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FUNCTIONAL AND STRUCTURAL DIFFERENCES IN FEBRILE MECHANISM BETWEEN RABBITS AND RATS

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SUMMARY

1. Febrile responsiveness of rabbits and rats to intravenous (I.V.) or intracerebroventricular injection of human recombinant interleukin-1α (IL-1α), human recombinant interleukin-1β (IL-1β) and prostaglandin E_2 was examined.

2. The I.V. injection of both IL-1α and IL-1β produced dose-dependent fever over a range of 0·05–2·0 μg/kg. A small dose of IL-1α (0·5 μg/kg) or IL-1β (0·5 μg/kg) produced a monophasic patterned fever in both rabbits and rats. A large dose (2·0 μg/kg) of IL-1α or IL-1β produced a biphasic fever in rabbits, but monophasic fever in rats. Febrile responses in rabbits induced by I.V. injection of IL-1α or IL-1β were significantly greater than those in rats induced by these same injections. Furthermore, in both species, the pyrogenicity of I.V. IL-1β was greater than that of IL-1α.

3. The intracerebroventricular injection of both IL-1α and IL-1β produced dose-dependent fever over a range of 0·2–20 ng. In rabbits and rats, the ventricular injections of IL-1α and IL-1β produced fever with almost the same pattern – monophasic, regardless of injection doses. Although febrile responsiveness of rabbits to ventricular injection of IL-1α was greater than that of rats, responsiveness to IL-1β was almost the same in both species. Pyrogenicity of ventricular IL-1β was greater than that of IL-1α. However, febrile responses in rats induced by ventricular injections of several doses (2–2000 ng) of prostaglandin E_2 were greater than those in rabbits.

4. The present results show that febrile responsiveness of rabbits to I.V. IL-1 is significantly greater than that of rats. However, fever sensitivity within the central nervous system (CNS) of rats is not lower compared with that of rabbits. Therefore, we considered that between the two species there exist structural differences in the organum vasculosum laminae terminalis (OVLT), which is currently believed to be the pathway of pyrogen to the CNS and/or the site of production of pyrogenic prostaglandins.

5. Histological examinations showed that the rabbit's OVLT has two vascular components with capillaries, one in the layer near the third ventricle and the other in the layer near the subarachnoideal space. However, the rat's OVLT only has a single component, in the layer near the subarachnoideal space. Furthermore,

perivascular spaces around capillaries of the rabbit's OVLT are more widely developed than those of the rats.

6. The present results suggest that differences in febrile response and sensitivity between rabbits and rats are attributed to structural differences of the OVLT between the two species.

INTRODUCTION

In recent years, considerable interest has been focused on the final steps involved in the pathogenesis of fever. According to current theory (Atkins, 1960) a variety of exogenous pyrogens, such as bacterial endotoxin, stimulate white blood cells including many kinds of reticuloendothelial cells, which then synthesize and release small mediator proteins called endogenous pyrogen (EP). Subsequently, EP stimulates the structures near the central nervous system to synthesize and release prostaglandins (Milton & Wendlandt, 1971), which act on the central nervous system (CNS) to induce fever. Now it is generally accepted that an active component of endogenous pyrogen is interleukin-1 (Dinarello, 1984). However, in recent advances of immunology, several kinds of cytokines released from various kinds of leukocytes, such as interferon (Dinarello, Bernheim, Duff, Le, Nagabhushan, Hamilton & Coceani, 1984), tumour necrosis factor (Dinarello, Cannon, Wolff, Bernhein, Beutler, Cerami, Figari, Palladino & O'Connor, 1986), and B cell stimulatory factor-2 (interleukin-6) (Helle, Brakenhoff, DeGroot & Aarden, 1988), have been found to be intrinsically pyrogenic. Also, these cytokines, as well as interleukin-1, induce some acute phase responses, and it is now generally recognized that fever and acute phase responses induced by cytokines constitute a primary host defence reaction (Kluger, Ringer & Anver, 1975; Kluger & Rothenberg, 1979).

For a long time it has been generally believed that EP directly stimulates the CNS to induce fever. However, since EP is a protein, its passage through the blood–brain barrier is likely to be limited. As for the pathogenesis of fever production, recently we proposed a new hypothesis (Morimoto, Murakami, Nakamori & Watanabe, 1987) that there are 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 via 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 and/or being produced within the blood–brain barrier. Subsequently, EP acts on structures within the blood–brain barrier to release prostaglandins which cause fever. Thus, in both mechanisms it is apparent that prostaglandins are involved in the central mechanism of fever production. At the present time, however, the sites of prostaglandin synthesis related to fever induction and the pathway of prostaglandin to the CNS still remain unknown.

