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EFFECT OF PROSTAGLANDIN E_2 ON THERMORESPONSIVE NEURONES IN THE PREOPTIC AND VENTROMEDIAL HYPOTHALAMIC REGIONS OF RATS

BY AKIO MORIMOTO, NAOTOSHI MURAKAMI AND TATSUO WATANABE

From the Department of Physiology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

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SUMMARY

1. We investigated the effect of microinjection of prostaglandin E_2 (PGE_2) into the preoptic (POA) or the ventromedial hypothalamic (VMH) region on rectal temperature in rats. Fever was induced by microinjection of PGE_2 into the POA or the VMH regions. The febrile responses induced by PGE_2 injected into the VMH region were significantly greater than those induced by injection into the POA region.

2. The effect of temperature on neuronal activity in the POA and the VMH regions was investigated by using slice preparations from rats. It was revealed that there exist many thermoresponsive neurones in the VMH region as well as in the POA region, and that the proportion of thermoresponsive neurones out of the total neurones examined in the VMH region was almost identical to that in the POA region. In addition, the warm-responsive neurones in the VMH region exhibited larger thermal coefficients than those in the POA region.

3. When PGE_2 was applied in a recording chamber where the tissue slice was perfused, most of the neurones in the VMH region which responded to PGE_2 showed a decrease in their firing rate, while those in the POA region showed an increase in their firing rate, regardless of their thermoresponsiveness. In the POA region, PGE_2 began to affect the activities of the warm-responsive neurones in the range of 5×10^{-7} to 7×10^{-6} M, whereas maximum responses were obtained between the concentrations of 5×10^{-6} and 5×10^{-5} M. In the VMH region, PGE_2 began to change the activities of the warm-responsive neurones in the range of 5×10^{-8} to 5×10^{-7} M, and the maximum effect of PGE_2 on the VMH warm-responsive neurones occurred between the concentrations of 8×10^{-7} and 4×10^{-5} M.

4. The present results show that neurones exhibit different responsiveness to PGE_2 and different sensitivity to PGE_2 between the POA and the VMH regions. Nevertheless, microinjection of PGE_2 into either the POA or the VMH region produces fever. Therefore, it is suggested that fever is produced by complex neuronal networks in the central nervous system.

INTRODUCTION

The preoptic (POA) region has been thought to be a region sensitive to microinjections of prostaglandin E_2 for producing fever (Stitt, 1973; Williams, Rudy, Yaksh & Viswanathan, 1977), although relatively little information is available concerning the febrile response to administration of prostaglandin E_2 into other brain regions. However, there is much evidence that the POA region is not the only site for producing fever. For example, Veale & Cooper (1975) reported that, after removal of the entire POA region of rabbits, an intravenous injection of endogenous pyrogen still produced fever of similar magnitude to that found in control rabbits. Similar results have been repeatedly shown in goats (Andersson, Gale, Hokfelt & Larsson, 1965), squirrel monkeys (Lipton & Trzcinka, 1976) and rats (Blatteis & Banet, 1986).

In our recent report (Morimoto, Murakami, Nakamori & Watanabe, 1988*a*), it was shown that microinjection of prostaglandin E_2 into the ventromedial hypothalamus (VMH) induces fever in rabbits. Furthermore, the febrile response induced by injection of prostaglandin E_2 into the VMH region is significantly greater than that caused by injection into the POA region. Therefore, at the present time, the VMH region, as well as the POA region, appears to play an important role in the pathogenesis of fever.

Since thermoresponsive neurones were found to be located in the POA region (Nakayama, Hammel, Hardy & Eisenman, 1963; Hardy, Hellon & Sutherland, 1964), these have been thought to play a role in regulation of body temperature (Hensel, 1981). Some of the previous studies showed that the intravenous injection or intrapreoptic injection of pyrogenic substances such as bacterial endotoxin (Cabanac, Stolwijk & Hardy, 1968; Wit & Wang, 1968; Eisenman, 1969; Nakayama & Hori, 1973), endogenous pyrogen (Schoener & Wang, 1975) or prostaglandin E (Schoener & Wang, 1976) inhibits the activities of the warm-responsive neurones and facilitates those of the cold-responsive neurones in the POA region. Those results were consistent with the simple theory that in fever inhibition of warm-responsive neurones and facilitation of cold-responsive neurones enhance heat production and attenuate heat loss, subsequently raising body temperature (Boulant, 1974). However, the possibility must be considered that when the pyrogenic substance was injected *in vivo*, fever may have been induced. It is still unclear whether the previous *in vivo* results are the causes or results of fever, because thermoresponsive neurones in the POA region respond to changes in deep and peripheral body temperature (Nakayama & Hardy, 1969). Our recent results, using the method of slice preparation from rats, showed that many of the neurones in the POA region respond to endogenous pyrogen and/or prostaglandin E_2 with a rise in their firing rate, regardless of their thermoresponsiveness, and that inhibitory action was rarely observed (Watanabe, Morimoto & Murakami, 1987*a*, *b*). This *in vitro* method had been chosen to exclude unknown factors arising from peripheral and/or central thermosensors and accompanying the febrile response. Therefore, it is at present unknown whether fever is in fact triggered by changing the activity of thermoresponsive neurones in the POA region specifically, and furthermore, whether thermoresponsive neurones actually take part in the development of fever.

