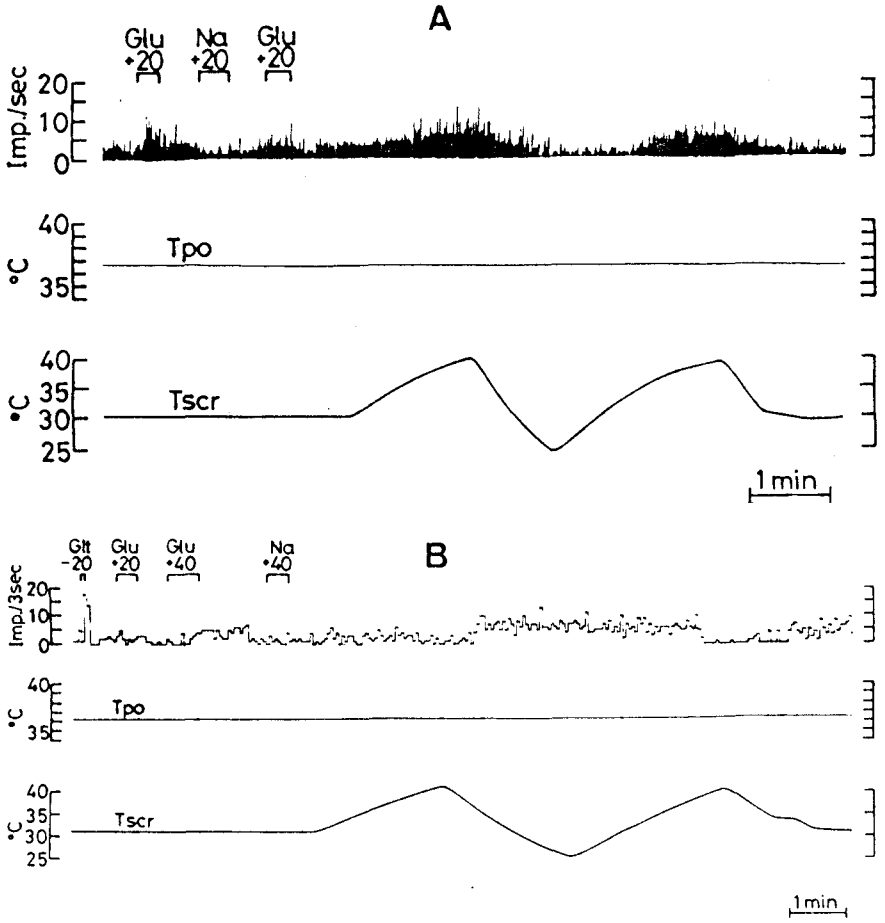
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Fig. 2

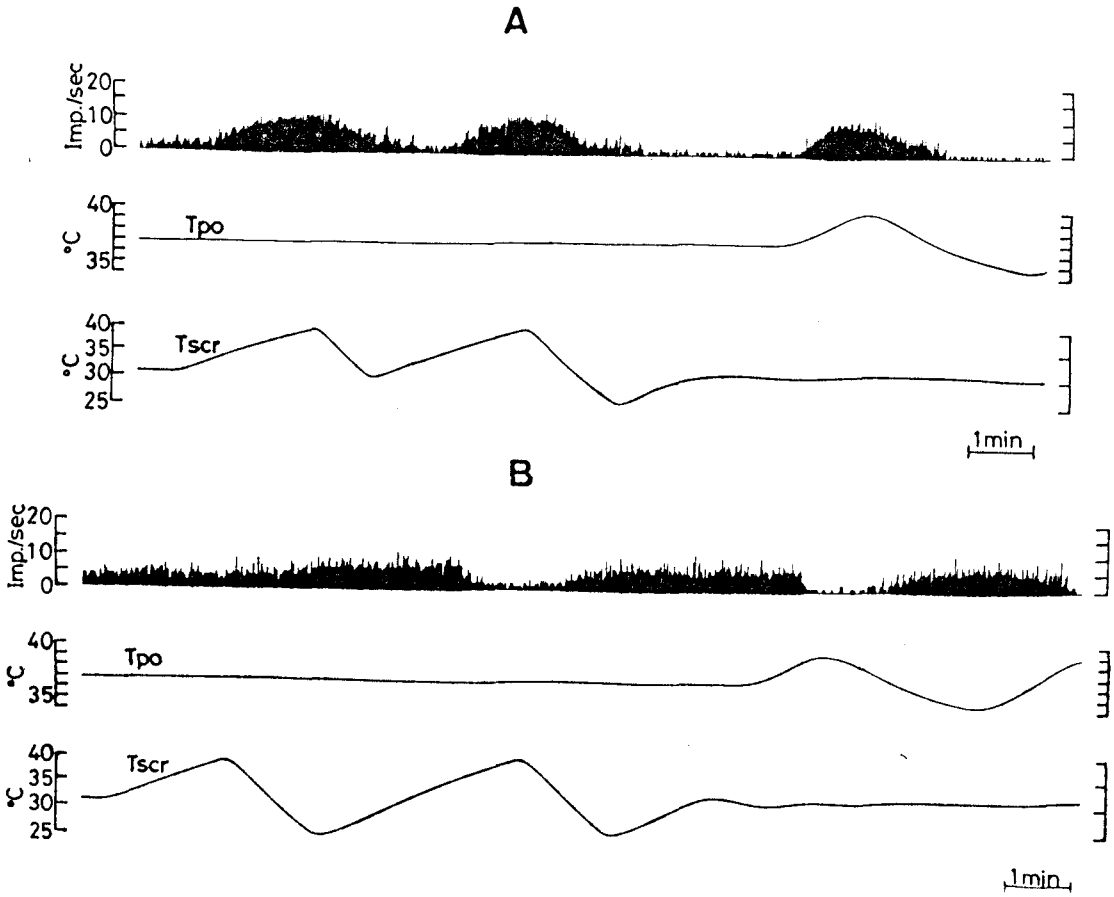
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TABLE I

