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MULTIPLE CONTROL OF FEVER PRODUCTION IN THE CENTRAL NERVOUS SYSTEM OF RABBITS

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SUMMARY

1. The effects of microinjection of prostaglandin D_2, E_2 and $F_{2\alpha}$ and of endogenous pyrogen on the rectal temperature of rabbits were extensively examined in sixty-eight brain regions and in the third cerebral ventricle.

2. Intracerebroventricular injection of both prostaglandins E_2 and $F_{2\alpha}$ produced dose-dependent fever over a range of 100–1000 ng. The selective brain regions, the nucleus broca ventralis, preoptic area, anterior hypothalamus and the ventromedial hypothalamus, responded to microinjections of a small dose (< 200 ng) of prostaglandins E_2 and $F_{2\alpha}$ by producing fever. Furthermore, the lateral hypothalamus, ventral thalamus, substantia nigra and the trigeminal nucleus were also sensitive to high concentrations of prostaglandins E_2 and $F_{2\alpha}$, fever being produced. It is likely that prostaglandin D_2 is not involved in fever induction.

3. The ventricular injection of endogenous pyrogen also produced fever. However, brain regions sensitive to microinjection of endogenous pyrogen were exclusively localized to regions near the organum vasculosum laminae terminalis (OVLT), such as the nucleus broca ventralis and the preoptic area. In contrast to the monophasic fever induced by prostaglandins E_2 and $F_{2\alpha}$, about 30 min after ventricular or cerebral injection of endogenous pyrogen the rectal temperature gradually started to rise and the fever was prolonged over 4 h.

4. We investigated the effect of an inhibitor of prostaglandin synthesis, sodium salicylate, on biphasic fever induced by intravenous injection of bacterial endotoxin. The microinjections of sodium salicylate into the bilateral regions near the OVLT suppressed the second peak but had no effect on the first peak.

5. The present study clarifies that there exist two separate mechanisms of induction of biphasic fever. Correlating with the first peak of biphasic fever, prostaglandins synthesized outside the blood–brain barrier act on multiple sites in the central nervous system to induce fever. Correlating with the second peak, endogenous pyrogen acts on regions near the OVLT to synthesize and release pyrogenic prostaglandins.

INTRODUCTION

In recent years, considerable interest has been focused on the final steps involved in the pathogenesis of fever. According to the current theory, fever is caused by an endogenous pyrogen (EP) (Atkins, 1960), recently known as interleukin 1 (Dinarello, 1984) which is released by monocytes in response to such pathogenic stimuli as bacterial endotoxin. It has been generally believed that EP induces fever by its action on the central nervous system (CNS). However, recently we proposed a new hypothesis (Morimoto, Murakami, Nakamori & Watanabe, 1987) that there exist two separate mechanisms of induction of biphasic fever, one outside and one inside the blood–brain barrier. The first phase is caused by EP acting on structures outside the blood–brain barrier, which subsequently synthesize and release prostaglandins, which, in turn, cause fever. The second phase is caused by EP entering into the cerebrospinal fluid across the circumventricular organ (Blatteis, Bealer, Hunter, Llanos, Ahokas & Mashburn, 1983) where the blood–brain barrier is absent. Subsequently, EP acts on structures within the blood–brain barrier to release prostaglandins (Dinarello & Bernheim, 1981) which cause fever. Thus, in both mechanisms, it is apparent that prostaglandins are involved in the central mechanism of fever production (Milton & Wendlandt, 1971; Feldberg & Gupta, 1973; Stitt, 1973; Morimoto *et al.* 1987).