Previous experiments (Stitt, Shamada & Bernheim, 1985; Morimoto, Watanabe, Ono, Sakata & Murakami, 1986) have shown that febrile responsiveness of rabbits to intravenous injection of EP is significantly greater than that of rats. However, the response of rats to intracerebroventricular (Stitt, 1986) or intrahypothalamic injection (Morimoto, Murakami, Nakamori & Watanabe, 1988*a*; Morimoto, Murakami & Watanabe, 1988*b*) of prostaglandin is greater than that of rabbits. This evidence leads us to speculate that between rabbits and rats there are functional

and/or structural differences in the interface of the blood–brain barrier existing between the circulation side and CNS side. As for the interface in the translation of the EP stimuli into fever, the organum vasculosum laminae terminalis (OVLT), a circumventricular organ, is currently believed to be the route of passage of EP to the CNS and/or the site which produces pyrogenic prostaglandin in response to EP (Blatteis, Bealer, Hunter, Llanos-Q, Ahokas & Mashburn, 1983; Stitt, 1985; Morimoto, Murakami, Nakamori & Watanabe, 1988*c*). Therefore, in the present study we investigated the structural difference in the OVLT between the rabbit and the rat. Furthermore, by comparing the differences in the febrile response and the structure of the OVLT between the two species, we suggest the mechanism by which pyrogens act on the CNS to induce fever.

METHODS

The animals used in this study were male New Zealand White rabbits weighing 3·0–4·0 kg and male albino rats (Wistar strain) weighing 230–300 g. The present study consisted of two experimental series. In the first, we examined febrile responses in rabbits and rats to intravenous or intracerebroventricular injection of human recombinant interleukin-1α, human recombinant interleukin-1β and prostaglandin E_2. In the second, structures of the OVLT of rabbits and rats were compared histologically.

Febrile responses in rabbits and rats

A total of fourteen animals, rabbits and rats, were used. Seven animals of each species had been implanted previously with a stainless-steel cannula (0·8 mm o.d.) that had been located in the third ventricle by standard stereotaxic techniques. This implantation was done under general anaesthesia (sodium pentobarbitone, 20 mg/kg, I.V. for rabbits and 50 mg/kg, I.P. for rats) at least 10 days before the start of the experiment.

On the day of the experiment, animals were minimally restrained in conventional stocks at the thermoneutral ambient temperature of rabbits (21 ± 1 °C) and rats (26 ± 1 °C) between 08.30 and 16.00 h. To avoid the effect of stress due to restraint, all had been well trained to adapt to the stocks. Throughout the experiment, rectal temperature was measured every minute with a copper–constantan thermocouple. The rectal temperature of each animal was allowed to stabilize for at least 90 min before any injections. Only animals whose body temperatures were stable and in the range of 38·0–39·2 °C were used to determine the effect of injection. Intravenous injections in rabbits were made into the marginal ear vein through a sterile needle (25 gauge). In rats, intravenous injections were performed through the cannula inserted into a superior caval vein, by the previously reported method (Morimoto *et al.* 1986). Intracerebroventricular injections were made through a stainless-steel needle (0·4 mm o.d.) attached to a polyethylene tube, and the volume infused was always 10 μl for rabbits and 5 μl for rats.

Human recombinant IL-1α and IL-1β were supplied by the Dainippon Pharmaceutical Co. Ltd, Japan, and by the Otsuka Pharmaceutical Co. Ltd, Japan, respectively. These interleukins had been produced by recombinant strains of *Escherichia coli*. That they were carefully produced and endotoxin-free was confirmed by a *Limulus* amoebocyte lysate test ($< 0{\cdot}05$ pg/μg protein). The biological activity which was assayed by thymocyte co-stimulation activity was 2×10^7 U/mg protein in both IL-1α and IL-1β. The molecular weight of IL-1α was 18000 and that of IL-1β was 17400. The isoelectric point (PI) of IL-1α was 5·3 and that of IL-1β was 6·9.

For injection, the IL-1 was dissolved in sterile saline. These solutions were divided into several vials and stored at −40 °C until used. We used each vial within 2 days after thawing and thus avoided having to repeat freezing and thawing. Prostaglandin E_2 was dissolved in sterile saline containing ethanol (2%). Injection doses of IL-1 and prostaglandin E_2 in each experimental group are described in the Results.

Microvasculature and histology of the OVLT in rabbits and rats

To investigate the microvasculature of the OVLT, the rabbits were killed by intravenous injection of a large dose of sodium pentobarbitone and the rats were killed by ether. The thorax was then opened and the brain perfused with saline via the thoracic aorta. Immediately after blood was washed out by perfusion of the saline, saline containing formaldehyde (4%) was perfused to fix the brain tissue and then gelatine carbon solution (5%, Rotering, FRG) was further perfused so that the brain vessels were filled with carbon. The brain was then removed and immersed in the same fixative at 4 °C for 7 days. Thereafter, the brain was cut into sections (100–150 μm) by a microslicer (Dohan EM, Kyoto, Japan). These sections were stained with Luxol Fast Blue and Alum Carmine. For histological observations, brains of both species were perfused with a glutaraldehyde (2%) and formaldehyde (2%) mixture in cacodylate buffer (0·1 M, pH 7·4). via the thoracic aorta. Then the brain was removed to take out the OVLT. OVLTs were post-fixed with osmic acid (2%) for 2 h, dehydrated with a graded acetone and embedded in an Epon 812. Serial sections (1 μm) were made with an LKB ultratome III and were stained with a Methylene Blue and Azur II mixture.

