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VENTROMEDIAL HYPOTHALAMUS IS HIGHLY SENSITIVE TO PROSTAGLANDIN E_2 FOR PRODUCING FEVER IN RABBITS

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SUMMARY

1. The febrile responses induced by intraventricular or intrapreoptic (bilateral) injections of prostaglandin E_2 (PGE_2) were investigated in the same group of rabbits. Both injections produced dose-dependent fever over a range of 100–2000 ng. However the magnitude of febrile responses induced by ventricular injections was significantly greater than those by intrapreoptic injections. This indicates that there exist regions more sensitive to PGE_2 than the preoptic region for producing fever.

2. To explore the regions sensitive to PGE_2, the effects of microinjection (1 μl) of PGE_2 (50 and 100 ng) on the rectal temperature were extensively examined in the forty regions of the brain stem. The results showed that the preoptic and anterior hypothalamic region, and the ventromedial hypothalamic region are highly sensitive to PGE_2 for producing fever.

3. The febrile responses to PGE_2 (50–1000 ng) microinjected into the preoptic region were compared with those induced by injection in the ventromedial hypothalamic region. Fever induced by injection in the ventromedial hypothalamic region was significantly greater than that by injection into the preoptic region.

4. Fever induced by PGE_2 injected into the ventromedial hypothalamic region was due to increased heat production in the cold environment (10 °C), while in 24 °C environment heat losses were reduced without significant changes in heat production.

5. The present results show that the ventromedial hypothalamic region is the most sensitive region to PGE_2 for producing fever.

INTRODUCTION

It is well known that fever is caused by the action on the central nervous system (CNS) of endogenous pyrogen, which is released from macrophages activated by exogenous pyrogen such as bacterial endotoxin. Since Milton & Wendlandt (1971) observed the strong pyrogenic action of intraventricular prostaglandin E_1, the prostaglandin E series has been generally believed to be the candidates for the final mediators in the CNS involved in the pathogenesis of fever. This hypothesis has been further substantiated by the fact that prostaglandin levels in the cerebrospinal fluid increase during fever (Feldberg & Gupta, 1973; Bernheim, Gilbert & Stitt, 1980). Furthermore, fevers induced by intravenous and intraventricular injection of

endogenous pyrogen are significantly suppressed by systemic and intraventricular injection of inhibitors of prostaglandin synthesis, respectively (Stitt & Bernheim, 1985; Morimoto, Murakami, Nakamori & Watanabe, 1987).

The preoptic and anterior hypothalamic (PO/AH) region has been thought to be a region sensitive to microinjections of prostaglandin E for producing fever (Stitt, 1973; Williams, Rudy, Yaksh & Viswanathan, 1977), although relatively little information is available about febrile response to administration of PGE into other brain regions. However, there is much evidence that the PO/AH region is not the only site for producing fever. For example, Veale & Cooper (1975) reported that after removal of the entire PO/AH 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). At the present time, therefore, we have not yet obtained a clear understanding of which region is most sensitive to prostaglandin E for producing fever.

In the present study, we first clarified that febrile responses induced by ventricular injection of prostaglandin E_2 are greater than those by intrapreoptic injection, which suggests the existence of regions more sensitive than the preoptic region. Furthermore, by exploring extensively sites sensitive to microinjections of prostaglandin E_2 in the brain stem, we found that the ventromedial hypothalamic region is the most sensitive region to prostaglandin E_2 for producing fever.

METHODS

The animals used in this study were male New Zealand white rabbits weighing 3·0–3·5 kg. The present study consisted of three experimental groups of animals. In Expt 1, each of the animals ($n = 7$) had been implanted previously with three stainless-steel tubes (1·0 mm o.d.), two located in the bilateral preoptic regions at co-ordinates AP 4, L 1·5, V 11 mm according to the rabbit brain atlas (Sawyer, Everett & Green, 1954) and one in the third ventricle, by standard stereotaxic techniques (Sawyer *et al.* 1954). In Expt 2, each of the animals ($n = 10$) had been implanted with four stainless-steel tubes in different regions of the brain stem. The regions where the tips of the tubes were located are shown in the Results. In Expt 3, each of the animals ($n = 7$) had been implanted with two stainless-steel tubes, one located in the preoptic region (right side) and the other in the ventromedial hypothalamic region (left side, AP 0, L 0·5, V 15 mm). These implantations were done at least 10 days before the start of the experiment under general anaesthesia (sodium pentobarbitone, 20 mg/kg, I.V.).