The preoptic and anterior hypothalamic (PO/AH) region has been believed to be the primary centre not only of body temperature regulation but also of fever production (Cooper, Cranston & Honour, 1967; Stitt, 1973; Williams, Rudy, Yaksh & Viswanathan, 1977). However, many previous results showed that after removal of the entire PO/AH region, fever is still observed with magnitude similar to that found in the control (Andersson, Gale, Hokfelt & Larsson, 1965; Veale & Cooper, 1975; Lipton & Trzcinka, 1976; Blatteis & Banet, 1986). Our recent study suggested (Morimoto, Murakami, Nakamori & Watanabe, 1988) that in the CNS there exist multiple sites responsible for fever control. In the present study, by exploring extensively the sites sensitive to microinjection of prostaglandins D_2, E_2 and $F_{2\alpha}$ and EP into sixty-eight brain regions throughout the brain stem, we clarified that many brain regions react to prostaglandins E_2 and $F_{2\alpha}$ by producing fever. However, EP acts exclusively in regions near the preoptic area and the organum vasculosum laminae terminalis (OVLT) to induce fever. Furthermore, microinjection of an inhibitor of prostaglandin synthesis, sodium salicylate, into the region near OVLT suppressed the second peak of biphasic fever induced by an intravenous injection of bacterial endotoxin, but had no effect on the first peak. The present study has clarified that, in the CNS, there are multiple control sites for fever production, and furthermore it has supported our hypothesis that in the CNS there exist two separate mechanisms of induction of biphasic fever.

METHODS

The animals used in this study were male New Zealand white rabbits weighing 3·0–4·0 kg. The present study consisted of three experimental groups of animals. In Expt 1, each animal ($n = 6$) had been implanted previously with one stainless-steel guide tube (1·0 mm o.d.) in the third ventricle for injection of pyrogenic substances. In Expt 2, each animal ($n = 18$) had been implanted

with four stainless-steel guide tubes in sixty-eight different regions throughout the brain stem and in the third ventricle, according to the rabbit brain atlas (Sawyer, Everett & Green, 1954), by standard stereotaxic techniques (Sawyer *et al.* 1954). In Expt 3, each animal ($n = 18$) had been implanted bilaterally (L, 1·0 mm in left and right sides) with two stainless-steel tubes in the regions near the preoptic area, anterior hypothalamic area or the ventromedial hypothalamic area. These implantations were done at least 10 days before the start of the experiment under general anaesthesia (sodium pentobarbitone, 20 mg/kg, I.V.). After completion of the experiment, the positions of tips of the guide tubes were histologically identified (Sawyer *et al.* 1954; Shek, Wen & Wisniewski, 1986) and the regions where the tips of the tubes were located are shown in the Results. Experimental schedule and injection methods were the same as in our preceding paper (Morimoto *et al.* 1988). For the cerebral injections, the volume was always 1 μl for prostaglandins and 2 μl for endogenous pyrogen. Each injection was performed over a period of 1 min. The injection doses in each experimental group are described in the Results.

Prostaglandins D_2, E_2 and $F_{2\alpha}$ (Ono) were dissolved in sterile saline containing 2% ethanol. The endogenous pyrogen (EP) used in this experiment was prepared from white blood cells of male rabbits (New Zealand white strain). The white blood cells were stimulated by lipopolysaccharide of *Salmonella typhosa* endotoxin (Difco). The general procedures have been described in detail elsewhere (Morimoto, Watanabe, Ono, Sakata & Murakami, 1986). Partial purification was achieved by ultrafiltration using two types of membranes (10YM10, 10XM50, Amicon), which removed all substances of molecular weight outside the range of 10000–50000. Consequently, 1·0 ml of this partially purified EP solution was derived from approximately $1{\cdot}5 \times 10^7$ white blood cells. The intravenous injection of 0·2 ml/kg of this EP solution produced monophasic fever (> 1 °C) in rabbits ($n = 4$). Furthermore, we confirmed that the intravenous injection of heat-treated EP (0·2 ml/kg), which had been inactivated by heating in a hot water bath at 60 °C for 30 min, did not cause fever in rabbits. This result demonstrated that this partially purified endogenous pyrogen was not contaminated with endotoxin. The bacterial endotoxin used in this study was lipopolysaccharide of *Salmonella typhosa* (Difco), which was dissolved in sterile saline at a concentration of 20 μg/ml. Sodium salicylate was dissolved in sterile saline at a concentration of 60 mg/ml.