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Response to Glucose & Na	Response to Tscr			
	Thermal		Non Thermal	Total
	W	C		
Facilitated by Glucose	9 (60 %)	5 (33 %)	1 (7 %)	15 (100 %)
Facilitated by Glucose & Na	1	0	0	1
No response	8 (15 %)	18 (35 %)	26 (50 %)	52 (100 %)
Total	18 (26 %)	23 (34 %)	27 (40 %)	68 (100 %)

TABLE II

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Response to Tscr	Response to Tpo			
	W	C	N	Total
W	10	0	3	13
C	0	16	0	16
N	2	2	12	16
Total	12	18	15	45

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RESPONSE OF LATERAL HYPOTHALAMIC NEURONS TO SCROTAL AND PREOPTIC THERMAL STIMULATION IN RATS

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Lateral hypothalamic neurons inhibited by electro-osmotic application of glucose responded to scrotal thermal stimulation in rats. These neurons received the temperature signals arising from the scrotum. The lateral hypothalamic neurons facilitated or inhibited by scrotal warming were mostly facilitated or inhibited by preoptic warming, respectively. Most of them received not only the scrotal thermal inputs but also thermal inputs from the preoptic area.

The amount of food intake which an organism consumes bears a clear relationship to the ambient temperature [11]. This finding suggests that peripheral temperature signals influence food intake. Further, it was reported that rats with anterior hypothalamic lesions [4] or capsaicin-desensitized rats that lose their thermoregulatory responses against heat [1] failed to reduce their food intake at high ambient temperature and increased it at low ambient temperature. In the preoptic area and anterior hypothalamus (POAH) are located the thermosensitive neurons which increase or decrease their firing rate to changes in local brain temperature [9]. Accordingly, we expected that POAH thermosensitive neurons might be involved not only in thermoregulation, but also in the regulation of food intake.

It is generally recognized that the neurons inhibited by glucose injected electro-osmotically by means of a micropipette into the lateral hypothalamus (LH), and those facilitated by glucose into the ventromedial hypothalamus (VMH) [10], take part in feeding control. In our previous papers [8, 13], we reported that glucose responsive neurons in LH and VMH responded to preoptic (PO) thermal stimulation.

In this study, the effect of scrotal thermal stimulation on LH neurons was observed in rats, to investigate whether LH glucose-responsive neurons receive not only central thermal signals but also peripheral thermal signals. Neuronal responses to peripheral thermal stimulation were compared with those to central thermal stimulation on the same LH neurons.

Thirty male Wistar rats weighing 310–420 g were used under anesthesia of 0.8

g/kg urethane and 60 mg/kg α-chloralose injected intraperitoneally. The methods of PO thermal stimulation, unit recording and chemical applications to LH neurons were the same as those described by Nakayama et al. [8]. Only additional procedures will be described in this paper.

Effects of scrotal thermal stimulation were observed at a constant preoptic temperature between 36 and 38°C; the scrotal temperature was changed between 25 and 42°C. The method of scrotal thermal stimulation was the same as that described by Nakayama et al. [7].

Usual experimental procedures were as follows. A neuron was tested to glutamate, glucose and Na. Subsequently, responses to scrotal and PO thermal stimulation were observed. The data of direct current effects were excluded by the criteria of Curtis and Koizumi [2]. In order to verify electrode positions, the Pontamine sky blue marking technique was used. Following each experiment, the rat was perfused with saline followed by 10% formalin. Frozen sections of the brain were cut at 50 μm to confirm placement of the electrode.

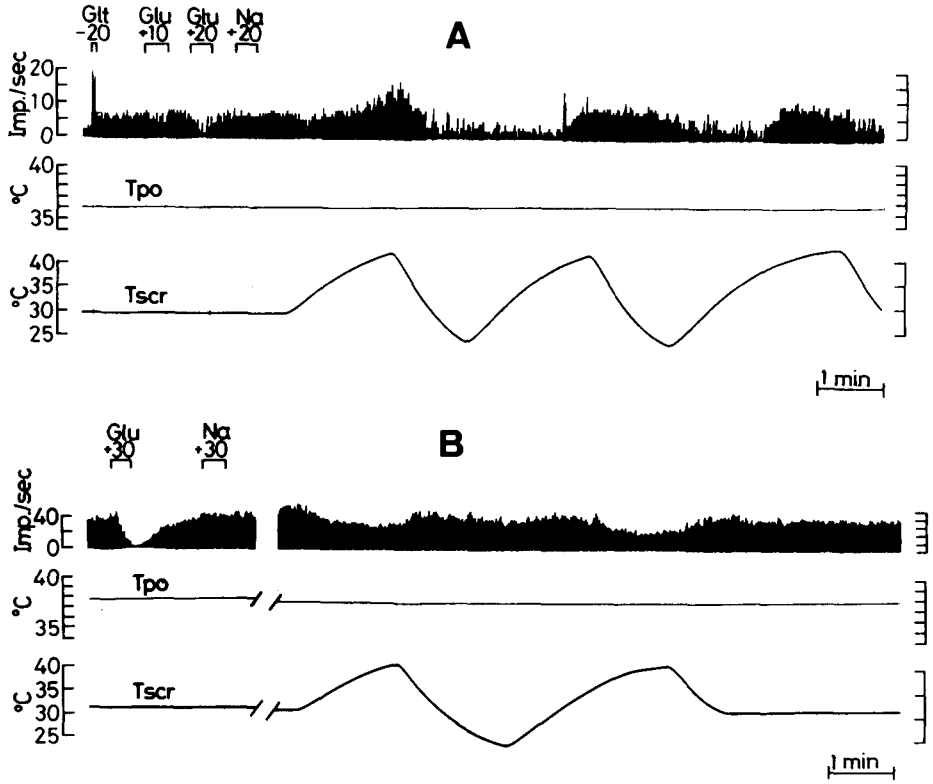

Fig. 1. Responses of two LH neurons to iontophoretically applied glutamate (Glt), glucose (Glu), Na, and to the scrotal temperature (Tscr) at constant preoptic temperature (Tpo). The numbers under the chemicals denote the order of flowing currents in nA.