On the day of the experiment, animals were minimally restrained in conventional rabbit stocks, at an ambient temperature of 21 ± 1 °C between 09.00 and 16.00 h. Throughout the experiment, the rectal temperature was measured every minute with a copper–constantan thermocouple. The rectal temperature in each animal was allowed to stabilize for at least 90 min before any injections were made. Furthermore, in Expt 3, the rectal and skin temperatures and oxygen consumption of each animal were simultaneously measured under two different ambient temperatures (10 ± 1 and 24 ± 1 °C). The skin temperature at the surface of the ear was also measured with a copper–constantan thermocouple. Oxygen consumption was measured with an oxygen analyser (Beckman, model 755) using an open system (air flow, 8 l/min). All data for the oxygen consumption and the rectal and skin temperatures were entered into a data acquisition control unit (Hewlett–Packard 3497A), and the digital values for each response were printed out. Intracerebral or intraventricular injections were made through a stainless-steel needle (0·6 mm o.d.) attached to a polyethylene tube. The volume injected was 1 μl for the cerebral injection and 2 μl for the ventricular injection. Each injection was performed for a period of 1 min. Prostaglandin E_2 was

dissolved in sterile saline containing ethanol (2%). Injection doses in each experimental group are described in the Results. The saline was also injected as the control 1 h before injection of prostaglandin E_2.

After the completion of each experiment, the animal was killed by a large dose of sodium pentobarbitone. The thorax was then opened and a 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 and served for histological identification of the tip of the stainless-steel tube (Sawyer *et al.* 1954; Shek, Wen & Wisniewski, 1986). The data were analysed for statistical significance using Student's *t* test for unpaired data.

RESULTS

Figure 1 shows changes in the rectal temperature after intraventricular or intrapreoptic microinjection of prostaglandin E_2. The injection dose into the third ventricle was 400 ng and that into the bilateral preoptic regions was 200 ng in each side, supposing that the total dose injected into the third ventricle was infused into the bilateral preoptic regions. Therefore, the total injection dose was the same

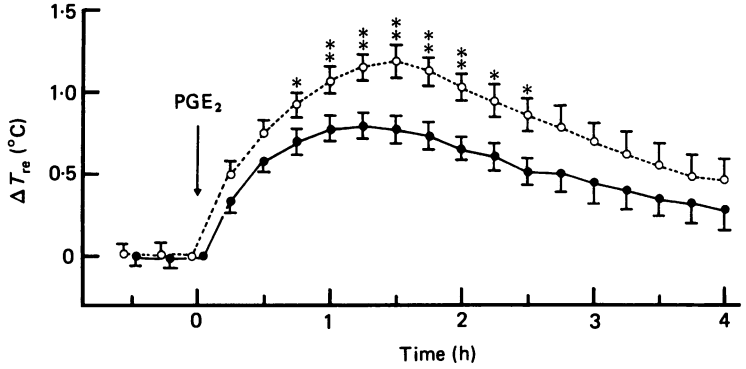

Fig. 1. Mean changes (mean ± S.E.M.) in rectal temperature (ΔT_{re}) in the same group of seven rabbits after intraventricular (○) and intrapreoptic (●) injection of prostaglandin E_2 (400 ng). Dose of intrapreoptic region was the total of bilateral injection dose (200 ng in each region of bilateral preoptic regions).

(400 ng) in both the intraventricular and the intrapreoptic injections. As shown in Fig. 1, in both cases, a typically monophasic fever was induced after injection of prostaglandin E_2. However, the magnitude in febrile response induced by ventricular injection was significantly greater than that induced by preoptic injection. Mean maximum rises in the rectal temperature for 4 h after ventricular or preoptic injection of several doses of prostaglandin E_2 over the range of 100–2000 ng are presented in Fig. 2. The injection-dose into each side of the bilateral preoptic regions was half of the injection dose into the ventricle. Therefore, doses injected into the preoptic region represent the total doses of bilateral injections. Both injections produced dose-dependent fever. However, it is clearly demonstrated that febrile responses induced by ventricular injection of prostaglandin E_2 were significantly greater than those by intrapreoptic injection. These results suggest the possibility that there exist regions more sensitive to prostaglandin E_2 than the preoptic region for producing fever.