RESULTS

The intracerebroventricular injection of prostaglandins E_2 and $F_{2\alpha}$ produced a dose-dependent fever over a range of 100–1000 ng. The febrile patterns induced by both prostaglandins E_2 and $F_{2\alpha}$ were almost the same; typically, a monophasic fever with a short latency. Intraventricular injections of doses less than 1000 ng of prostaglandin D_2 did not affect the rectal temperature. However, about 30 min after injection of a dose greater than 10 μg of prostaglandin D_2, the rectal temperature gradually started to rise and the fever was significantly prolonged. Figure 1*A* shows changes in the rectal temperature after intraventricular injections of prostaglandins D_2 (20 μg), E_2 (200 ng) and $F_{2\alpha}$ (200 ng). The intraventricular injection of EP (20 and 50 μl) also induced fever. As shown in Fig. 1*B*, about 30 min after intraventricular injection of EP the rectal temperature gradually started to rise and the fever was prolonged over 4 h. In addition, intraventricular injection of heat-treated EP (50 μl), which had been inactivated by heating in a hot water bath at 60 °C for 30 min, did not cause fever.

To explore the regions sensitive to prostaglandins E_2, $F_{2\alpha}$ and D_2, the effect of microinjection with each prostaglandin (200 and 1000 ng) on the rectal temperature was extensively examined in sixty-eight different regions throughout the brain stem and the third ventricle. Figures 2*A*, 3*A* and 4*A* show the brain regions illustrating the distribution of injection sites. Also indicated is the sensitivity to prostaglandins E_2 (Fig. 2*A*), $F_{2\alpha}$ (Fig. 3*A*) and D_2 (Fig. 4*A*), which evoked a criterion level of effect

(increase greater than 0·5 °C in rectal temperature within 3 h); ○ indicates the sites not sensitive to 1000 ng, ▲ indicates the sites sensitive to 1000 but not to 200 ng, and ● indicates the sites sensitive to both 200 and 1000 ng.

As shown in Figs 2*A* and 3*A*, many brain regions react to injection with prostaglandins E_2 and $F_{2\alpha}$ by producing fever. In each case of febrile response induced by microinjection of prostaglandins E_2 and $F_{2\alpha}$, the fever was initiated

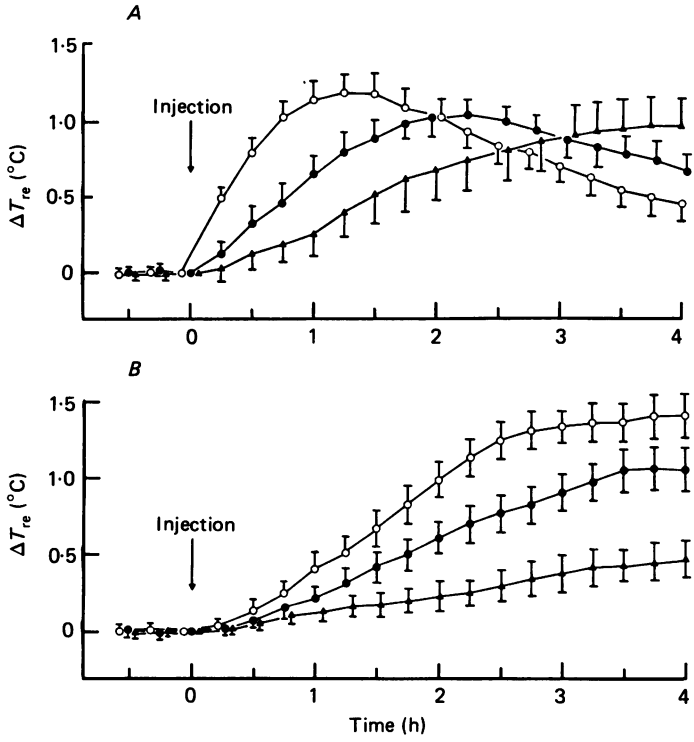

Fig. 1. *A*, mean changes (mean ± S.E. of mean) in rectal temperature (ΔT_{re}) in the same group of six rabbits after intracerebroventricular injections of prostaglandin D_2 (▲, 20 μg), prostaglandin E_2 (○, 200 ng) and prostaglandin $F_{2\alpha}$ (●, 200 ng). *B*, mean changes (mean ± S.E. of mean) in rectal temperature (ΔT_{re}) in the same group of six rabbits after intracerebroventricular injection of endogenous pyrogen (EP) (○, 50 μl; ●, 20 μl) and heated EP (▲, 50 μl).

within a short period after injection. The febrile pattern was monophasic, similar to the fever induced by intraventricular injection. Mean maximum rises in rectal temperature after injection of several doses of prostaglandins E_2 and $F_{2\alpha}$ are shown in Fig. 2*B* and Fig. 3*B*, respectively. It appears that the regions of nucleus broca ventralis, preoptic area, anterior hypothalamus and the ventromedial hypothalamus are highly sensitive to prostaglandins E_2 and $F_{2\alpha}$. Moreover, the lateral hypothalamus, ventral thalamus, substantia nigra and the trigeminal nucleus respond to high concentrations of prostaglandins E_2 and $F_{2\alpha}$ by producing fever.