TABLE I

NUMBERS OF LH NEURONS CLASSIFIED BY THEIR RESPONSES TO GLUCOSE, Na AND TO Tscr

W, facilitated by warming; C, inhibited by warming.

Response to glucose and Na	Response to Tscr			
	Thermal		Non-thermal	Total
	W	C		
Inhibited by glucose	8 (38%)	12 (57%)	1 (5%)	21 (100%)
Inhibited by Na	1	1	1	3
Facilitated by glucose	0	1	0	1
Facilitated by Na	0	0	1	1
No response	15 (33%)	8 (17%)	23 (50%)	46 (100%)
Totals	24 (33%)	22 (31%)	26 (36%)	72 (100%)

The present findings are based on observations of 72 LH neurons. The results are summarized in Table I. The neurons were classified according to their responses to glucose, Na and scrotal thermal stimulation. Twenty-one out of 72 neurons were inhibited by glucose and 46 neurons had no response to glucose. In the 5 other neurons, 3 were inhibited by both glucose and Na, one was facilitated by glucose and one was facilitated by both glucose and Na. The responses of neurons inhibited or facilitated by both glucose and Na were attributed to the action of Na of the sol-

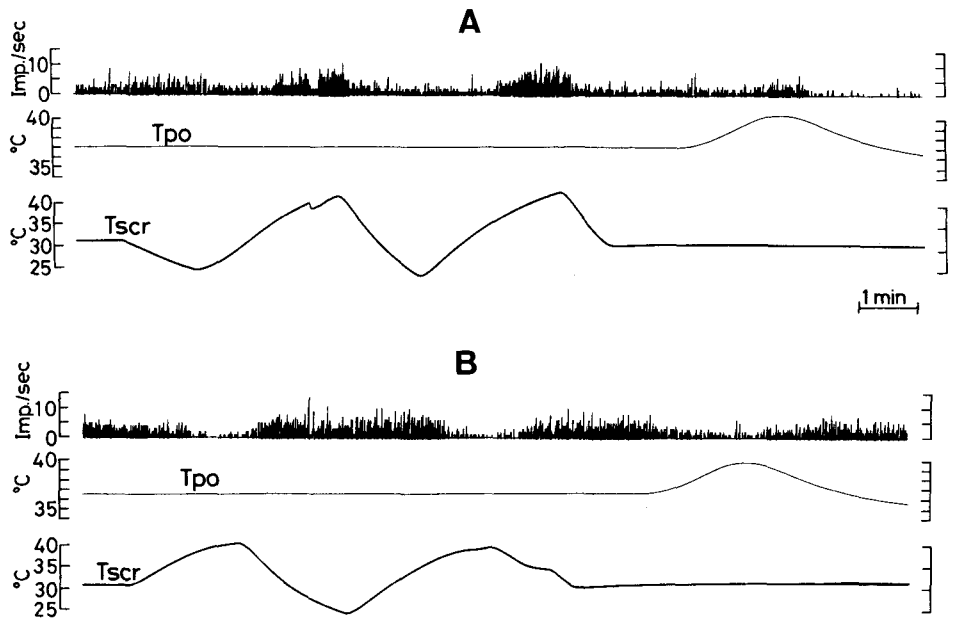

Fig. 2. Responses of two LH neurons to Tscr and Tpo.

TABLE II

NUMBERS OF LH NEURONS CLASSIFIED BY THEIR RESPONSES TO Tscr AND Tpo

W, facilitated by warming; C, inhibited by warming; N, not responsive to thermal stimulation.

Response to Tscr	Response to Tpo			
	W	C	N	Total
W	11	2	3	16
C	3	9	5	17
N	2	0	14	16
Totals	16	11	22	49

vent. Out of 72 neurons, 24 were facilitated by scrotal warming, 22 were inhibited by scrotal warming, and 26 were not changed by scrotal thermal stimulation. Sixty-four percent of LH neurons responded to scrotal thermal stimulation. Out of 21 neurons inhibited by glucose, 20 (95%) responded to scrotal thermal stimulation. Of these neurons, 8 were facilitated and 12 were inhibited by scrotal warming. Twenty-three out of the 46 neurons which had no response to glucose were not influenced by scrotal thermal stimulation; 15 were facilitated and 8 were inhibited by scrotal warming.

Examples of neuronal responses inhibited by glucose are shown in Fig. 1. These neurons did not respond to Na. Fig. 1A shows the neuron whose firing rate was increased by scrotal warming and Fig. 1B shows another neuron whose firing rate was decreased by scrotal warming.

The changes in firing rate of all neurons which responded to scrotal temperature change occurred within the range of 32–41°C. The scrotal temperature change needed to produce full change in the neuronal activity varied from less than 1 to 6°C, and was usually less than 4°C.

We observed responses to PO thermal stimulation in 49 neurons out of 72 in LH. The results are summarized in Table II and typical examples of these responses are shown in Fig. 2.