RESULTS

Figure 1 shows changes in the rectal temperature of rabbits (Fig. 1*A*) and rats (Fig. 1*B*) after intravenous (I.V.) injection of IL-1α. In Fig. 1*A* and *B*, the I.V. injection of a small dose of IL-1α (0·5 μg/kg) produced a monophasic patterned fever in both rabbits and rats. A large dose (2·0 μg/kg) produced biphasic fever in rabbits, but only monophasic fever in rats. The time to the peak of the monophasic fever and to the first peak of the biphasic fever of rabbits was 60–80 min, and the second peak in the biphasic fever occurred at 180–210 min. However, in rats, the first peak occurred earlier at 20–30 min. Results in Fig. 1 show that febrile responsiveness of rabbits is greater than that of rats.

Mean maximum rise in the rectal temperature of rabbits and rats after I.V. injections of several doses of IL-1α or IL-1β over the range of 0·05–2·0 μg/kg are presented in Fig. 2. Both injections of IL-1α and IL-1β produced dose-dependent fever. However, it is clearly demonstrated that febrile responses in rabbits induced by both intravenous injections of IL-1α and those of IL-1β were significantly greater than those in rats induced by respective injections. Comparing the pyrogenicity between the interleukins, IL-1β was more pyrogenic than IL-1α. In addition, we examined the effects of higher intravenous doses of 20 μg/kg of IL-1α or IL-1β on the rectal temperature of rats ($n = 4$). Intravenous injections of 20 μg/kg of IL-1α still produced only monophasic fever, but those of IL-1β (20 μg/kg) produced biphasic fever.

Figure 3 shows changes in the rectal temperature of rabbits and rats after intracerebroventricular injection of 2 ng of IL-1α (Fig. 3*A*), 2 ng of IL-1β (Fig. 3*B*) and 200 ng of prostaglandin E_2 (Fig. 3*C*). In both species, the ventricular injections of IL-1α or IL-1β produced only monophasic fever with almost the same pattern. Although febrile responses in rabbits to ventricular IL-1α were greater than those in rats, the ventricular injection of IL-1β produced almost the same magnitude of fever in both species. On the other hand, as shown in Fig. 3*C*, rats showed greater febrile response to ventricular prostaglandin E_2 than rabbits. Comparing the febrile pattern induced by ventricular injection of prostaglandin E_2, the time to peak of fever in rats was shorter than that in rabbits, just as that observed in febrile responses induced by intravenous injection of IL-1s.

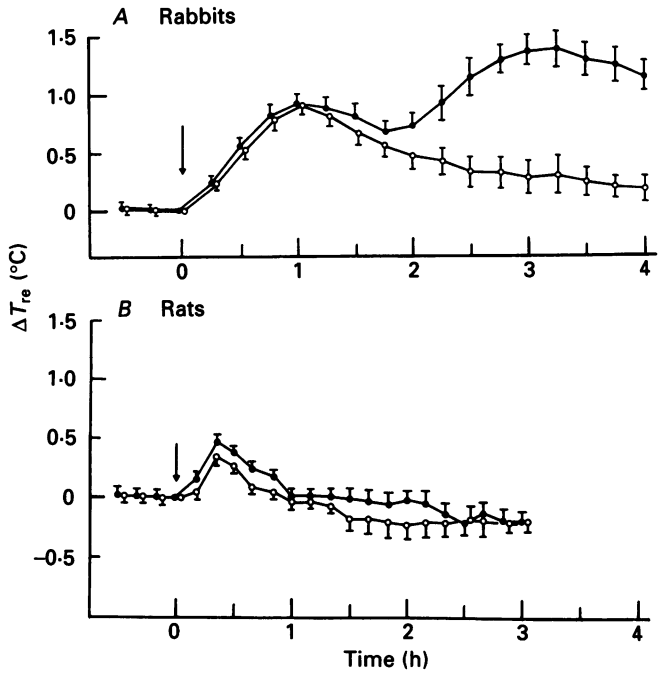

Fig. 1. Mean changes (mean ± S.E.M.) in rectal temperature (ΔT_{re}) in the same group of seven rabbits (*A*) and seven rats (*B*) after intravenous injection (arrows) of human recombinant interleukin-1α: ○, 0·5 μg/kg; ●, 2·0 μg/kg.