A high concentration of prostaglandin D_2 (1000 ng) also produced fever, although

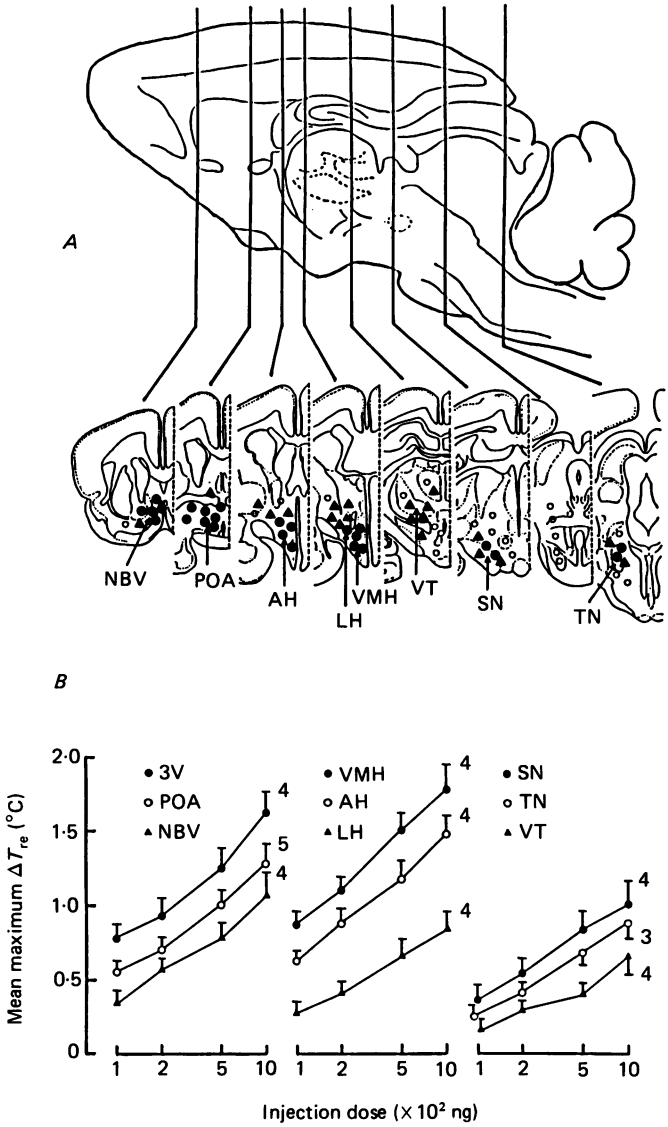

Fig. 2. *A*, the rabbit brain regions illustrating the distribution of injection sites. Also indicated is the sensitivity of each site to prostaglandin E_2, based upon the dose of prostaglandin E_2 which evoked a criterion level of effect (increase greater than 0·5 °C in rectal temperature within 3 h). ○, sites not sensitive to 1000 ng, ▲, sites sensitive to 1000 but not to 200 ng; ●, sites sensitive to both 1000 and 200 ng. *B*, Mean maximum rise (mean ± S.E. of mean) in rectal temperature (ΔT_{re}) after injection of prostaglandin E_2 over a range of 100–1000 ng into selective brain regions. Abbreviations: 3V, third ventricle; NBV, nucleus broca ventralis; POA, preoptic area; AH, anterior hypothalamus; VMH, ventromedial hypothalamus; LH, lateral hypothalamus; VT, ventral thalamus; SN, substantia nigra; TN, trigeminal nucleus. Numbers to the right of curves represent number of experiments.

the sensitive sites are exclusively localized in the preoptic area, anterior hypothalamus and the ventromedial hypothalamus. As shown in Fig. 4*B*, the febrile pattern was almost identical to that induced by intraventricular injection (Fig. 1*A*). However, the injections of doses lower than 1000 ng into any other regions did not affect the rectal temperature.

