Circumventricular organs and fever

YOSHIMI TAKAHASHI, PAULINE SMITH, ALISTAIR FERGUSON, and QUENTIN J. PITTMAN

1Neuroscience Research Group, Department of Physiology and Biophysics, Faculty of Medicine, The University of Calgary, Calgary, Alberta T2N 4N1; and 2Department of Physiology, Queen’s University, Kingston, Ontario, Canada K7L 3N6

Takahashi, Yoshimi, Pauline Smith, Alistair Ferguson, and Quentin J. Pittman. Circumventricular organs and fever. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1690–R1695, 1997.—We have examined the roles of three circumventricular organs, the area postrema, the subfornical organ, and the organum vasculosum of the lamina terminalis (OVLT), as possible access points for circulating pyrogens to cause fever. In conscious, unrestrained rats prepared with telemetry devices, intracerebroventricular cannulas, and intravenous catheters, body temperature was monitored after intravenously administered lipopolysaccharide and, on a different occasion, after intra- cerebroventricular prostaglandin E1. Lipopolysaccharide-induced fevers in sham control lesioned rats were indistinguishable from those observed in animals with lesions of the area postrema, the OVLT, or the tissue immediately adjacent to this structure (peri-OVLT). In contrast, rats with lesions of the subfornical organ displayed reduced fevers. In none of the groups of lesioned animals were prostaglandin E1 fevers reduced. Thus lesions did not interfere with central thermogenic pathways responsive to prostaglandin. Our results indicate that subfornical organ neurons respond to circulating pyrogens and through their efferent projections activate central pathways involved in fever.

area postrema; subfornical organ; organum vasculosum of the lamina terminalis; lipopolysaccharide; prostaglandin; cytokine

Although pyrogens such as lipopolysaccharide (LPS) and interleukins (ILs) can elicit fever when administered either intravenously, intraperitoneally, or centrally (intracerebroventricularly), it is now generally accepted that the blood-brain barrier precludes general access of pyrogens into the brain (23). Attention has thus focused on how the pyrogenic signal reaches the brain to cause activation of the appropriate neural pathways. Recently, evidence has been provided that the subdiaphragmatic vagus may be activated by the pyrogenic cytokine IL-1 in the gut to cause fever (26, 30), sickness behavior (15), and activation of the hypothalamic-pituitary-adrenal axis (7). Interestingly, c-fos activation in the brain in response to intraperitoneal LPS is completely blocked by subdiaphragmatic vagotomy, whereas that due to intravenous LPS is only minimally affected (29). Thus it is possible that there are several means by which peripheral immune signals can activate the central nervous system (CNS), depending on the specific location of the immune challenge.

Over 10 years ago, the potential sensory role of the circumventricular organs (CVOs), which lie outside of the blood-brain barrier, in transducing the pyrogenic signal to the brain was recognized (2). The organum vasculosum of the lamina terminalis (OVLT) is one structure that may be an access point for pyrogens to the brain (1, 32), as in several species, fever can be elicited after injection of pyrogens directly into the structure. In response to intravenously administered LPS, cytokine mRNA and protein levels increase in the OVLT (18, 19). Similarly, the immediate early gene c-fos, is activated in this structure after pyrogens are given peripherally (9, 22, 24, 29). Several investigators have examined the consequences of lesions to the OVLT on fever generation; in sheep (3) and guinea pigs (2), lesions of the OVLT suppress fever, whereas similar lesions in rats have been reported to enhance fever (27).

As circulating pyrogens activate many different central functions and pathways, evidence that the OVLT responds with increased c-fos or IL-1β synthesis in response to peripheral pyrogens is not, in itself, evidence for its involvement in fever. Few studies have verified that central thermoregulatory pathways remain intact after OVLT lesions and it has been suggested (26) that OVLT lesions may impinge on sites in the ventromedial preoptic area (VMPO) that are reported to be exquisitely sensitive to prostaglandin E2 (PGE2); furthermore, the fact that, in rats, lesions of the OVLT can be associated with elevated fevers (27) raises the possibility that this structure actually responds to circulating antipyretics that may be generated after exposure to LPS (12). It is also possible that lesions to the OVLT may have produced damage in descending pathways from another CVO, the subfornical organ (SFO); this structure is known to project to the hypothalamus via axons passing very close to the OVLT (10, 17). It is interesting that c-fos immunoreactivity, increased protein synthesis, and increased levels of IL-1β are also found in the SFO after systemically administered pyrogens (20, 24, 31). To the best of our knowledge, the possible involvement of the SFO as a structure important in febrile responses to peripheral pyrogens has not been determined.