In many LH neurons, the direction of responses to PO thermal stimulation was the same as that to scrotal thermal stimulation. The neurons facilitated by scrotal warming were mostly facilitated by PO warming (Fig. 2A), and the neurons inhibited by scrotal warming were mostly inhibited by PO warming (Fig. 2B). Almost all neurons not responsive to scrotal thermal stimulation did not respond to PO thermal stimulation.

The scrotum of the rat makes a good preparation to investigate the effects of peripheral warming and cooling on unit discharges in the central nervous system [7] as well as thermoregulatory responses. With regard to unit studies, scrotal thermal stimulation in rats influenced the electrical activities of the preoptic and hypothalamic thermosensitive neurons [7]. Scrotal thermal stimulation induces thermoregulatory responses not only in rats [6] but also in various other spieces [3, 5,

12]. In this paper, we studied whether LH neurons were influenced by peripheral thermal stimulation as well as PO thermal stimulation.

The present study revealed that 64% of LH neurons responded to scrotal thermal stimulation. It is worth mentioning that 20 out of 21 neurons inhibited by glucose responded to scrotal thermal stimulation. This indicates that almost all LH neurons inhibited by glucose receive the thermal inputs from the scrotal thermoreceptors. All homeotherms that have been studied eat more in a cold environment than in a hot environment [11]. It is likely that the LH neurons inhibited by glucose are involved in an activation of feeding behavior. Our results in unit studies are compatible with the fact that food intake is influenced by the ambient temperature. In neurons inhibited by glucose, the neurons inhibited by scrotal warming were observed more frequently than those facilitated by warming. However, it is as yet unknown how LH glucose-responsive neurons facilitated by scrotal warming take part in feeding control in a cold environment.

The scrotal temperature that produces changes in the firing rate in LH neurons is similar to that in PO thermosensitive neurons reported by Nakayama et al. [7]. However, it is an open question whether the thermal afferents from the scrotum project directly or via PO thermosensitive neurons to LH. For the present, it is reasonable to say that LH neurons receive thermal input from both PO and scrotum.

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Dynamic Response of Preoptic and Hypothalamic Neurons to Scrotal Thermal Stimulation in Rats

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Abstract. Activities of preoptic and anterior hypothalamic (POAH) neurons were recorded in anesthetized rats in response to scrotal thermal stimulation. Thirteen out of 54 neurons responsive to changes of scrotal temperature (T_{scr}) showed dynamic responses. Four of these neurons increased and 3 neurons decreased their firing rates responding dynamically to warming but not to cooling. Six other neurons were inhibited by scrotal cooling only. These dynamic responses were produced even by temperature changes as slow as 2° C/min and only when the scrotum was warmed or cooled in the T_{scr} range above 35° C. These dynamic responses are suggested to be a result of signal processing in supraspinal structures including POAH itself.

Key words: Preoptic area – Hypothalamus – Temperature-sensitive neuron – Scrotal temperature – Dynamic response

Introduction

Thermal afferent pathways from the scrotum to the central nervous system were extensively investigated in rats. The primary thermoafferent fibers from the scrotal skin show dynamic/static responses with a unilateral receptive field of 1 mm diameter (Hellon et al. 1975). The static activity/temperature curve is bell-shaped with a peak at 42° C for warm receptors and at 23–28° C for cold receptors. Dorsal horn neurons are reported to show a variety of different responses to scrotal thermal stimuli, dynamic/static as well as purely dynamic or static (Hellon and Misra 1973a; Neya and Pierau 1980).

Neurons responsive to scrotal thermal stimulation were also found in the midbrain raphe nuclei (Jahns 1976), thalamus (Hellon and Misra 1973b; Jahns 1975; Schingnitz and Werner 1980), somatosensory cortex (Hellon et al. 1973) and preoptic area and anterior hypothalamus (POAH) (Nakayama et al. 1979). A major difference between the activities of dorsal horn neurons and those of supraspinal structures is that dynamic responses were often observed in the former but were rare in the latter. So far, dynamic responses of POAH neurons to thermal stimulation of any part of the body have not been reported. However this report will demonstrate that some POAH neurons show dynamic responses to T_{scr} changes. We will use the word "dynamic", but the dynamic responses of POAH neurons are quite different from those observed in the primary afferent fibers and the dorsal horn neurons, mainly in the point that the POAH neurons are dynamically sensitive even to such slow rates of temperature change that produce no dynamic responses in the primary fibers or dorsal horn neurons.

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Methods

Male Wistar rats weighing between 300 and 400 g were anesthetized with urethane given i.p. in a dose of 1–1.5 g per kg. The rats were mounted stereotaxically with the heads fixed according to the Pellegrino and Cushman coordinate system (1967). Two stainless steel guide tubes (O.D. 0.9 mm) were implanted into the right half of the brain at locations 2.0 mm and 5.0 mm lateral (L) to the midline, 7.0 mm anterior (A) and were inserted to a depth (D) of 3.0 mm from the stereotaxic zero point. The medial tube was used as water perfused thermode for conductive warming or cooling of the brain tissue and the lateral tube contained a thermocouple to measure the brain temperature.

For bilateral thermal stimulation, the scrotal skin was clipped, depilated and placed lightly against a rectangular thermode which was covered by a thin film of liquid paraffin to make good contact. Two perfusion thermodes, each with a working area of 30 × 15 mm, were mounted rigidly in a synthetic resin frame and separated from each other by a 2 mm air gap. Each thermode had its own water supply. The water temperature was changed between 25 and 45° C by manual setting of the heating current. T_{scr} was measured by a thermocouple cemented to the surface of each thermode which was in contact with the skin.