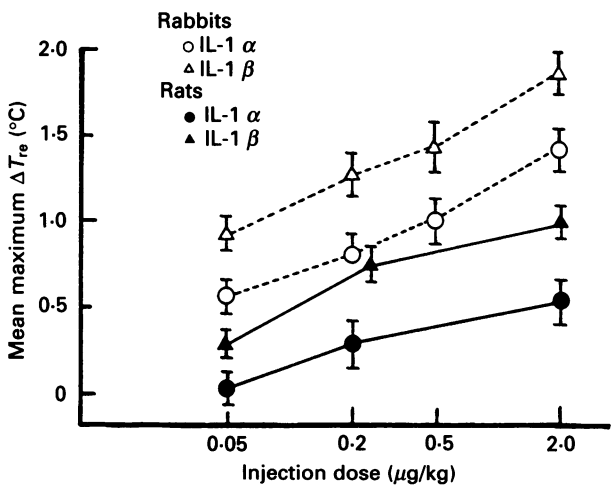

Fig. 2. Mean maximum rise (mean ± S.E.M.) in rectal temperature (ΔT_{re}) in the same group of seven rabbits and seven rats after intravenous injection of human recombinant inteleukin-1α (IL-1α) and IL-1β of several doses.

Mean maximum rise in the rectal temperature of rabbits and rats after intracerebroventricular injections of several doses of IL-1α or IL-1β over the range of 0·2–20 ng are presented in Fig. 4*A*. Injections of both IL-1α and IL-1β produced dose-dependent fever. Although febrile responsiveness in rabbits to ventricular

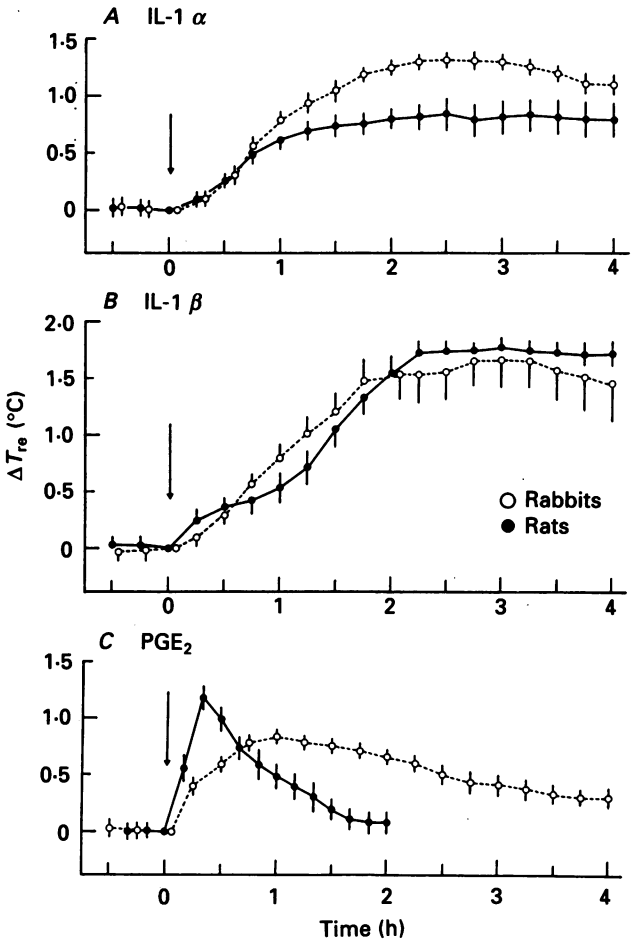

Fig. 3. Mean changes (mean ± S.E.M.) in rectal temperature (ΔT_{re}) in the same group of seven rabbits (○) and seven rats (●) after intracerebroventricular injection of human recombinant interleukin-1α (IL-1α) (2 ng, *A*), IL-1β (2 ng, *B*), and prostaglandin E_2 (PGE_2, 200 ng, *C*).

injections of IL-1α was greater than that in rats, responsiveness to IL-1β was almost the same in both species. Furthermore, it is apparent that pyrogenicity of IL-1β is greater than that of IL-1α. Figure 4*B* shows the mean maximum rise in the rectal temperature of rabbits and rats after intracerebroventricular injections of several doses of prostaglandin E_2 over the range of 2–2000 ng. The febrile responsiveness in rats to ventricular prostaglandin E_2 was greater than that in rabbits.

Figure 5 shows sagittal and horizontal sections (100–150 μm in thickness) of the organum vasculosum laminae terminalis (OVLT) of rabbit and rat brains in which

vessels had been filled with carbon solution. *A* and *B* are lower magnification and *C* and *D* higher magnification photomicrographs of the sagittal sections of the OVLT of a rat (*A* and *C*) and rabbit (*B* and *D*). *E* and *F* are horizontal sections from a rat (*E*) and rabbit (*F*). As shown in Fig. 5, the rabbit's OVLT has two vascular components with many capillaries, one in the layer near the third ventricle and the other one in the layer near the subarachnoideal space. However, the rat's OVLT has only a single vascular component in the layer near the subarachnoideal space.