Despite the evidence alluded to above, it is often overlooked that decerebrate animals, with connections between forebrain and hindbrain structures severed, are still capable of developing fevers (16). Furthermore, disruption of descending projections from structures anterior to the hypothalamus (i.e., OVLT or SFO) did not affect c-fos induction in the paraventricular nucleus after intravenous IL-1β (5). Such observations would suggest that there are midbrain or brain stem sites also capable of transducing the peripheral pyrogenic signal and eliciting a febrile response. Although vagal afferent signals would presumably use such brain stem sites as the nucleus of the solitary tract, it is possible that the area postrema (AP), a CVO sensitive to many circulating substances (6), could mediate the peripheral pyrogenic actions of circulating LPS. There is considerable
evidence for c-fos activation and enhanced IL-1β mRNA activity in this structure (9, 22, 24, 29, 31) after peripheral injection of various pyrogens.

In light of this evidence for a possible involvement in fever of other CVOs in addition to the OVLT, we have carried out experiments designed to test the hypothesis that the SFO and AP are structures important in transducing circulating peripheral pyrogenic signals to brain structures involved in fever generation. We have also reexamined the role of the OVLT in this regard. To ensure that any effects on fever were not due simply to disruption of brain thermogenic pathways, we also tested the responses of lesioned animals to the central pyrogen mediator PGE₁. Our data do not indicate an obligatory role for any one CVO in fever generation in the rat, but point to a possible involvement of the SFO in responding to circulating pyrogens.

METHODS

Adult, male Sprague-Dawley rats (300-350 g body wt) were purchased from Charles River, QC, Canada. All experiments were carried out in accordance with guidelines set and approved by Animal Care Committees of The University of Calgary and Queen's University.

AP lesions. Under pentobarbital sodium anesthesia (65 mg/kg ip), the rats were placed in a stereotaxic head holder, the head was flexed, and the cisterna magna was opened to permit access to the fourth ventricle. Under stereotaxic control, rats were given electrolytic (Kathley Instruments 225 Current Source) 0.5 mA for 20 s) lesions of the AP using a monopolar, parylene C-insulated tungsten electrode (Micro-Probe) with a tip diameter of 100 µm. Other rats received sham lesions in which the electrode was placed in the AP but no current was passed. The midline incision was then sutured and the animals were allowed to recover for a minimum of 1 wk. During this time, the animal’s health was carefully monitored, and palatable foods were placed in the cages to stimulate appetite and prevent undue weight loss. At the end of this 1-wk recovery period, animals were shipped from Kingston to Calgary and following a further 7-day rest period, a second surgery was performed. Under pentobarbital sodium anesthesia (65 mg/kg), rats were stereotaxically directed at the lateral ventricle, a jugular intravenous catheter filled with sterile, heparinized saline, and an intraperitoneal temperature telemetry device (model VMHF, Mini-Mitter, Sunriver, OR) for remote body core temperature measurements. The animals were then given a further 1-wk recovery period before commencement of the experiments. All rats were housed under a 12:12-h light-dark cycle and given water and pelleted rat food ad libitum.

SFO lesions. SFO-lesioned rats were treated identically to those in the AP study except that electrolytic lesions were made in the SFO using a current of 0.5 mA delivered for 30 s; peri-SFO lesions were made by lesioning adjacent tissue in the hippocampal commissure, and, in controls, the electrode was lowered into the brain near the SFO, but no current was passed. Stereotaxic coordinates for the SFO lesions were midline, 0.8 mm anterior to bregma, 4.5 mm below dura.