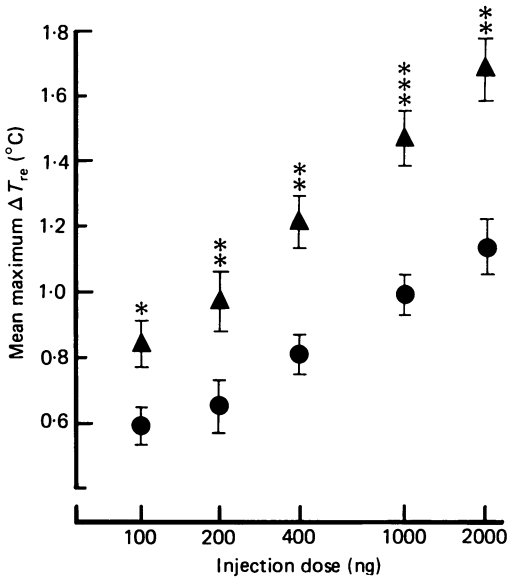

Fig. 2. Mean maximum rise (mean ± S.E.M.) in rectal temperature (ΔT_{re}) in the same group of seven rabbits after intaventricular (▲) and intrapreoptic (●) injection of prostaglandin E_2 of several doses. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

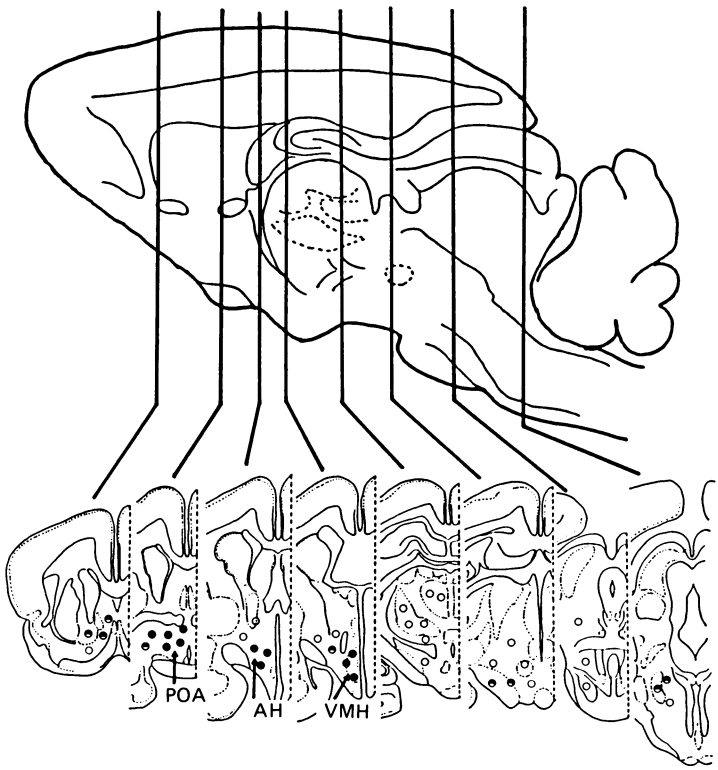

Fig. 3. The rabbit brain regions illustrating the distribution of injection sites. Also indicated is the sensitivity to prostaglandin E_2 of each site, based upon the dose of prostaglandin E_2 which evoked a criterion level of effect (above 0·5 °C increase in rectal temperature within 4 h). ○, sites not sensitive to 100 ng. ◓, sites sensitive to 100 but not to 50 ng. ●, sites sensitive to both 100 and 50 ng. Abbreviations: POA, preoptic area; AH, anterior hypothalamus; VMH, ventromedial hypothalamus.