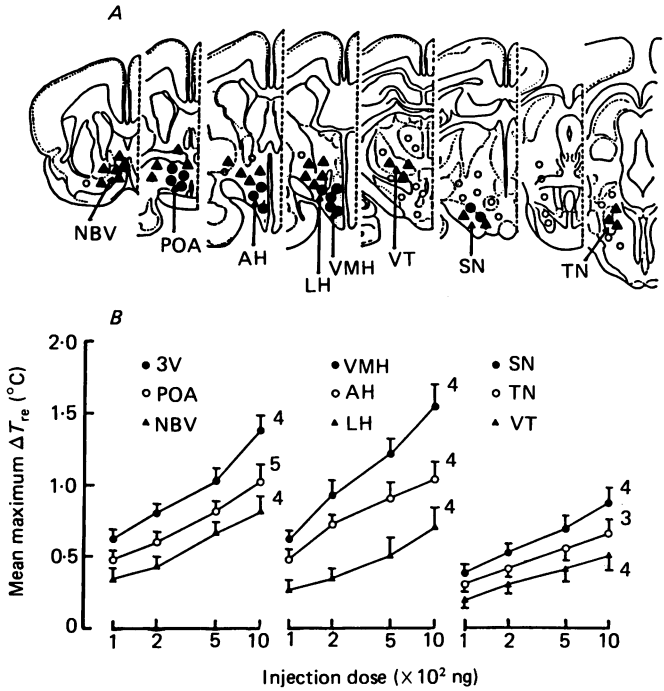

Fig. 3. *A*, the rabbit brain regions illustrating the distribution of injection sites. Also indicated is the sensitivity of each site to prostaglandin $F_{2\alpha}$, based upon the dose of prostaglandin $F_{2\alpha}$ which evoked a criterion level of effect (increase greater than above 0·5 °C in rectal temperature within 3 h). ○, sites not sensitive to 1000 ng; ▲, sites sensitive to 1000 but not to 200 ng; ●, sites sensitive to both 1000 and 200 ng. *B*, mean maximum rise (mean ± S.E. of mean) in rectal temperature (ΔT_{re}) after injection of prostaglandin $F_{2\alpha}$ over a range of 100–1000 ng into selective brain regions. For abbreviations see the legend for Fig. 2. Numbers to the right of curves represent number of experiments.

In order to determine the regions sensitive to EP, the effect of microinjection of EP (2 μl) on the rectal temperature was examined. Figure 5*A* illustrates the distribution of injection sites and those sensitivities to EP. The sensitivity to EP was determined by the criterion level of effect in which 2·0 μl of EP induced increases greater than 0·5 °C (▲) and 1 °C (●) in the rectal temperature within 4 h; ○ indicates the insensitive sites. As shown in Fig. 5*A*, only the nucleus broca ventralis, preoptic area and the anterior hypothalamus are sensitive to EP. In contrast to the fever induced by prostaglandins E_2 and $F_{2\alpha}$, about 30 min after injection of EP, the body temperature gradually started to rise and the fever was significantly prolonged

(Fig. 5*B*). This pattern was almost identical to that induced by intraventricular injection of EP.

We also investigated the effect of an inhibitor of prostaglandin synthesis, sodium salicylate, on febrile response induced by intravenous endotoxin (2 μg/kg). At the rising phases of the first and the second peak of the biphasic fever, sodium salicylate