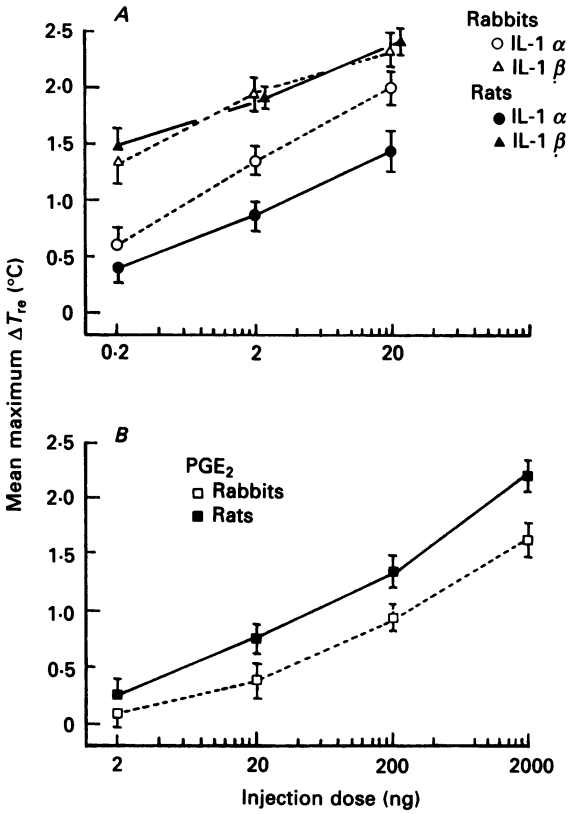

Fig. 4. Mean maximum rise (mean ± S.E.M.) in rectal temperature (ΔT_{re}) in the same group of seven rabbits and seven rats after intracerebroventricular injection of several doses of human recombinant interleukin-1 α (IL-1 α) and IL-1 β (*A*) and prostaglandin E_2 (PGE_2, *B*).

Figure 6 *A* and *B* shows sagittal sections (1 μm in thickness) of the OVLT of a rat (*A*) and rabbit (*B*). The rabbit has many perivascular spaces around the capillaries in both layers of the OVLT. Furthermore, comparing perivascular spaces of the rabbit with those of the rat, it can be seen that perivascular spaces of the rabbit are more widely developed than those of the rat. *C* and *D* are horizontal sections of a rabbit's OVLT. The perivascular spaces near the third ventricle are significantly developed. Furthermore, as shown in Fig. 6*D*, the ependymal cells covering the OVLT facing the third ventricle differ from normal cells covering the third ventricle, having a lack of cilia.