The former part of the present study has shown that the febrile response induced by microinjection of prostaglandin E_2 into the VMH region is significantly greater than that induced by injection into the POA region in rats. Given that fever is triggered by resetting neuronal activity of thermoresponsive neurones (Boulant, 1974), it is inferred that thermoresponsive neurones also exist in the VMH region, as well as in the POA region. Therefore, in the latter part of the present study, we investigated whether or not thermoresponsive neurones exist in the VMH region by using slice preparations from rats. The result revealed that there are many thermoresponsive neurones in the VMH region, and the proportion of thermoresponsive neurones of the total neurones examined in the VMH region was almost identical to that in the POA region. However, the warm-responsive neurones in the VMH region demonstrated a higher thermal coefficient than those in the POA region. Furthermore, when prostaglandin E_2 was applied on the neurones in the POA or VMH region, most of the neurones which responded to prostaglandin E_2 showed an increase in firing rate in the POA region and a decrease in the VMH region, regardless of their thermoresponsiveness.

The present results clarify that there exist many thermoresponsive neurones both in the POA and in the VMH region. However, the direction of the neuronal response to prostaglandin E_2 in these regions was quite different, and our results are not in accordance with the simple theory believed previously. This indicates that there are complex neuronal networks for fever production and that the central mechanism of fever production is not explained by the simple theory that fever is processed by inhibition of warm-responsive neurones and facilitation of cold-responsive neurones.

METHODS

The animals used in this study were male Wistar strain rats weighing 250–300 g. The present study consisted of two experimental series (experiments 1 and 2). In experiment 1, we examined how microinjection of prostaglandin E_2 into the POA or the VMH region affects the body temperature in rats. In experiment 2, the effect of changes in tissue temperature and prostaglandin E_2 on the neuronal activity in the POA and the VMH regions was investigated, using slice preparations of those regions of the rat.

Microinjection of prostaglandin E_2 into the POA and the VMH regions

Each of the animals ($n = 6$) had been implanted previously with two stainless-steel tubes (0·8 mm o.d.), one located in the POA region at co-ordinates AP 2, L 1·5, V 8·5 mm and the other in the VMH region at co-ordinates AP 0·4, L 1·0, V 9 mm according to the rat brain atlas (Pellegrino, Pellegrino & Cushman, 1979) by standard stereotaxic techniques. This implantation was done at least 10 days before the start of the experiment under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.P.). Each rat had been handled sufficiently to make it adapt to manipulation by experimenters. Furthermore, all animals had been well trained to adapt to restraint and thus avoid the effect of stress due to such restraint.

On the day of the experiment, rats were minimally restrained in a small wooden board in which they could freely move their extremities except their tails which were fixed by adhesive plaster (1 cm in width) to rods horizontally attached to the board. Throughout the experiment, the rectal temperature (T_{re}) was measured every minute by a copper–constantan thermocouple. All experiments were performed at an ambient temperature of 26 ± 1 °C which is the thermoneutral temperature of rats. The rectal temperature of each rat was allowed to stabilize for a period of 90 min before injections. Only rats whose body temperatures were stable and in the range of 38·0–39·2 °C were used to determine the effect of the injection of prostaglandin E_2, because Szekely

& Szelenyi (1979) reported that the maximal febrile response is strongly affected by body core temperature at the time of injection. Intracerebral injections were made through a stainless-steel needle (0·4 mm o.d.) attached to a polyethylene tube. The injection doses of prostaglandin E_2 ranged from 25 to 200 ng, and the volume injected was always 1 μl.