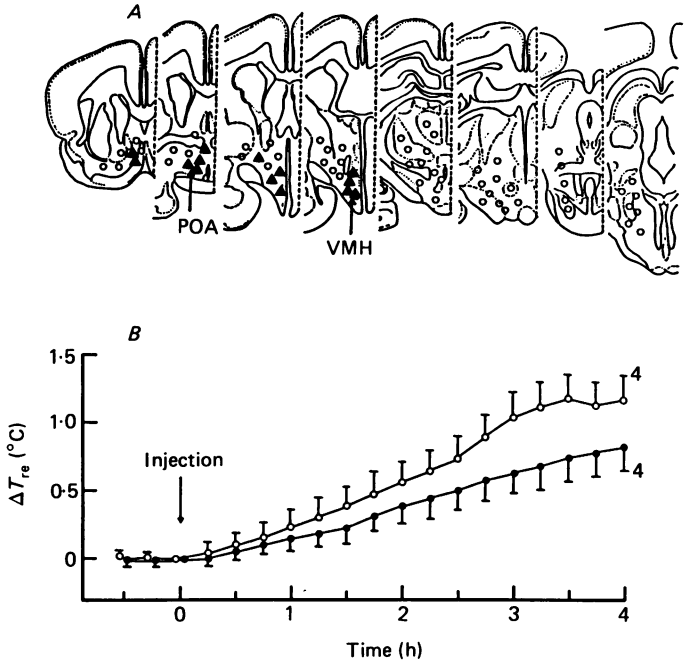

Fig. 4. *A*, the rabbit brain regions illustrating the distribution of injection sites. Also indicated is the sensitivity of each site to prostaglandin D_2 based upon 1 μg of prostaglandin D_2 evoking a criterion level of effect (increase of greater than 0·5 °C in rectal temperature within 4 h). ○, sites not sensitive; ▲, sites sensitive to 1000 ng. *B*: mean changes (mean ± S.E. of mean) in rectal temperature (ΔT_{re}) after injection of prostaglandin D_2 into the selective brain regions. For abbreviations see the legend for Fig. 2. Numbers to the right of curves respresent number of experiments.

(60 μg in 1 μl) was bilaterally and simultaneously injected into the regions where the injections of prostaglandins and/or EP produced significant fever (Fig. 6*A*). In Fig. 6*A* filled symbols indicate the regions where antipyretic action of sodium salicylate was observed. Figure 6*B* shows the effect of sodium salicylate or saline, as a control, injected into regions shown by filled symbols in Fig. 6*A*, on the febrile response induced by intravenous endotoxin. The microinjections of sodium salicylate into the regions near the nucleus broca ventralis and the preoptic area, which are also regions localized near the organum vasculosum laminae terminalis (OVLT; Fig. 6*A*), suppressed the second peak but had not effect on the first peak. The injections into the other regions had no effect on either the first or the second peak of biphasic fever.

DISCUSSION

Since Milton & Wendlandt (1971) observed the strong pyrogenic action of intraventricular prostaglandin E_1, prostaglandins have been generally believed to be possible final mediators in the CNS involved in pathogenesis of fever. In the present study, the pyrogenicities of intraventricular and intracerebral injections of

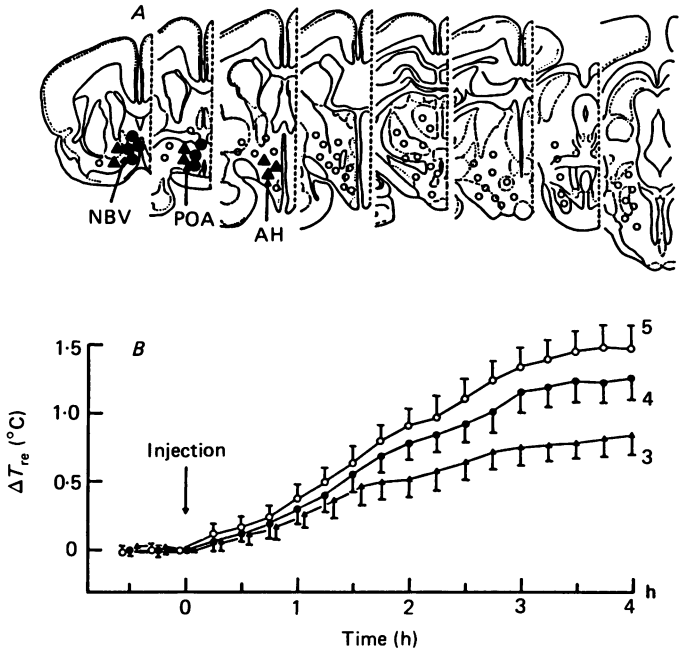

Fig. 5. *A*, the rabbit brain regions illustrating the distribution of injection sites. Also indicated is the sensitivity to endogenous pyrogen of each site based upon 2 μl of endogenous pyrogen evoking a criterion level of effect. ○, sites not sensitive; ▲, sites sensitive (increase greater than 0·5 °C in rectal temperature within 4 h); ●, sites sensitive (increase greater than 1·0 °C in rectal temperature within 4 h). For abbreviations see the legend for Fig. 2. *B*, mean changes (mean ± S.E. of mean) in rectal temperature (ΔT_{re}) after injection of endogenous pyrogen into selective brain regions. Numbers to the right of curves represent number of experiments.