Recording of neuronal discharges was made from the left half of the POAH at stereotaxic coordinates of A: 6.8 to 8.0 mm, L: 0.7 to 1.2 mm, D: 1.0 to −3.0 mm. A piece of bone was removed from the left half of the skull and the underlying dura was removed to insert a recording electrode. The skull opening was filled with warm Ringer-agar. Glass microelectrodes were filled with Pontamine sky blue dissolved in 0.5 M Na acetate. The dye was deposited by iontophoresis to mark the tip position. Extracellular neuronal discharges were amplified conventionally, counted by a rate meter and displayed on an ink chart recorder together with T_{hy} and T_{scr}.

To test the mechanical responses of each unit, the skin areas of the scrotum, tail, lower extremities and trunk were stimulated by a small brush. Following each experiment the rat was perfused with saline followed by 10% formalin. Frozen sections of the brain were cut at 100 μm to confirm the position of the electrode.

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Table 1. Numbers of preoptic and anterior hypothalamic (POAH) neurons showing dynamic responses to scrotal warming or cooling and their static responses to scrotal (T_{scr}) and hypothalamic temperature (T_{hy})

Dynamic response to thermal stimulation of the scrotum	Static response to thermal stimulation of the scrotum			Response to thermal stimulation of the POAH				Total
	Warm	Cold	Insensitive	Warm	Cold	Insensitive	?	
Warm-facilitation	0	0	4	1	0	2	1	4
Warm-inhibition	1	0	2	1	0	2	0	3
Cold-inhibition	4	1	1	3	0	2	1	6
Total	5	1	7	5	0	6	2	13

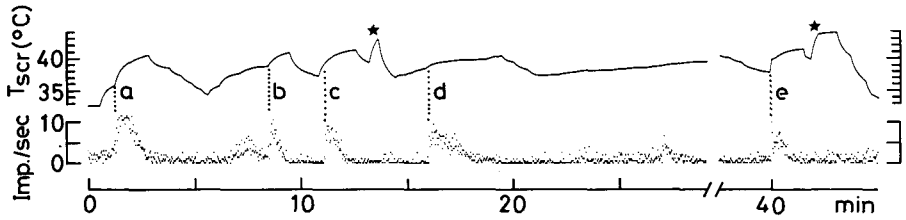

Fig. 1a—e
Activity of a POAH neuron (rate meter readings) which dynamically increased its firing rate in response to scrotal warming. Dotted lines (**a—e**) indicate dynamic responses. Stars: scrotal warming which produced no dynamic responses

Results

We obtained 54 POAH neurons responsive to scrotal thermal stimulation. Thirteen of these neurons showed clearly dynamic responses.

General Features. The dynamic responses were divided into three types (Table 1); "warm-facilitation", "warm-inhibition" and "cold-inhibition". In neurons showing the dynamic responses of "warm-facilitation" and "warm-inhibition" the activity was transiently increased and decreased, respectively, by warming the scrotum. Neurons of these types demonstrated no dynamic responses during scrotal cooling. The dynamic "cold-inhibition" was a transient decrease of the firing rate to scrotal cooling only. More than half of the dynamically responsive neurons (7/13) exhibited no static response to T_{scr} changes. The dynamic responses were independent of the rate of T_{scr} change and observed during T_{scr} changes as slow as 2° C/min. All the dynamic responses were observed only when the T_{scr} change, either warming or cooling, was made above 35° C. Sensitivity to T_{hy} was determined for 11 dynamically responsive neurons: Five were warm-sensitive and the other 6 neurons were insensitive to T_{hy}. Similar to the results in the previous paper of Nakayama et al. (1979), all temperature responsive POAH neurons including those with only dynamic response were insensitive to mechanical stimulation of the peripheral skin. Both dynamic and non-dynamic neurons were scattered widely in the POAH.

Warm-Facilitation. The neuron shown in Fig. 1 responded to T_{scr} rise with transient increase in its firing rate ("a" to "e" in Fig. 1). The threshold T_{scr} above which temperature changes became effective was 36° C, thus, the T_{scr} rise during the first 1 min in Fig. 1, did not produce any dynamic response. On the other hand, T_{scr} changes above 39° C (marked with stars in Fig. 1) also had no effect. The "dynamic" responses, seen in Fig. 1 in the limited range of T_{scr} between 36 and 39° C seem not to be produced by a bell-shaped characteristic of the thermoresponsive input to the neuron, since the firing rate never increased as the T_{scr} was falling and since the response was weakened during the very slow rise in T_{scr} (0.3° C/min, between 21 and 28 min in Fig. 1). The neuron responded dynamically to the T_{scr} change of 3° C/min (d) to about the same degree as to faster T_{scr} changes (a, b, c and e).

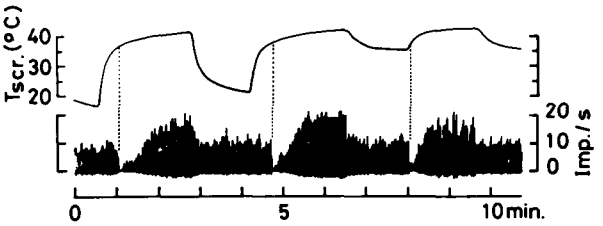

Fig. 2. Activity of a POAH neuron (rate meter readings) which dynamically decreased its firing rate in response to scrotal warming. Dotted lines indicate dynamic responses

Warm-Inhibition. Figure 2 shows an example of the activity of a POAH neuron showing a dynamic "warm-inhibition". The firing rate of about 10 imp/s was suddenly reduced almost to zero when the T_{scr} exceeded 37° C. Then, the activity gradually increased to a new level of 20 imp/s in about 30 s and retained that level as long as T_{scr} was high. Transient inhibition during warming would be the typical response of cold-receptors in the skin. This neuron, however, is statically warm-responsive to T_{scr}.