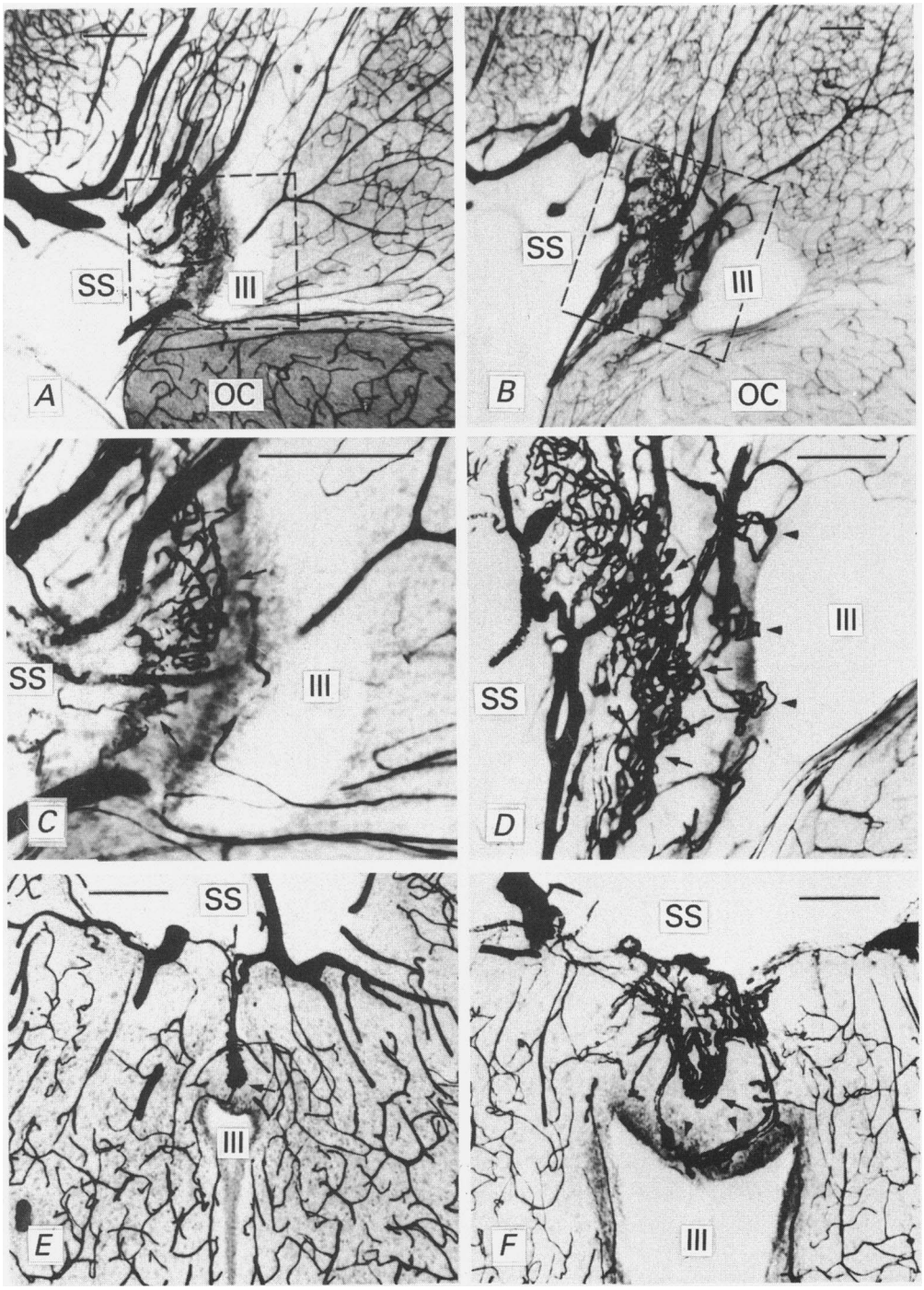

Fig. 5. Photomicrographs of sagittal and horizontal sections (100–150μm in thickness) of the organum vasculosum laminae terminalis (OVLT) of rats (*A*, *C* and *E*) and rabbits (*B*, *D* and *F*), in which vessels were filled with carbon. *A–D*: sagittal sections; *E* and *F*: horizontal sections. Scale bars represent 200 μm in each case. In *A* and *B*, the OVLT is located in the dashed square. Arrows and arrow-heads indicate capillaries. Abbreviations: III, third ventricle; OC, optic chiasma; SS, subarachnoideal space.

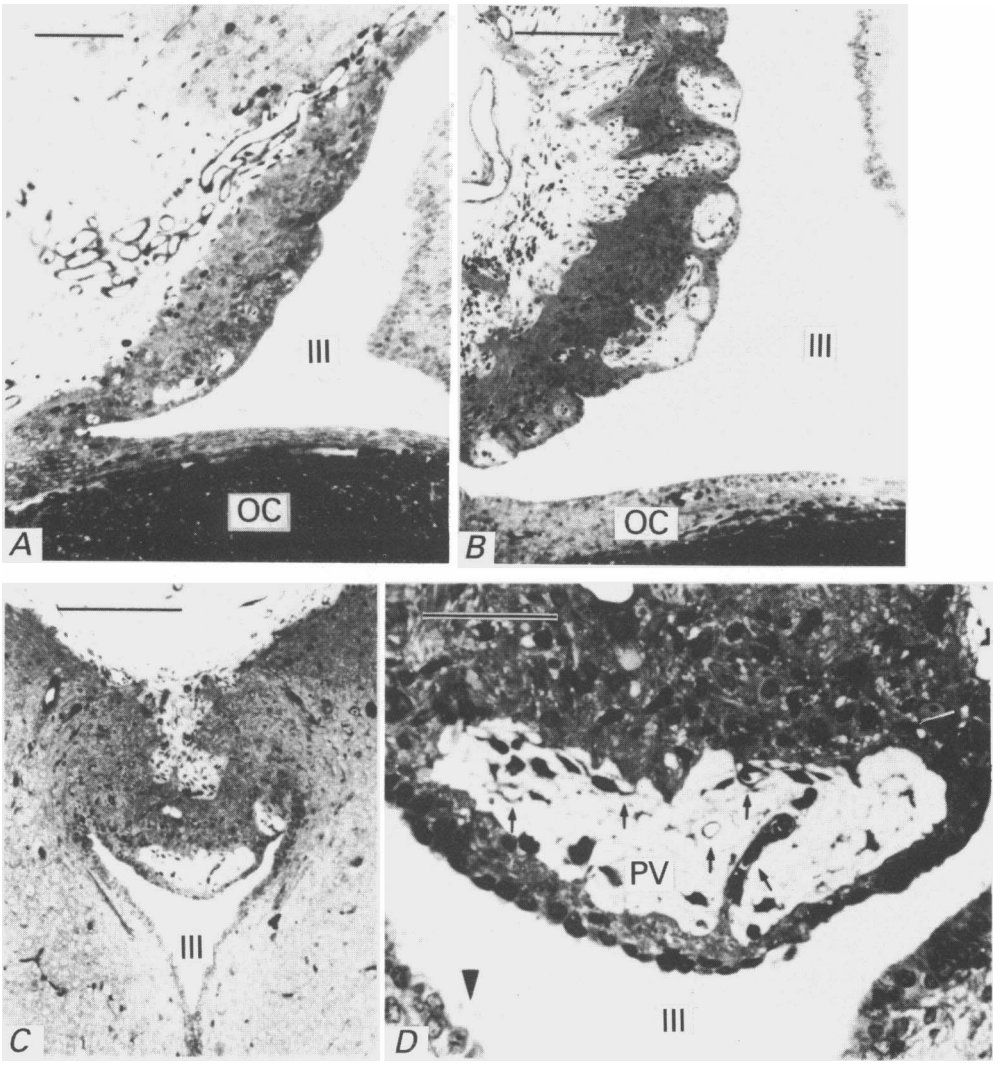

Fig. 6. *A* and *B*, photomicrographs of sagittal sections (1 μm in thickness) of the organum vasculosum laminae terminalis of rat (*A*) and rabbit (*B*). *C* and *D*, photomicrographs of horizontal sections (1 μm in thickness) of the organum vasculosum laminae terminalis of rabbit. Scale bars represent: 100 μm (*A*), 200 μm (*B*), 200 μm (*C*) and 20 μm (*D*). Arrow indicates capillary, arrow-head indicates cilia. Abbreviations: III, third ventricle; PV, perivascular space; OC, optic chiasma.