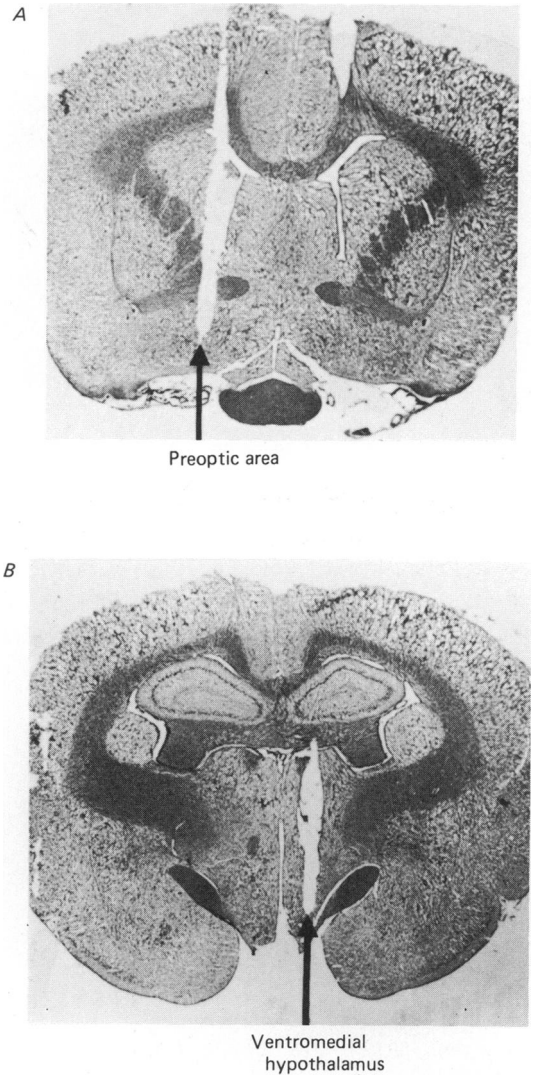

Fig. 4. Haematoxylin-eosin staining sections indicated (arrows) by tip of cannula in preoptic area (*A*) and ventromedial hypothalamus (*B*).

Figure 3 shows the forty brain regions of ten rabbits, in which the effects of microinjection of prostaglandin E_2 (50 and 100 ng) on the rectal temperature were investigated. We determined the sensitive sites for injecting prostaglandin E_2 by the criterion that the maximum rise in the rectal temperature should be above 0·5 °C. As shown in Fig. 3, the sites near the preoptic area, anterior hypothalamic region and the ventromedial hypothalamic region were observed to be highly sensitive to prostaglandin E_2 in both 50 and 100 ng injection doses. The saline (1 μl) as the control injected into any brain regions 1 h before injection of prostaglandin E_2 did not affect the rectal temperature.

To compare the sensitivities to microinjections of prostaglandin E_2 of the preoptic region and the ventromedial hypothalamic region, we have examined the febrile response induced by the same injection dose of prostaglandin E_2 using the same group of rabbits. The positions of the tips of the stainless-steel tubes, which were histologically identified (Fig. 4), are summarized in Fig. 5*A*. Figure 5*B* shows the