Cold-Inhibition. An example of POAH neurons having the characteristics of dynamic "cold-inhibition" is shown in Fig. 3. This neuron was statically excited by scrotal warming, which can be seen in the records between 10 and 15 min. The firing rate increased from 10 to 50 imp/s in an operating range between 35 and 38° C. Step decreases of T_{scr}, when induced in the temperature range above 36° C, transiently inhibited unit activity ("a", "b" and "c" in Fig. 3). This inhibition was followed by gradual recovery of the activity to the previous

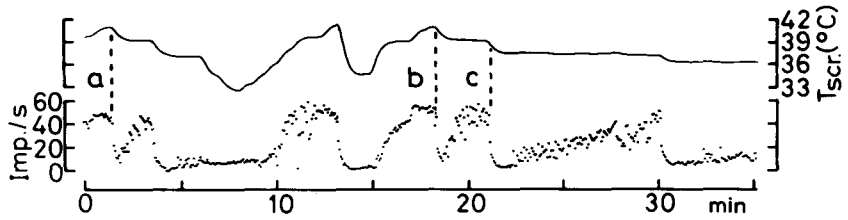

Fig. 3a—c
Activity of a POAH neuron (rate meter readings) which was statically warm responsive to T_{scr} and dynamically decreased its firing rate in response to scrotal cooling. Dashed lines (**a—c**) indicate dynamic responses

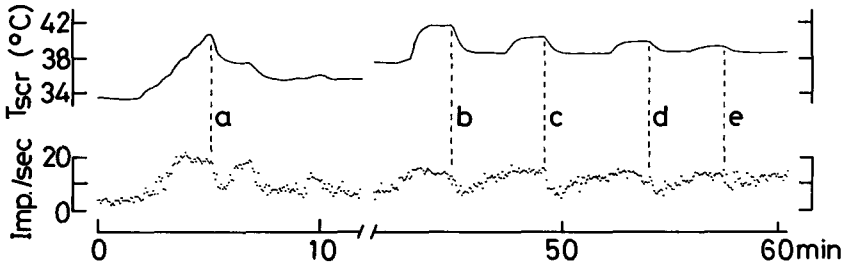

Fig. 4a—e
Activity of a POAH neuron tested by means of step changes of T_{scr}. This neuron was statically warm responsive to T_{scr} and dynamically decreased its firing rate in response to scrotal cooling. Dashed lines (**a—e**) indicate dynamic responses

level. It is worth mentioning that the duration of this recovery was prolonged at lower T_{scr} levels. This can be seen by a comparison of the responses "b" and "c" in Fig. 3. When T_{scr} was decreased from 41 to 39° C (b), the decrease in firing rate was followed by a quick recovery within about 1 min. On the other hand, it took more than 15 min to recover after T_{scr} was decreased from 39 to 37° C (c).

Figure 4 gives dynamic responses of another neuron showing "cold-inhibition". This neuron was statically excited by scrotal warming and its firing rate increased from 5 to 15 imp/s in an operating range between 34 and 38° C. This is shown in the first 10 min of the recording in which warming from 34 to 42° C increased firing rate which levelled off above 38° C. Cooling from 42 to 38° C caused a dynamic inhibition. Between 45 and 60 min, T_{scr} was decreased by 3 (b), 2 (c), 1 (d) and 0.5° C (e) to a constant temperature of 39° C, with a rate of 6, 4, 2 and 1° C/min, respectively. The dynamic responses to the first three stimuli (b, c and d) were approximately the same. The firing rate was reduced from 15 to 7 imp/s and then gradually increased again to the initial high level within 1.5 min. A dynamic response with a similar time course was observed in the case of the slowest temperature fall (e). However, the magnitude of the firing rate reduction was considerably smaller. Rewarming of the scrotum after the restoration of activity produced no effect.

Discussion

The results presented here show dynamic responses of POAH neurons to T_{scr} changes, which can be divided into three types: i) warm-facilitation, ii) warm-inhibition and iii) cold-inhibition.

These responses were produced only when the scrotum was warmed or cooled in the temperature range above 35 °C. Most scrotal cold receptors are not active above 35° C (Iggo 1969; Hellon et al. 1975). Thus, only the input from scrotal warm receptors might be involved in the generation of POAH dynamic responses. The dynamic responses of POAH neurons differ from those of skin receptors. Facilitation during a rise and inhibition during a fall of temperature are typical dynamic responses of warm receptors of the scrotal skin. In the POAH, "warm-facilitation" (= facilitation during T_{scr} rise) and "cold-inhibition" (= inhibition during T_{scr} fall) never appeared on one and the same neurons. Similarly, in POAH neurons an inhibition during T_{scr} rise ("warm-inhibition") was never accompanied by an excitation during a fall of T_{scr} (so-called "cold-facilitation") as observed in skin cold-receptors. Additionally, the adaptation times of the POAH dynamic responses, which are 30 s or longer, are considerably greater than those of the skin warm receptors, which are in the order of 8 s (Hellon et al. 1975).

Information from the receptors seems to be processed to a great extent in the spinal cord (Hellon and Mitchell 1975). However, the dorsal horn neurons did not show a dynamic response to temperature changes of 4° C/min or slower while rates of 2° C/min elicited dynamic responses in POAH neurons. In particular, inhibitory response such as "warm-" and "cold-inhibition" in POAH neurons are not reported for the dorsal horn neurons. These differences suggest that the dynamic response of POAH neurons is a result of further signal processing somewhere in the supraspinal structures including POAH itself.

POAH neurons responsive to temperature changes of the peripheral skin have previously been described (Wit and Wang 1968; Hellon 1970, 1972; Boulant and Bignall 1973; Boulant and Hardy 1974). Dynamic responses have not been reported in these studies in which large skin areas were stimulated and the rate of the temperature change was slow. In fact, a survey of the published data did not yield dynamic responses comparable to our results. This might result from the fact that convergence of signals from skin thermoreceptors of various parts of the body occurred in these experiments. Thus, a possible dynamic component might have been smoothened temporally and/or spatially during simultaneous stimulation of wide areas of the trunk. On the other hand, POAH neurons showed no dynamic responses to heating or cooling restricted to the tail in rats (Knox et al. 1973). Therefore, we conclude from our results that the dynamic response of POAH neurons may be specific for the warm-receptor input from the scrotum.