DISCUSSION

The present results show that febrile responsiveness of rabbits to intravenous injection of both IL-1α and IL-1β was significantly greater than that of rats. However, ventricular injections of IL-1β produced almost the same magnitude of fever in both species, although febrile response in rabbits to ventricular IL-1α was greater than that in rats. On the other hand, rats showed greater febrile response to

ventricular injection of prostaglandin E_2 than rabbits. Therefore, febrile sensitivity within the CNS of rats does not seem to be lower compared with that of rabbits. In the present study, febrile responsiveness of rabbits and rats to ventricular IL-1s or prostaglandin E_2 was compared at the same injection doses. However, more precise study on febrile sensitivity within the CNS may be necessary, because the effects of intracerebral or intraventricular injection depend on concentration and/or total dose of injecting substance. These results lead us to consider that, between rabbits and rats, there exist functional and/or structural differences in the OVLT, which is currently believed to be the pathway of pyrogen to the CNS and the site of production of pyrogenic prostaglandins (Blatteis *et al.* 1983; Stitt, 1985; Morimoto *et al.* 1988*c*).

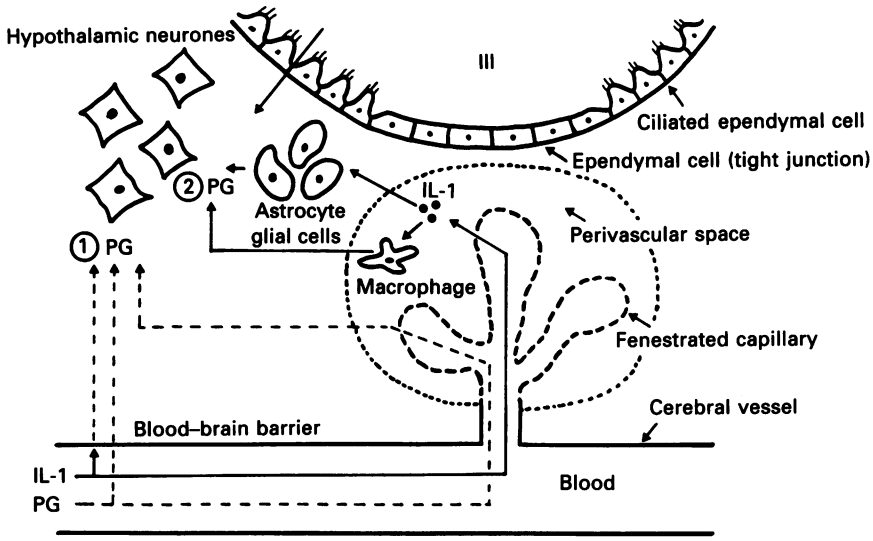

Fig. 7. Schema of the current hypothesis of the involvement of the organum vasculosum laminae terminalis in fever production in the rabbit. Abbreviations: IL-1, interleukin-1; PG, prostaglandin; III, third ventricle.

Weindl & Rochester (1969) reported that when horseradish peroxidase, with a molecular weight of about 40000, is given intravenously, it does not enter typical brain tissues, except in the OVLT, where it crosses the fenestrated endothelium of capillaries and enters into the perivascular spaces, reaching the neuropil. Therefore, we can speculate that smaller molecular substances, such as prostaglandin or IL-1, can penetrate into the perivascular spaces to reach the neuropil in the same way.

In Fig. 7, we schematically summarize the involvement of the OVLT in the genesis of biphasic fever production in rabbits. Skarnes, Brown, Hull & McCracken (1981) showed that concentration of prostaglandin E in the blood of the carotid artery in sheep increases during the first phase of the biphasic fever but not during the second phase. On the other hand, Rotondo, Abul, Milton & Davidson (1988) have recently shown that the plasma level of prostaglandin E_2 concentration of the blood obtained from rabbit's ear vein was elevated during both phases of the biphasic fever. Furthermore, ventricular injection of inhibitors of prostaglandin synthesis had no effect on the first phase of biphasic fever, but systemic injection suppressed it (Morimoto *et al.* 1987; Hashimoto, Bando, Iriki & Hashimoto, 1988). Hence, it is

considered that prostaglandins synthesized outside the blood–brain barrier easily enter into the perivascular spaces across the fenestrated capillaries of the OVLT to act on the CNS involved in fever control. Therefore, the higher sensitivity of rabbits to I.V. IL-1 is considered to be attributed to widely developed fenestrated capillaries and perivascular spaces of the OVLT, although the possibility cannot be excluded that the lower response in rats to intravenous IL-1 might be due to the lower concentration of prostaglandins released in the circulation. Furthermore, it is possible that prostaglandins synthesized and released from specific and/or non-specific regions, as well as the OVLT (Stitt, 1985), near the CNS (Dinarello & Bernheim, 1981) enter into the CNS across the normal blood–brain barrier (Dascombe & Milton, 1979) to act on multiple sites for fever control (Morimoto *et al.* 1988*a*, *c*).