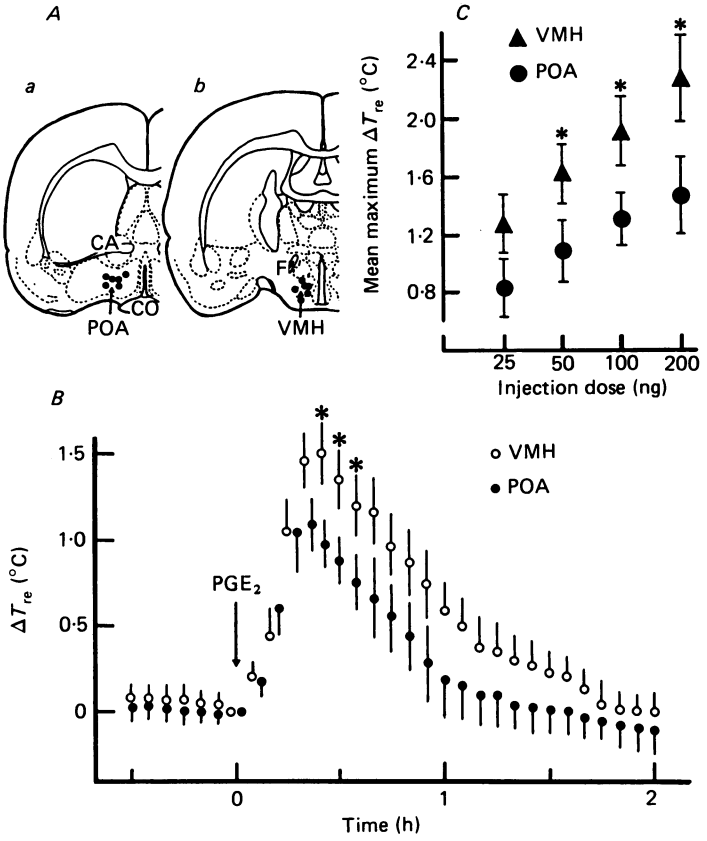

Fig. 1. *A*, the filled circles represent the positions of the injection sites in the brain regions of the same group of six rats. Abbreviations: POA, preoptic region; VMH, ventromedial hypothalamic region; CA, commissura anterior; CO, chiasma opticum; F, fornix. *B*, mean changes (mean ± S.E.M.) in rectal temperature ($\triangle T_{re}$) in the same group of six rats after intrapreoptic (POA, ●) and intraventromedial hypothalamic (VMH, ○) injection of prostaglandin E_2 (50 ng). *C*, mean maximum rise in rectal temperature ($\triangle T_{re}$) in the same group of six rats after intrapreoptic (POA, ●) and intraventromedial hypothalamic (VMH, ▲) injection of differing doses of prostaglandin E_2. *$P < 0·05$.

After the completion of the experiment, the animal was killed by a large dose of sodium pentobarbitone. The thorax was then opened and formaldehyde solution (10%) was perfused via cardiac puncture. The brain was removed, frozen in Freon chilled with liquid N_2 and cut into sections (28 μm) in a cryostat. These sections were stained with Haematoxylin and Eosin which allowed histological identification of the location of tip of the stainless-steel tube. The data were analysed for statistical significance by Student's *t* test for unpaired data.

Slice preparation and unitary recording

Single-unit activity was recorded in tissue slices of the hypothalamus which were prepared from male Wistar strain rats, weighing 200–250 g. Detailed descriptions of slice preparation and unit recording methods have been reported previously (Hori, Nakashima, Hori & Kiyohara, 1980;

Kelso, Perlmutter & Boulant, 1982; Watanabe, Morimoto & Murakami, 1986). Several slices (400 μm thick) including the POA or the VMH region were obtained by using a guillotine-type razor-blade slicer (Hotta Rika, model YH-10S). One hour pre-incubation time in a pre-incubation chamber (37 °C) filled with Krebs–Ringer solution was allowed for recovery of the slices from the damage due to preparation. The Krebs–Ringer solution had the following composition (in mM at

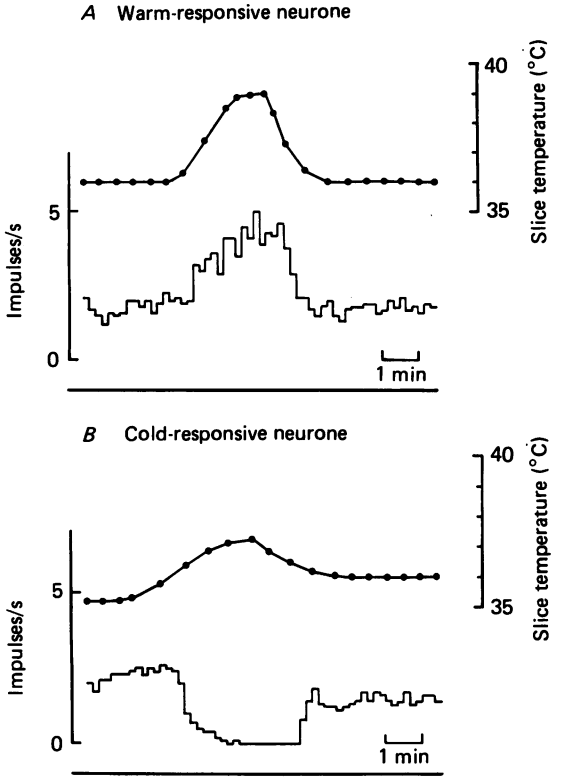

Fig. 2. Representative example of a warm- and a cold-responsive neurone in the ventromedial hypothalamic (VMH) region.

pH 7·4): NaCl, 124; KCl, 5; KH_2PO_4, 1·24; $MgSO_4$, 1·3; $CaCl_2$, 2·6; $NaHCO_3$, 26; and glucose, 10, equilibrated with a gas mixture (95% O_2 and 5% CO_2). Thereafter, the slice was subjected to unit recording in a recording chamber perfused with Krebs–Ringer solution as mentioned above. The temperature of the tissue slice, varied by control of the temperature of the Krebs–Ringer solution in the recording chamber, was continuously measured, and single-unit activity was extracellularly recorded with a glass microelectrode. Prostaglandin E_2 was applied to the perfusate through a side route by a microsyringe pump (Infors AG CH-4015), a procedure called 'bath application'. The concentration of prostaglandin E_2 in the recording chamber was determined as described previously (Watanabe *et al.* 1987*a*, *b*). After the end of the experiment, different brain regions were histologically identified by Haematoxylin and Eosin staining.