When speculating about the physiological function of the POAH neurons showing dynamic responses, a first explanation might be that they could be used for detecting the

direction of ambient temperature changes. Another possibility is that the dynamic, thermoresponsive neurons belong to the effector mechanism. In fact, under similar experimental conditions as in the present study, the respiratory rate of anaesthetized rats increased with an initial overshoot, with a time course similar to that of the response of the dynamic warm-facilitatory POAH neuron, when T_{scr} rose and exceeded a threshold of about 37° C. Further, with slight step decreases in T_{scr} above the threshold, the respiratory rate was observed to decrease to the normal level and then to return gradually to an elevated level again (unpublished observations). These responses resemble the responses of the "cold-inhibitory" POAH neurons. Also reported was a transient reaction of arterial blood pressure to step changes in T_{scr} (Neya and Pierau 1976) and initial transient skin vasodilations in unshorn rams when the scrotum was heated (Hales and Hutchinson 1971).

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EFFECTS OF PREOPTIC THERMAL STIMULATION ON THE VENTROMEDIAL HYPOTHALAMIC NEURONS IN RATS

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The effects of temperature on food intake were observed on activities of 54 ventromedial hypothalamic neurons in rats in response to iontophoretically applied glucose and to preoptic thermal stimulation. The major response was that 16 neurons out of 22 facilitated by glucose in the satiety center were facilitated by a rise in preoptic temperature.

Since the proposal of the thermostatic theory [3], evidence has been accumulated which has shown the effect of temperature on food intake in various animal species [2, 3, 9]. In goat, for example, local warming of preoptic and anterior hypothalamic area (POAH) or exposure to a hot environment resulted in a decrease of food intake, while the opposite thermal stimulations produced reversed responses [2].

On the other hand, microelectrode studies have revealed the existence of thermosensitive neurons in POAH [4, 5], neurons inhibited by glucose in the lateral hypothalamus (LH), and neurons facilitated by glucose in the ventromedial nucleus of the hypothalamus (VMH) [7].

Anand et al. [1] recorded single unit activities in the satiety and feeding centers. The discharge frequencies of these units, however, were not influenced by local heating, indicating that the neurons per se are not sensitive to temperature.

Recently, we found that the LH neurons inhibited by glucose were facilitated by local cooling of the preoptic area (PO) [10]. In the present study, the effect of PO thermal stimulation on the VMH neurons in rats was studied.

The experimental arrangement was similar to that described in our previous paper [10]. Male Wistar rats weighing 270–390 g were used with anesthesia of 0.8 g/kg urethane and 60 mg/kg α-chloralose injected intraperitoneally. When necessary, maintenance doses were injected subsequently. Stereotaxic coordinates of the thermode for conductive thermal stimulation were 8.0 mm anterior, 2.0 mm right to the midline and -3.0 mm vertical according to the Pellegrino and Cushman atlas [8]. Those for the thermocouple were 5.0 mm, 2.0 mm and -3.0 mm. In the preliminary test, the temperature at this location was found to be identical with that of the PO (8.0 mm, 1.0 mm to the left and -3.0 mm). By perfusing the thermode with

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water of controlled temperature, the PO temperature was changed between 32 and 43 °C. The VMH temperature changed 0.7 °C when the PO temperature was changed 1.0 °C.

The recording electrode, which contained 2% Pontamine sky blue in 0.5 M sodium acetate, was glued to a 3-barrelled glass pipette. The tip of the recording electrode extended beyond the orifice of the pipette by 10–30 μm. Each pipette was filled with 2 M monosodium-L-glutamate (pH 8.0) and 0.5 or 1 M glucose in 0.9% NaCl and 0.9% NaCl. The chemicals were applied to the neuron iontophoretically or electro-osmotically by flowing currents of the order of nA through the pipettes. The direct current effects were excluded, because the flowing currents used were so small and the responses obtained were not coincident in time course with the current flow. Single unit discharges were recorded from the VMH at stereotaxic coordinates of 5.0–6.4 mm, 0.5–1.1 mm to the left and −2.6 to −4.1 mm in the hypothalamus. The electrode was replaced when glutamate failed to increase discharge frequency. Neurons responding to glutamate were subjected to further studies. Glucose was applied by flowing current of +5 to +50 nA for 15–60 sec. After a recovery period of 20–60 sec, sodium was applied in the same way. Subsequently, responses to PO thermal stimulation were observed.

Iontophoretic current signals, unit discharges and PO temperature were tape-recorded. Simultaneously, the number of impulses per sec was counted on-line and recorded by an ink-writer. Room temperature was 23–27 °C. The rectal temperature was maintained at 37–38.5 °C by intermittent infrared radiation. Position of the electrode was verified by Pontamine sky blue marking technique.

TABLE I

NUMBER OF VENTROMEDIAL HYPOTHALAMIC NEURONS CLASSIFIED BY THEIR RESPONSES TO GLUCOSE, SODIUM AND TO PREOPTIC TEMPERATURE

Neuronal response to Glucose and Na	Response to preoptic thermal stimulation				Total
	Facilitated by warming	Facilitated by cooling	Facilitated during warming and cooling	No response	
Facilitated by glucose	16	4	0	2	22 (41%)
Facilitated by glucose and sodium	1	2	0	0	3 (6%)
Inhibited by glucose and sodium	0	0	1	1	2 (3.5%)
Inhibited by sodium	1	0	1	0	2 (3.5%)
No response	9	6	0	10	25 (46%)
Total	27	12	2	13	54

By histological examination, 54 electrode tips were identified in the VMH. These neurons were classified according to their responses to glucose, sodium and PO thermal stimulation, as shown in Table I. A tendency for each class of neurons to be localized within specific areas of the VMH was not observed. Twenty-two neurons (41%) were facilitated and 25 (46%) were not influenced by glucose. Glucose effect had a mean latency of onset of 17 sec and a mean after-effect of 30 sec.