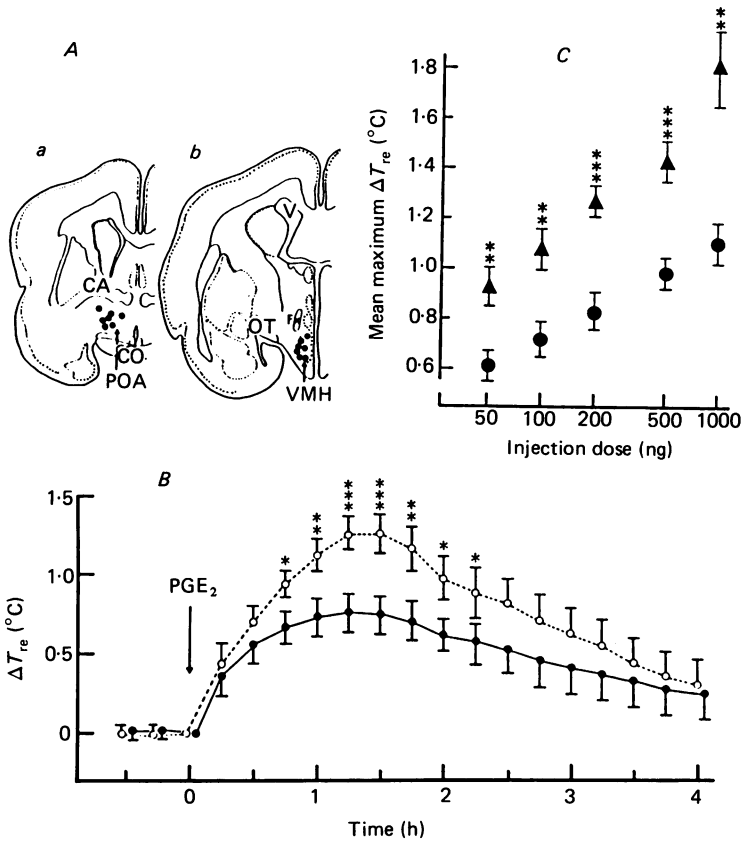

Fig. 5. *A*, the position of the injection sites of brain regions in the same group of seven rabbits. Abbreviations: POA, preoptic area; VMH, ventromedial hypothalamus; CA, anterior commissure; CO, optic chiasma; OT, optic tract; V, ventricle. *B*, mean changes (mean ± S.E.M.) in rectal temperature (ΔT_{re}) in the same group of seven rabbits after intrapreoptic (●) and intraventromedial hypothalamic (○) injection of prostaglandin E_2 (200 ng). *C*, mean maximum rise in rectal temperature (ΔT_{re}) in the same group of seven rabbits after intrapreoptic (●) and intraventromedial hypothalamic (▲) injection of prostaglandin E_2 of several doses. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

febrile responses after intrapreoptic or intraventromedial hypothalamic injection of 200 ng of prostaglandin E_2. The febrile responses induced by intraventromedial hypothalamic injection are significantly greater than those by intrapreoptic injection. Mean maximum rise in the rectal temperature for 4 h after intrapreoptic or intraventromedial hypothalamic injection of several doses of prostaglandin E_2 over the range of 50–1000 ng are presented in Fig. 5*C*. It is apparent that febrile

responses induced by intraventromedial hypothalamic injection of prostaglandin E_2 are significantly greater than those produced by intrapreoptic injection.

The changes in the rectal and skin temperatures and the oxygen consumption during fever induced by intraventromedial hypothalamic injection of prostaglandin E_2 at the different ambient temperature are shown in Fig. 6. At an ambient temperature of 24 ± 1 °C, skin temperature decreased significantly during the rising phase of the rectal temperature, while oxygen consumption did not change. In contrast, at an ambient temperature of 10 ± 1 °C, oxygen consumption increased during the rising phase of the rectal temperature without significant changes in the skin temperature.

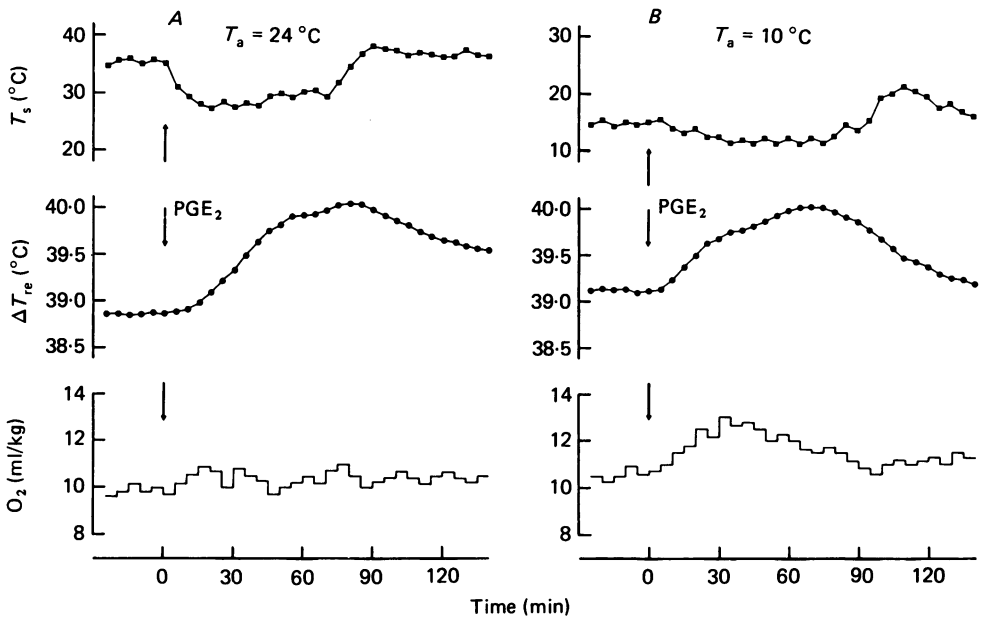

Fig. 6. The changes in the rectal (ΔT_{re}) and ear skin (T_s) temperatures and oxygen consumption during fever induced by intraventromedial hypothalamic injection of prostaglandin E_2 (PGE_2, 200 ng) at different ambient temperatures (T_a). *A*, $T_a = 24$ °C, *B*, $T_a = 10$ °C.