RESULTS

Effect of microinjection of prostaglandin E_2 into the POA or VMH region on body temperature

Figure 1*A* summarizes the positions of the tips of the stainless-steel tubes. Figure 1*B* depicts the changes in rectal temperature (ΔT_{re}) of rats ($n = 6$) after intrapreoptic

or intraventromedial hypothalamic injections of prostaglandin E_2 at 50 ng. As shown in Fig. 1*B*, prostaglandin E_2 produced a monophasic fever with a rapid onset when injected into either the POA or the VMH region. The peak of the fever occurred between 20 and 30 min after injections. However, the febrile responses induced by prostaglandin E_2 injected into the VMH region were significantly greater than those induced by the injection of prostaglandin E_2 into the POA region. Figure 1*C* shows the mean maximum rise in the rectal temperature for 2 h after intrapreoptic or intraventromedial hypothalamic injection of several doses of prostaglandin E_2 over the range of 25–200 ng. In Fig. 1*C*, it is apparent that the febrile response induced by intraventromedial hypothalamic injection of prostaglandin E_2 is significantly greater than that induced by intrapreoptic injection.

Neuronal responses of the POA and VMH regions to thermal stimulation and prostaglandin E_2

Eighty-nine neurones in the POA region were examined for responsiveness to changes in slice temperature and changes in the concentration of prostaglandin E_2. Of the eighty-nine neurones in the POA region, fifty-five neurones increased their firing rates with a rise in slice temperature (i.e. warm-responsive neurones), three neurones decreased their firing rates with a rise in slice temperature (i.e. cold-responsive neurones), and thirty-one neurones were thermally insensitive neurones and did not respond to the temperature change. The criterion for warm- or cold-responsiveness in each neurone was based on a thermal coefficient of 0·5 or −0·5 impulses/(s °C) respectively. In addition, the effects of changes in slice temperature on neuronal activity were also investigated in sixty-two neurones in the VMH region. However, we could investigate the effect of prostaglandin E_2 on only forty-three neurones out of the sixty-two neurones examined in the VMH region. Figure 2 shows two types of neurones in the VMH region that change their firing rate with a change in slice temperature. In Fig. 2*A*, one neurone increases its firing rate with a rise in slice temperature, while the other neurone shown in Fig. 2*B* decreases its firing rate with a rise in slice temperature. Among the neurones examined in the VMH region, forty-one were warm-responsive, three were cold-responsive, and eighteen were thermally insensitive. In Table 1, we classified the neurones in the POA and VMH regions by their thermal coefficients. The percentages of warm-responsive, cold-responsive and thermally insensitive neurones in the VMH region were similar to those in the POA region. Most of the warm-responsive neurones (forty-five out of fifty-five) in the POA region had thermal coefficients of 0·5–0·9 impulses/(s °C), while more than half of the warm-responsive neurones had a thermal coefficient above 1·0 impulses/(s °C) in the VMH region. This indicates that the warm-responsive neurones in the VMH region possess a greater thermal coefficient than those in the POA region. After each neurone was examined for thermoresponsiveness, prostaglandin E_2 was added to the perfusate, keeping the slice temperature constant. Injection of prostaglandin E_2 terminated after changes in the firing rates had reached a constant level. Several different concentrations of prostaglandin E_2 were applied in turn to each neurone in the same manner.

Figure 3 shows a warm-responsive neurone of the POA region whose activity increased dose dependently when prostaglandin E_2 was applied into the culture of

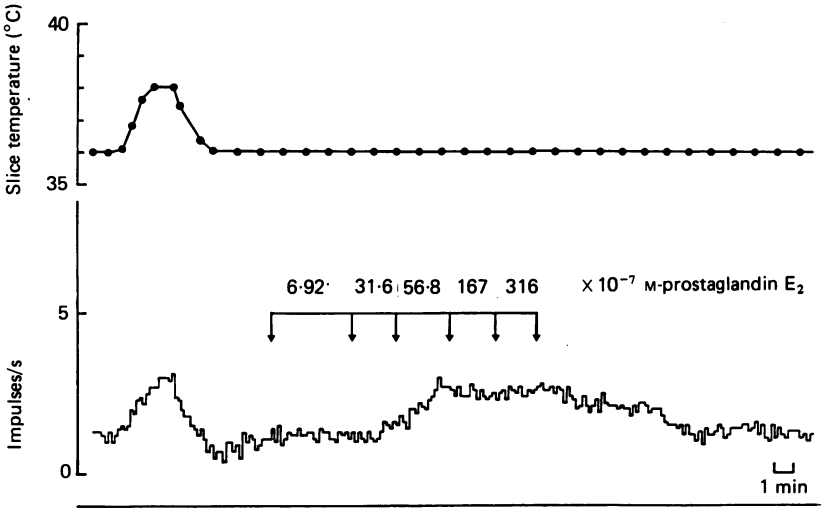

Fig. 3. Representative example of a warm-responsive neurone in the preoptic region with a bath application of prostaglandin E_2. Concentrations of prostaglandin E_2 are expressed in molar concentrations.