Out of 22 neurons facilitated by glucose, 16 were facilitated by PO warming. The responses of one of the 16 neurons are shown in Fig. 1A. The discharge frequency increased from 2.5 to 5.0 per sec on the application of glucose with a latency of 24 sec. After cessation of iontophoretic current, the discharge returned to the control

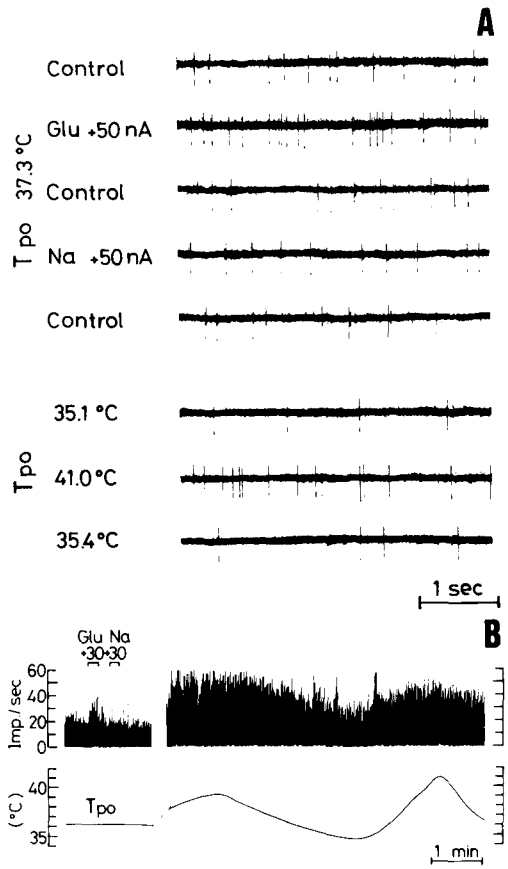

Fig. 1. Responses of two ventromedial hypothalamic neurons in rats to iontophoretically applied glucose (Glu), Na and to preoptic temperature (T_{po}). In A, the second tracing is 37 sec after the beginning of glucose application, the third tracing 120 sec after the cessation of glucose application, the fourth tracing 18 sec after the beginning of Na application, and the fifth tracing 15 sec after the cessation of Na application.

level in 75 sec. The neuron did not respond to sodium. On thermal stimulation, the discharge frequency increased to 4.0/sec at a PO temperature of 41 °C and decreased to 1.0/sec at 35.1 °C. In another neuron (Fig. 1B), the discharge frequency increased from 21 to 36 per sec on application of glucose with +30 nA, returned to the control level 3 sec after the iontophoresis, and was not influenced by sodium with +30 nA. At PO temperatures of 34.3 °C and 41 °C, the discharge frequencies were 29.8 and 55.0 imp./sec, respectively. Similar responses to glucose, sodium and PO thermal stimulation were observed in the rest of 14 neurons.

Four neurons facilitated by glucose were facilitated by PO cooling. One of these neurons increased discharge frequency from 3.6 to 5.6 per sec with glucose +50 nA, and did not respond to sodium +50 nA at PO temperature of 36.8 °C. Discharge frequency decreased to 2.0/sec at a PO temperature of 39.3 °C and increased to 6.0/sec at 35.8 °C.

Three neurons were facilitated both by glucose and sodium. One of them was facilitated by PO warming and 2 were facilitated by PO cooling. Two neurons were inhibited both by glucose and Na. One of them increased discharge frequency, but only during PO warming and cooling. A similar transient response to PO thermal stimulation was observed in one of 2 neurons inhibited by sodium. Nine neurons out of 25 which did not respond either to glucose or to sodium were facilitated by PO warming and 6 were facilitated by PO cooling.

Thus, the neuronal response most frequently observed in the VMH is the facilitation both by glucose and by PO warming. As the temperature of the VMH could not be kept at a constant level during PO thermal stimulation, the possibility cannot be ruled out that the activities of VMH neurons are directly influenced by local temperature. But as was mentioned, neuronal activities of the LH and VMH were not influenced by local temperature [1]. Another possibility might be suggested, therefore, that the VMH neurons are influenced by signals from the PO thermosensitive neurons. Our previous paper revealed that LH neurons inhibited by glucose are facilitated by PO cooling [10]. The responses of LH neurons are thus opposite to those of VMH neurons. The latency of onset of glucose effect was 17 sec in the VMH neurons and 3–4 sec in the LH neurons. The after-effect of glucose lasted longer in the VMH neurons than in the LH neurons, 30 sec in the former and less than 6 sec in the latter. The reasons for these differences are not clear.

Food intake increased and decreased when rats were exposed to ambient temperatures of 19–24 °C and 34 °C, respectively [3]. According to Spector et al. [9], PO heating increased and decreased food intake when the ambient temperature was 25 °C and 35 °C, respectively. They concluded that there is no single temperature that uniquely governs the level of food intake. In this regard, it is interesting to note that the PO thermosensitive neurons receive projections from peripheral thermoreceptors in rat [6]. It seems highly probable, therefore, that temperature signals from the cutaneous receptor are conveyed to the PO thermosensitive neurons, which in turn influence activities of the hypothalamic satiety and feeding centers.

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