DISCUSSION

Based on previous results, the preoptic and anterior hypothalamic (PO/AH) region has been believed to be a target specifically sensitive to injection of prostaglandin E in rabbits (Stitt, 1973) and rats (Williams *et al.* 1977). However, many previous reports showed that after lesion or removal of the PO/AH region animals still responded to intravenous injection of endotoxin and endogenous pyrogen (Andersson *et al.* 1965; Veale & Cooper, 1975; Lipton & Trzcinka, 1976; Blatteis & Banet, 1986), or intraventricular injection of prostaglandin (Lipton & Trzcinka, 1976), for producing fever of almost the same magnitude as that observed in the control group. These results indicate that in the CNS there is multiple control of fever production. The first part of the present results further stresses this idea. It

also suggests that there exists a region(s) more sensitive to prostaglandins than the preoptic region for producing fever, because ventricular injection of prostaglandin E_2 produced greater fever than intrapreoptic injection.

Recently we have clarified that prostaglandin related to the fever induction is synthesized both inside and outside the blood–brain barrier (Morimoto *et al.* 1987). However, according to the recent theory of Stitt (1985, 1986), the site of release of endogenous prostaglandin in response to circulating endogenous pyrogen is the organum vasculosum laminae terminalis (OVLT) and, furthermore, the OVLT, rather than the PO/AH, is a site sensitive to prostaglandin for producing fever. As Stitt speculates (1985), within 3–6 days after lesion of the OVLT, the regions near the lesioned sites may become irritative or inflammatory, and subsequently sensitize the action of endogenous pyrogen. However, even about 3 weeks after lesion, the febrile response to endogenous pyrogen still remains at the level of that before the lesion, although it is inferred that the site of OVLT lesion might be replaced by granulation tissues and that the site sensitive to prostaglandin and of release of prostaglandin were markedly destroyed. Therefore, the possibilities cannot be excluded: (1) that increased prostaglandins in the circulation (Skarnes, Brown, Hull & McCracken, 1981) enter into the cerebrospinal fluid across the blood–brain barrier (Dascombe & Milton, 1979), and (2) that prostaglandin is synthesized and released from specific and/or non-specific regions (Dinarello & Bernheim, 1981), as well as the OVLT, within the CNS.

In Stitt's results using rats (1986), the fever-sensitivity of the OVLT to prostaglandin is greater than that of the PO/AH. However, he did not compare the febrile responses induced by prostaglandin injected into the OVLT with those induced by injection into the third ventricle. The OVLT is located medially and rostal to the PO/AH, and this area is also near the anterior wall of the third ventricle. Therefore it is doubtful that prostaglandin which might have been injected into the OVLT could flow into the third ventricle. Subsequent to the flow of prostaglandin into the third ventricle, as the present study showed, a greater febrile response was induced. Furthermore, the OVLT of the rat is more sensitive to prostaglandin, on a dose-comparable basis, than the ventromedial hypothalamic region of the rabbit reported in our present results. However, since the febrile responsiveness to prostaglandin injected into the rat's PO/AH is significantly greater than that to prostaglandin injected into rabbit's PO/AH (Stitt, 1986), the fever-sensitivity to prostaglandin between rat's and rabbit's brains can not be simply compared.

In the present study, we have discovered that the ventromedial hypothalamic region is the most sensitive site to prostaglandin E_2 for producing fever. With regard to the involvement of the ventromedial hypothalamic region in thermoregulation, we have already proved that metabolic activity of this region increases during exposure to cold (Morimoto & Murakami, 1985) and local electrical stimulation of this region induces a total pattern of cold defence responses (i.e. increase in heat production and decrease in heat loss; Perkins, Rothwell, Stock & Stone, 1981; Morimoto, Murakami, Ono, Watanabe & Sakata, 1986). Therefore, it is inferred that the ventromedial hypothalamic region plays important roles in the body temperature regulation not only during exposure to cold but also during fever. Furthermore, the present findings show that the changes in thermoregulatory outputs which occurred

during fever induced by injection of prostaglandin E_2 into the ventromedial hypothalamic region were different under different ambient temperatures. This excludes the possibility that prostaglandin E_2 injected into the ventromedial hypothalamic region is acting as a non-specific efferent pathway stimulant or suppressant. The ability of ambient temperature to modify the manner in which fever was developed in response to prostaglandin E_2 injection into the ventromedial hypothalamic region is consistent with the idea that thermal balance is well maintained during fever.

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