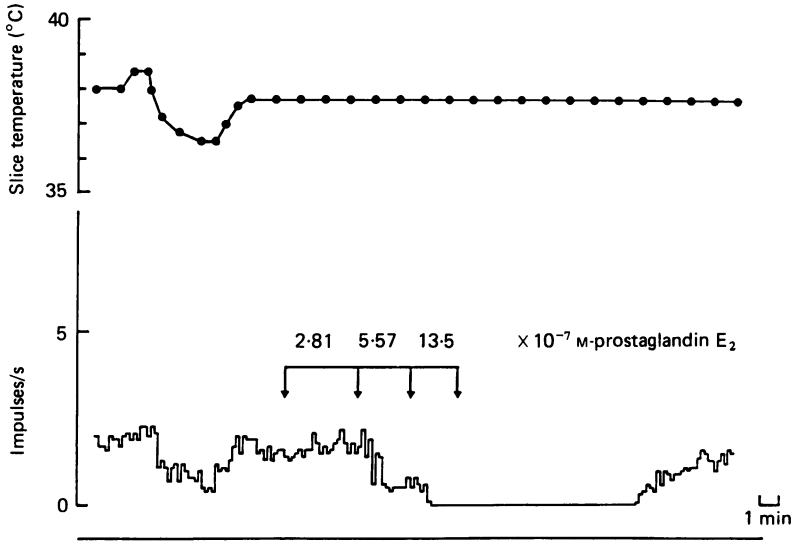

Fig. 4. Representative example of a warm-responsive neurone in the ventromedial hypothalamic region with a bath application of prostaglandin E_2. Concentrations of prostaglandin E_2 are expressed in molar concentrations.

the POA slice. As the concentration of prostaglandin E_2 in the chamber increased stepwise in the range from $6{\cdot}92 \times 10^{-7}$ to $3{\cdot}16 \times 10^{-5}$ M, the firing rate gradually increased and eventually reached a stationary discharge rate. Figure 4 shows a warm-responsive neurone of the VMH region which decreases its firing rate with the application of prostaglandin E_2 into the chamber. The stepwise increase in the

TABLE 1. Classification of the neurones in the preoptic and the ventromedial hypothalamic regions, based on their thermal coefficients

	Cold-responsive neurone	Thermally insensitive neurone	Warm-responsive neurone		
Preoptic region					
Thermal coefficient (impulses/(s °C))	< -0.5	$-0.4 \sim 0.4$	$0.5 \sim 0.9$	$1.0 \sim 1.9$	$2.0 <$
Number of neurones (total = 89)	3 (3·37 %)	31 (34·83 %)	45	9	1
				(61·80 %)	
Ventromedial hypothalamic region					
Thermal coefficient (impulses/(s °C))	< -0.5	$-0.4 \sim 0.4$	$0.5 \sim 0.9$	$1.0 \sim 1.9$	$2.0 <$
Number of neurones (total = 62)	3 (4·84 %)	18 (29·03 %)	18	18	5
				(66·13 %)	

TABLE 2. Summary of the responses of neurones in the preoptic and ventromedial hypothalamic regions to bath-applied prostaglandin E_2

	Direction of thermal response	Number of neurones: Warm-responsive neurone	Thermally insensitive neurones	Cold-responsive neurones
Preoptic region				
Prostaglandin E_2	↑	34	13	1
	→	18	16	2
	↓	3	2	0
Total		55	31	3
Ventromedial hypothalamic region				
Prostaglandin E_2	↑	1	0	0
	→	10	3	0
	↓	17	9	3
Total		28	12	3

concentration of prostaglandin E_2, ranging from $2{\cdot}81 \times 10^{-7}$ to $1{\cdot}35 \times 10^{-6}$ M, inhibited the firing rate of the warm-responsive neurone.

Figure 5 summarizes the dose–response relationships between neuronal activity of warm-responsive neurones in the POA and VMH regions, and prostaglandin E_2 concentration. Prostaglandin E_2 increased the activity of warm-responsive neurones