prostaglandin E_2 are almost identical to those of prostaglandin $F_{2\alpha}$. However, the latency to onset and time to peak of febrile response induced by prostaglandin $F_{2\alpha}$ seem to be longer than those induced by prostaglandin E_2. As compared with the previous results (Stitt & Bernheim, 1985; Stitt, Shimada & Bernheim, 1985; Morimoto *et al.* 1986), the time course of febrile response induced by prostaglandin E_2 closely resembles that of fever induced by intravenous injection of EP or endotoxin in rabbits. Intraventricular and intracerebral injection of prostaglandin D_2 produced hyperthermia when given at a high dose, but the patterns of febrile responses were quite different from those induced by prostaglandins E_2 and $F_{2\alpha}$. Forstermann, Heldt & Hertting (1983) reported that ventricular prostaglandin D_2

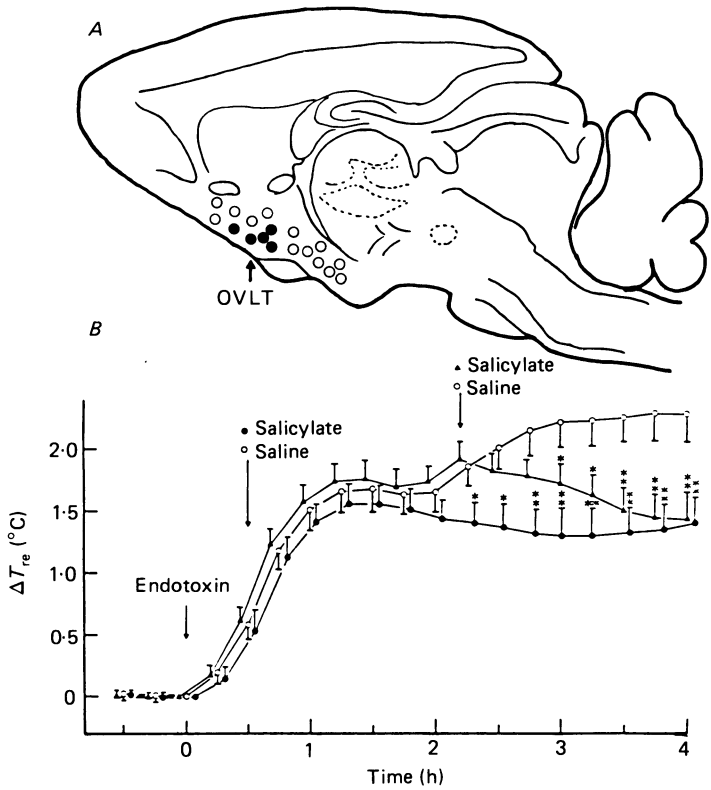

Fig. 6. *A*, the rabbit brain region illustrating the distribution of bilateral injection sites. Also indicated is the sensitivity of respective sites to sodium salicylate (60 μg in each side). ○, regions not sensitive to sodium salicylate; ●, regions where antipyretic action of sodium salicylate was observed. OVLT, Organum vasculosum laminae terminalis. *B*, mean changes (mean ± S.E. of mean) in rectal temperature (ΔT_{re}) after intravenous injection of bacterial endotoxin (2 μg/kg) into the rabbits ($n = 5$) which were bilaterally implanted with guide-tubes at the regions denoted by ● in Fig. 6*A*. Note that the bilateral injections of sodium salicylate (60 μg in each side) suppressed the second peak (●, ▲) of biphasic fever but had no effect on the first peak (●). Saline injection as a control (○) did not affect either the first or the second peak. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

produced hyperthermia at a dose of 20 μg, while 0·2 μg did not affect the body temperature. However, the concentration of prostaglandin D_2 necessary to induce hyperthermia is significantly higher than the concentration detected in the cerebrospinal fluid during fever (Feldberg & Gupta, 1973; Bernheim, Gilbert & Stitt, 1980). Therefore, it is likely that prostaglandin D_2 is not involved in pathogenesis of naturally occurring fever. Moreover, the hypothermic effects induced by prostaglandin D_2 in rats (Ueno, Narumiya, Ogorochi, Nakayama, Ishikawa & Hayaishi, 1982) were not observed in rabbits.