OVLT lesions. Adult rats under pentobarbital sodium anesthesia were given electrolytic (Grass, DC Constant Current Lesion Maker, 1.5 mA for 30 s) lesions in the OVLT (+0.8 mm anterior to bregma; on the midline and 8.3 mm below dura) or just adjacent to this structure. Other control rats received sham lesions in which the electrode was lowered in the vicinity of the OVLT and no current was passed. As these experiments were all carried out in Calgary, rats were prepared with the Mini-Mitter telemetry device and the indwelling intravenous catheter at the same time.

Experimental protocol. During at least a 1-wk postoperative period, animals were monitored daily, and palatable solutions were available to encourage resumption of normal eating and drinking. All rats lost weight as a result of the surgeries and lesions, and animals with SFO or AP lesions were polydipsic. Nonetheless, all animals displayed stable eating and drinking patterns and appeared healthy before experiments commenced. This was a minimum of 3 wk after AP and SFO lesions and at least 1 wk after all other surgical interventions. All experiments were carried out in a temperature-controlled room (22°C) in which the rats had been placed for at least 12 h before they were subjected to experimentation. Baseline temperature recordings were taken for a minimum of 1 h, after which all rats were given injections of 30 µg/kg lipopolysaccharide iv (LPS; Escherichia coli, L-3755, Sigma) in sterile saline between 1000 and 1200. Body core temperature was recorded for a further 6 h. Approximately 1 wk later, each rat was subjected to a second experimental protocol in which body temperature was again measured and 30 µg/kg of PGE₁ in 5 µl sterile saline was given intracerebroventricularly through a 27-gauge stainless steel needle. Temperature was recorded for a further 2 h.

On termination of the experiments, all rats were deeply anesthetized with pentobarbital sodium (65 mg/kg ip), then perfused through the heart with 0.9% saline followed by 4% formal saline, and postfixed in 10% Formalin for 3 days. Frozen coronal sections were taken and stained with a Nissl stain. An individual blind to the experimental protocol and to the temperature data evaluated the sections for placement of lesions and assigned animals into lesioned, perilesioned, or control (sham lesioned) groups on the basis of histological examination.

All data are reported and plotted as means ± SE. Temperature data were evaluated by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls’s pairwise comparisons for statistical comparison of lesioned, perilesioned, and control groups. After identification of significant differences between experimental groups, further ANOVAs were carried out to reveal significance at key time points. In addition, fever indexes (FI), calculated as change in degrees Celsius per hour (Δ°C/h) after injection of LPS or PGE, were obtained for each animal, and differences between controls and experimental groups were evaluated by an unpaired Student’s t-test for the AP groups and by an ANOVA for the OVLT and SFO groups. Similar tests were used to compare baseline temperatures. Statistical significance was set at P = 0.05.

RESULTS

Examples of sham and effective lesions for each site are presented in Fig. 1. With respect to AP lesions, all animals that were placed in the “lesioned” group were devoid of AP tissue, but in all cases the adjacent nucleus of the solitary tract was intact. None of the sham-lesioned animals from this group showed damage to either the AP or the adjacent tissue aside from traces of the electrode tract. Animals with lesions of the SFO all showed a complete absence of SFO tissue with slight damage to adjacent tissue in the hippocampal commissure. Peri-SFO lesioned animals had lesions similar in size, but some SFO tissue remained and the adjacent
hippocampal commissure was more extensively damaged. Sham-lesioned animals (controls) had evidence of electrode tracks in the commissure, but in all cases the SFO was intact and undamaged. With respect to the OVLT, animals that were considered lesioned showed destruction of tissue on the midline at locations similar to those described previously as sites where lesions interfered with fever generation (2). Lesions, however, did not obliterate the VMPO, an area highly responsive to PGE (25). In addition, in this area we identified a number of “misses” in which tissue adjacent to the OVLT, but sparing both OVLT and VMPO tissue, was destroyed; these animals were considered to have lesions in the peri-OVLT area. The remaining sham-lesioned animals exhibited no damage other than a faint scar from the electrode tract.

AP lesions. At the time of experimentation, basal body temperatures of rats with lesions of the AP (36.9 ± 0.20°C; n = 10) were identical (P > 0.05) to those of control, sham-lesioned rats (37.1 ± 0.12°C; n = 7). Fevers resulting from intravenous injection of 30 µg/kg LPS are shown in lesioned and control animals in Fig.