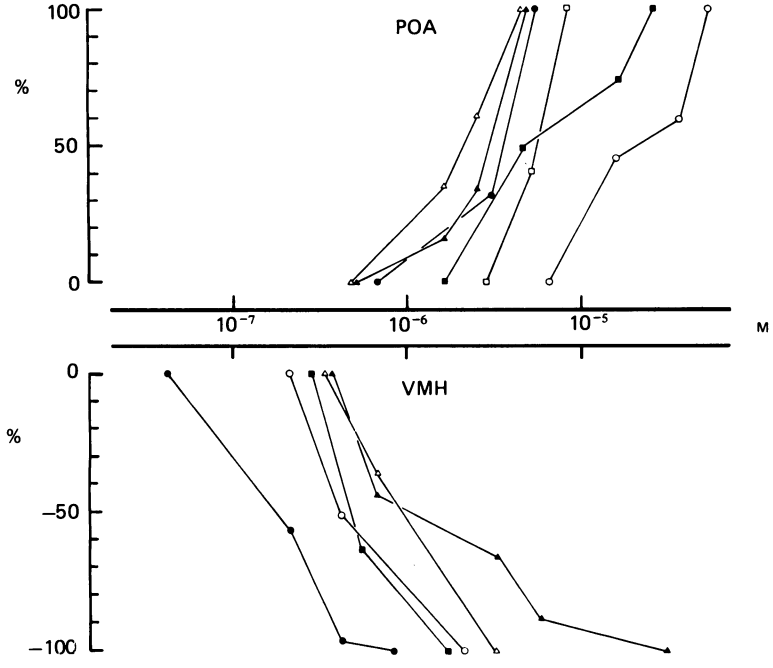

Fig. 5. Dose–response relationship when warm-responsive neurones in the preoptic (POA) and the ventromedial hypothalamic (VMH) regions changed their activities with application of prostaglandin E_2. Response was expressed as percentage changes. Maximum change in firing rate from the basal value was considered as 100% change in response.

located in the POA region, while decreasing that of those in the VMH region. When the firing rate of a neurone did not continue to change during stepwise increases in the concentration of prostaglandin E_2 applied, this was taken as the maximum response. To obtain a dose–response relationship, the maximum change from the basal firing rate was considered to be 100%. Then, changes in the firing rates from the basal level, induced by selected doses of prostaglandin E_2, were divided by the 100% value in order to determine the percentage change in the firing rate of the neurone. These percentages were plotted against the concentration of prostaglandin E_2. In the case of the POA region, prostaglandin E_2 starts to affect the activities of the warm-responsive neurones in the range of 5×10^{-7} to 7×10^{-6} M. The maximum responses were obtained between the concentrations of 5×10^{-6} and 5×10^{-5} M. Meanwhile, the VMH warm-responsive neurones decreased their firing rates with a prostaglandin E_2 concentration in the range of 5×10^{-8} to 5×10^{-7} M. The maximum effects of prostaglandin E_2 on warm-responsive neurones occurred between the concentrations of 8×10^{-7} and 4×10^{-5} M.

Table 2 summarizes changes in response to prostaglandin E_2 and the number of neurones investigated on the effect of prostaglandin E_2 in the POA and the VMH regions. In Table 2, a large population (62%) of warm-responsive neurones in the POA region responded to prostaglandin E_2 with an increase in their firing rate. Furthermore, in thermally insensitive neurones, prostaglandin E_2 increased the firing rates in thirteen of the thirty-one neurones. One of three cold-responsive neurones showed a facilitatory response to prostaglandin E_2. When prostaglandin E_2 was applied in the VMH region, more than 50% of the warm-responsive, cold-responsive and the thermally insensitive neurones decreased their firing rates.

DISCUSSION

Since Milton & Wendlandt (1970) observed the strong pyrogenic action of prostaglandin E, the prostaglandin E series has been thought to be a candidate for the causative agent of the development of fever. Furthermore, the target site of prostaglandin E for producing fever has been considered to be localized exclusively in the POA region (Stitt, 1973; Williams *et al.* 1977). However, recent reports have shown that the organum vasculosum laminae terminalis (Stitt & Shimada, 1986) and the VMH region (Morimoto *et al.* 1988*a*) are more sensitive sites to prostaglandin E_2 for inducing fever than the POA region when prostaglandin E_2 is locally injected. Furthermore, it is now suggested that there exist multiple control mechanisms for fever production in the central nervous system (Morimoto, Murakami, Nakamori & Watanabe, 1988*b*). The present results show that fever was induced by microinjection of prostaglandin E_2 into either the POA or the VMH region in rats, as previously reported using rabbits (Morimoto *et al.* 1988*a*). Furthermore, febrile responses induced by intraventromedial hypothalamic injection of prostaglandin E_2 were significantly greater than those by intrapreoptic injection. This confirms that these two brain regions, POA and VMH, are closely related to the pathogenesis of fever.

It is well known that thermoresponsive neurones are concentrated in the POA region (Nakayama *et al.* 1963; Hardy *et al.* 1964) and these neurones have been thought to play an important role in fever production (Boulant, 1974). Previous studies using the method of slice preparations (Hori *et al.* 1980; Kelso *et al.* 1982; Watanabe *et al.* 1986) demonstrated that there exist many thermoresponsive neurones in the POA region. Additionally, our recent report showed that prostaglandin E_2 facilitates the neuronal activity of most of the neurones responding to prostaglandin E_2, although the direction of the response to prostaglandin E_2 could not be classified by thermal responsiveness of neurones of the POA region (Watanabe *et al.* 1987*a*, *b*). Since a febrile response was induced by microinjection of prostaglandin E_2 into the VMH region in the present results, thermoresponsive neurones and the neurones sensitive to prostaglandin E_2 might be anticipated to exist also in the VMH region. In the present study, the effects of thermal stimulation and prostaglandin E_2 on the neuronal activity in the VMH region were compared to those in the POA region. The present results show that there exist thermoresponsive neurones in the VMH region, both warm- and cold-responsive neurones, and that the proportion of the thermoresponsive neurones in the VMH region was almost identical