Comparing the febrile pattern between rabbits and rats, the time to peak of fever in rats induced by I.V. injection of IL-1 was shorter than that in rabbits. However, when prostaglandins were injected into the third ventricle (in the present results) or into the hypothalamic region (Morimoto *et al.* 1988*a*, *b*), time to peak in rats was also shorter than that in rabbits. Stitt *et al.* (1985) compared febrile responses of guinea-pigs that had body weight similar to that of rats. They concluded that characteristics of the fever–response curves in rabbits and guinea-pigs are similar and both are markedly different from those of rats, and that body mass does not seem to be a major determinant in the dynamics of the febrile response in these species. Therefore, the difference in the time to peak of fever between rabbits and rats is thought to be due to the difference in the central mechanism of fever production.

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. However, many previous results (Andersson, Gale, Hokfelt & Larsson, 1965; Veale & Cooper, 1975; Lipton & Trzcinka, 1976; Blatteis & Banet, 1986) showed that after removal of the entire PO–AH region, fever is still observed with magnitude similar to that found in the control. In our recent report, we showed that many brain sites are sensitive to prostaglandin E_2 for producing fever (Morimoto *et al.* 1988*c*). Among these regions, the ventromedial hypothalamic regions of rabbits and rats are more sensitive than the PO–AH regions (Morimoto *et al.* 1988*b*, *c*). Furthermore, Stitt (1986) reported that, in rats, the OVLT is more sensitive to prostaglandin E_1 than the PO–AH regions. Now it is suggested that in the CNS there exist multiple sites which respond to prostaglandin with fever. In contrast to many regions sensitive to prostaglandin, those sensitive to microinjection of endogenous pyrogen (EP) are very small and localized exclusively near the preoptic area and the OVLT (Cooper, Cranston & Honour, 1967; Morimoto *et al.* 1988*c*). Moreover, this evidence indicates that these regions may synthesize prostaglandins in response to EP more than do other regions insensitive to EP. Concerning the second peak of the biphasic fever, we suggested that pyrogen within the blood–brain barrier acts on structures of the CNS to synthesize and release prostaglandins which, in turn, cause fever (Morimoto *et al.* 1987). Therefore, the regions near the sites where the 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 supported by the result (Morimoto *et al.* 1988*c*) that sodium salicylate injected into the region near the OVLT suppressed the second peak of the biphasic fever but had no effect on the first peak.

In the present results, when a large dose of IL-1 was intravenously injected, biphasic fever appeared in rabbits, while monophasic fever appeared in rats. Therefore, in the case of the second peak of the biphasic fever in rabbits, it is speculated that IL-1 enters into the perivascular space across the fenestrated capillaries of the OVLT and then acts on reticuloendothelial cells in the perivascular spaces of the OVLT or on the astrocytes and microglial cells near the OVLT, producing prostaglandins (Fontana, Kristensen, Dubs, Gemsa & Weber, 1982), which, in turn, cause fever. Therefore, when an inhibitor of prostaglandin synthesis was injected into the third ventricle (Morimoto *et al.* 1987; Hashimoto *et al.* 1988) or locally into the regions near the OVLT (Morimoto *et al.* 1988*c*), the second peak was suppressed. However, the ependymal cells covering the OVLT facing the third ventricle differ from the normal cells, not only in their lack of cilia but also in having tight junctions which prevent the free movement of substances to and from the third ventricle (Weindl & Joynt, 1969). On the other hand, where the ventricle is lined by typical ciliated ependymal cells, substances move to and from the ventricle (Weindl & Joynt, 1969). Therefore, pyrogenic substances or an inhibitor of prostaglandin synthesis injected into the third ventricle penetrate the wall with ciliated ependymal cells and enter the CNS. Furthermore, it is possible that astrocytes and microglial cells near the OVLT newly synthesize and release an IL-1-like substance in response to pyrogen within the CNS (Fontana *et al.* 1982; Fontana, Weber & Dayer, 1984). In the present results, however, a markedly large dose of IL-1β (20 μg/kg) injected intravenously induced biphasic fever in rats, while the same dose of IL-α induced monophasic fever. From a consideration of the present results that pyrogenicity of IL-β is significantly stronger than that of IL-1α in both cases of intravenous and intracerebroventricular injections, it may be possible that, even in rats, a very small amount of IL-1β enters into the CNS producing the second peak of fever.

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