According to some previous results, the PO/AH region has been believed to be a target specifically sensitive to injection of prostaglandin E in rabbits (Stitt, 1973) and rats (William *et al.* 1977). However, other reports showed that after lesion or removal of the PO/AH region animals still responded to intravenous injection of

endotoxin or EP (Andersson *et al.* 1965; Veale & Cooper, 1975; Lipton & Trzcinka, 1976; Blatteis & Banet, 1986) or intraventricular injection of prostaglandin (Lipton & Trzcinka, 1976) by producing fever of a magnitude comparable to that observed in the control group. These results indicate that in the CNS there are multiple sites responsible for fever control.

We have reported in a preceding paper (Morimoto *et al.* 1988) that prostaglandin E_2 produces fever more readily in the ventromedial hypothalamic region than in the PO/AH region. Furthermore, from the present results, it seems that many brain regions respond to microinjections of prostaglandins E_2 and $F_{2\alpha}$ by producing fever, although the responsiveness of the selective regions varies somewhat with injection dose. Accordingly, it is believed that in the CNS there are multiple sites which respond to prostaglandins E_2 and $F_{2\alpha}$ with fever production. However, when an inhibitor of prostaglandin synthesis, sodium salicylate, was injected into brain regions where pyrogenic action of prostaglandins was observed, we observed no effect on the first peak of the biphasic fever induced by intravenous injection of endotoxin. Our recent report (Morimoto *et al.* 1987) clarified that prostaglandin synthesis involved in fever induction occurs both inside and outside the blood–brain barrier; it thus seems that the first peak is caused by action of EP on structures outside the blood–brain barrier, causing synthesis and release of pyrogenic prostaglandins.

In contrast to the many regions sensitive to prostaglandins E_2 and $F_{2\alpha}$, those sensitive to microinjections of EP were very small and localized exclusively near the preoptic area and the OVLT. The sites sensitive to EP obtained from the present data are comparable to those observed by Cooper, Cranston & Honour (1967). We also noticed that to produce a magnitude of fever identical to that induced by cerebral injection of EP (2·0 μl), the dose of ventricular injection needs to be greater than 10 times that of cerebral injection, suggesting a very small number of action sites.

Based on our recent hypothesis (Morimoto *et al.* 1987), concerning the second peak of the biphasic fever, EP entering and/or produced within the blood–brain barrier acts on structures of the CNS to synthesize and release prostaglandins which, in turn, cause fever. Therefore, the regions near the sites where pyrogenic action of EP microinjections was observed are considered to synthesize pyrogenic prostaglandins in response to central EP during the second peak. This hypothesis is further confirmed by the fact that sodium salicylate injected into these regions suppressed the second peak of the biphasic fever, but had no effect on the first peak. Cranston & Rawlins (1972) also reported that 3 h after intravenous infusion of EP, microinjection of sodium salicylate produced antipyresis when given into the preoptic area, in a region almost identical to that shown in the present results. However, since EP is a protein, its passage through the blood–brain barrier is likely to be limited. Therefore, it seems likely that EP enters into these regions across the OVLT, one of the circumventricular organs where the blood–brain barrier is absent (Blatteis *et al.* 1983; Stitt, 1985), or that astrocytes and microglia existing near these regions release an EP-like substance (Fontana, Kristensen, Dubs, Gemsa & Weber, 1982; Fontana, Weber & Dayer, 1984). Moreover, the sites sensitive to EP do not always accord with the sites sensitive to prostaglandin E_2 or $F_{2\alpha}$. This evidence indicates that the regions near the preoptic area and the OVLT may synthesize prostaglandins in response to EP more than do other regions insensitive to EP.

In conclusion, we have established our hypothesis that there exist two separate mechanisms of induction of biphasic fever, one inside and one outside the blood–brain barrier. During the first peak of the biphasic fever, prostaglandins synthesized outside the blood–brain barrier act on multiple sites in the CNS to induce fever. In contrast, during the second peak, EP entering and/or produced near the OVLT acts on structures near the regions of the preoptic area and the OVLT, within the blood–brain barrier, to synthesize and release pyrogenic prostaglandins.

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