to that in the POA region. However, when the thermal coefficient of the thermoresponsive neurones in the VMH region was compared with that in the POA region, the warm-responsive neurones in the VMH region showed a higher thermal coefficient than those in the POA region. There are several reports that the VMH region plays an important role in thermoregulation, especially regulation of non-shivering thermogenesis (Shimazu & Takahashi, 1980; Perkins, Rothwell, Stock & Stone, 1981; Niijima, Rohner-Jeanrenaud & Jeanrenaud, 1984). Therefore, these thermoresponsive neurones in the VMH region might be related to development of the thermal response. Indeed, electrical stimulation of the VMH region is reported to elicit cold defence responses in rabbits (Morimoto, Murakami, Ono, Watanabe & Sakata, 1986).

Furthermore, the present study showed that most of the neurones in the VMH region which responded to prostaglandin E_2 react with a decrease in firing rate, while those in the POA region react with an increase in firing rate. However, in neither region could we classify the direction of the neuronal response to prostaglandin E_2, based on thermoresponsiveness of the neurones. Thus, the direction of neuronal responses to prostaglandin E_2 in the two regions is quite different. Moreover, prostaglandin E_2 is considered to act simultaneously on the neurones in both the POA and the VMH regions during naturally occurring fever, because the concentration of prostaglandin E_2 in the cerebrospinal fluid increases during fever (Feldberg & Gupta, 1973; Philipp-Dormston & Siegert, 1974; Bernheim, Gilbert & Stitt, 1980). Therefore, it is suggested that there are complex neuronal networks between the POA and VMH regions involved in the development of fever.

Additionally, from the present results on the responses of neurones examined in the POA and the VMH regions to prostaglandin E_2, the neurones in the VMH region respond to prostaglandin E_2 at lower concentrations than those in the POA region. These *in vitro* results are in good accordance with the *in vivo* febrile responses induced by microinjection of prostaglandin E_2, because fever is induced by injections of lower concentrations in the VMH region than in the POA region.

It has been repeatedly shown that the activation of the VMH region is concerned with the development of non-shivering thermogenesis (Shimazu & Takahashi, 1980; Perkins *et al.* 1981; Niijima *et al.* 1984). Therefore, provided that the activation of the non-shivering thermogenesis was responsible for the induction of fever which was produced by the microinjection of prostaglandin E_2 into the VMH region, the neurones in the VMH region may be expected to be facilitated by prostaglandin E_2. However, in the present study, prostaglandin E_2 clearly inhibited the activity of the neurones in the VMH region. Therefore, it remains to be elucidated whether these discrepancies indicate that the mechanism for induction of fever might be different from that for induction of non-shivering thermogenesis.

REFERENCES

ANDERSSON, B., GALE, C. C., HOKFELT, B. & LARSSON, B. (1965). Acute and chronic effects of preoptic lesions. *Acta physiologica scandinavica* **65**, 45–60.

BERNHEIM, H. A., GILBERT, T. M. & STITT, J. T. (1980). Prostaglandin E levels in third ventricular cerebrospinal fluid of rabbits during fever and changes in body temperature. *Journal of Physiology* **301**, 69–78.

BLATTEIS, C. M. & BANET, M. (1986). Autonomic thermoregulation after separation of the preoptic area from the hypothalamus in rats. *Pflügers Archiv* **406**, 480–484.

BOULANT, J. A. (1974). The effect of firing rate on preoptic neuronal thermosensitivity. *Journal of Physiology* **240**, 661–669.

CABANAC, M., STOLWIJK, J. A. J. & HARDY, J. D. (1968). Effect of temperature and pyrogens on single-unit activity in the rabbit's brain stem. *Journal of Applied Physiology* **24**, 645–652.

EISENMAN, J. S. (1969). Pyrogen-induced changes in the thermosensitivity of septal and preoptic neurons. *American Journal of Physiology* **216**, 330–334.

FELDBERG, W. & GUPTA, K. P. (1973). Pyrogen fever and prostaglandin activity in cerebrospinal fluid. *Journal of Physiology* **228**, 41–53.

HARDY, J. D., HELLON, R. F. & SUTHERLAND, K. (1964). Temperature-sensitive neurones in the dog's hypothalamus. *Journal of Physiology* **175**, 242–253.

HENSEL, H. (1981). *Thermoreception and Temperature Regulation*. London: Academic Press.

HORI, T., NAKASHIMA, T., HORI, N. & KIYOHARA, T. (1980). Thermo-sensitive neurons in hypothalamic tissue slices in vitro. *Brain Research* **186**, 203–207.

KELSO, S. R., PERLMUTTER, M. N. & BOULANT, A. (1982). Thermosensitive single-unit activity of in vitro hypothalamic slices. *American Journal of Physiology* **242**, R77–84.

LIPTON, J. M. & TRZCINKA, G. P. (1976). Persistence of febrile response to pyrogens after PO/AH lesions in squirrel monkeys. *American Journal of Physiology* **231**, 1638–1648.

MILTON, A. S. & WENDLANDT, S. (1970). A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *Journal of Physiology* **207**, 76–77*P*.

MORIMOTO, A., MURAKAMI, N., NAKAMORI, T. & WATANABE, T. (1988*a*). Ventromedial hypothalamus is highly sensitive site to prostaglandin E_2 for producing fever in rabbits. *Journal of Physiology* **397**, 259–268.

MORIMOTO, A., MURAKAMI, N., NAKAMORI, T. & WATANABE, T. (1988*b*). Multiple control of fever production in the central nervous system of rabbits. *Journal of Physiology* **397**, 269–280.

MORIMOTO, A., MURAKAMI, N., ONO, T., WATANABE, T. & SAKATA, Y. (1986). Stimulation of ventromedial hypothalamus induces cold defense responses in conscious rabbits. *American Journal of Physiology* **250**, R560–566.

NAKAYAMA, T., HAMMEL, H. T., HARDY, J. D. & EISENMAN, J. S. (1963). Thermal stimulation of electrical activity of single units of the preoptic region. *American Journal of Physiology* **204**, 1122–1126.

NAKAYAMA, T. & HARDY, J. D. (1969). Unit responses in the rabbit's brain stem to changes in brain and cutaneous temperature. *Journal of Applied Physiology* **27**, 848–857.

NAKAYAMA, T. & HORI, T. (1973). Effect of anesthetic and pyrogen on thermally sensitive neurons in the brainstem. *Journal of Applied Physiology* **34**(3), 351–355.

NIIJIMA, A., ROHNER-JEANRENAUD, F. & JEANRENAUD, B. (1984). Role of ventromedial hypothalamus on sympathetic efferents of brown adipose tissue. *American Journal of Physiology* **247**, R650–654.

PELLEGRINO, L. J., PELLEGRINO, A. S. & CUSHMAN, A. J. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd edn. New York: Plenum.

PERKINS, M. N., ROTHWELL, N. J., STOCK, M. J. & STONE, T. W. (1981). Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. *Nature* **289**, 401–402.

PHILIPP-DORMSTON, W. K. & SIEGERT, R. (1974). Prostaglandins of the E and F series in rabbit cerebrospinal fluid during fever induced by Newcastle disease virus, E. coli endotoxin, or endogenous pyrogen. *Medical Microbiological Immunology* **159**, 279–284.

SCHOENER, E. P. & WANG, S. C. (1975). Leukocytic pyrogen and sodium acetylsalicylate on hypothalamic neurons in the cat. *American Journal of Physiology* **229**, 185–190.

SCHOENER, E. P. & WANG, S. C. (1976). Effects of locally administered prostaglandin E_1 on anterior hypothalamic neurons. *Brain Research* **117**, 157–162.

SHIMAZU, T. & TAKAHASHI, A. (1980). Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose tissue. *Nature* **284**, 62–63.

STITT, J. T. (1973). Prostaglandin E_1 fever induced in rabbits. *Journal of Physiology* **232**, 163–179.

STITT, J. T. & SHIMADA, S. G. (1986). Febrile sensitivity of the OVLT region in rats, to microinjections of PGE. *Proceedings of the International Union of Physiological Sciences* **XVI**, 24.

SZEKELY, M. & SZELENYI, Z. (1979). Endotoxin fever in the rat. *Acta physiologica academiae scientiarum hungaricae* **53**, 265–277.

VEALE, W. L. & COPPER, K. E. (1975). Comparison of sites of action of prostaglandin E and leucocyte pyrogen in brain. In *Temperature Regulation and Drug Action*, ed. LOMAX, J., SCHONBAUM, E. & JACOB, J., pp. 218–226. Basel: Karger.

WATANABE, T., MORIMOTO, A. & MURAKAMI, N. (1986). Effect of amine on temperature-responsive neuron in slice preparation of rat brain stem. *American Journal of Physiology* **250**, R553–559.

WATANABE, T., MORIMOTO, A. & MURAKAMI, N. (1987*a*). Effects of endogenous pyrogen and prostaglandin E_2 on hypothalamic neurons in rat brain slices. *Canadian Journal of Physiology and Pharmacology* **65**, 1382–1388.

WATANABE, T., MORIMOTO, A. & MURAKAMI, N. (1987*b*). Effect of PGE_2 on preoptic and anterior hypothalamic neurons using brain slice preparation. *Journal of Applied Physiology* **63**(3), 918–922.

WILLIAMS, J. W., RUDY, T. A., YAKSH, T. L. & VISWANATHAN, C. T. (1977). An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. *Brain Research* **120**, 251–262.

WIT, A. & WANG, S. C. (1968). Temperature-sensitive neurons in preoptic/anterior hypothalamic region: actions of pyrogen and acetylsalicylate. *American Journal of Physiology* **215**, 1160–1169.