Fig. 1. Photomicrographs of coronal sections illustrating the area postrema (AP; A1), subfornical organ (SFO; B1), and organum vasculosum of the lamina terminalis (OVLT; C1) and typical lesions of respective sites (A2, B2, C2). Scale bar is 500 µM.
In the sham-lesioned control rats, typical LPS-induced fever was seen, with core temperature beginning to rise 1 h after injection and reaching a maximum of ~1.7°C, 2.5 h after injection. The lesioned animals exhibited a similar profile of fever, although the initial peak was marginally lower. ANOVA revealed no significant difference between the groups (P > 0.05). Fever indexes calculated for each group indicated that there were no significant differences (P > 0.05) in the magnitude of the fever as a function of the lesion. With respect to prostaglandin fever magnitude, lesioned animals showed delayed defervesce between 1 and 1.5 h after the PGE (P < 0.05, F = 12.000, Fig. 2B). Fever indexes were not significantly different in the two groups.

SFO lesions. Basal core temperatures of the lesioned (37.4 ± 0.22°C; n = 5), perilesioned (37.2 ± 0.11°C, n = 7), and control (37.3 ± 0.12°C; n = 13) groups were identical. Figure 3 shows fever responses to LPS in SFO-lesioned, perilesioned, and control animals. These groups of animals showed fever profiles that were slightly different from those of the rats in the AP group in that they displayed a 0.5°C temperature rise associated with the injection procedure and handling. Fever profiles of the SFO control and perilesioned rats were markedly different from those of the lesioned rats (P < 0.05; F = 22.642). Whereas the control and perilesioned animals displayed robust, 1.5°C fevers peaking at ~2.5 h postinjection, the lesioned rats at this time displayed only a ~0.5°C fever. Fever indexes over 6 h did not differ, but over 4 h that of the lesioned rats was significantly less than for the control and perilesioned rats (P < 0.05; F = 20.116). In contrast to these differences in LPS fevers, prostaglandin fevers in these three groups of animals were virtually superimposable with no statistical differences (P > 0.05 for both fever curves and fever indexes) between them (Fig. 3B).

OVLT lesions. Three groups of animals were examined for their febrile response; these were divided into OVLT-lesioned, sham-lesioned, and peri-OVLT-lesioned animals. Fevers due to LPS were virtually superimposable in all three groups, and the fever indexes were identical (P > 0.05; Fig. 4A). Prostaglandin fevers in the three groups were somewhat variable, although there was no significant difference between the fever indexes in the three groups (Fig. 4B). The peri-OVLT groups displayed a slightly higher fever (P < 0.05; F = 13.550) than did the other two groups.

**DISCUSSION**

These data indicate that discrete electrolytic lesions of the AP and the OVLT do not interfere with fever induced by intravenously injected LPS. In contrast,
lesions of the SFO resulted in attenuated fevers. The latter occurred in the absence of interference with brain thermogenic pathways responsive to PGE, the presumed end mediator of fever, as PGE-induced thermogenesis was unaltered in these same animals. Thus our data suggest that the SFO, a structure responsive to many circulating substances (6), may participate in the host defense reaction.

Many of the effects of LPS on body temperature are thought to be mediated by PGE within the brain (4). Therefore, in all lesioned animals, we evaluated their responses to intracerebroventricular PGE1 to differentiate between effects of the lesion in interfering with transduction of the LPS-induced pyrogenic signal to the brain and possible effects on the animal’s ability to activate a thermogenic response involving increased metabolism and decreased heat loss. With respect to all groups of animals, PGE1 fevers were not reduced by the lesions, indicating that central thermogenic pathways responsive to this substance (and presumably to LPS-activated prostaglandin) were intact. It was of interest that AP-lesioned rats showed a slightly delayed defer- vescence after PGE and peri-OVLT lesioned animals showed a slightly elevated fever peak; we do not have an explanation for these small differences.

In light of a substantial body of literature implicating the OVLT as an entry site for LPS, we were surprised that discrete lesions of this structure did not alter febrile responses. Thus our data add to the confusion in the literature in which OVLT lesions have reduced in many species (1) and augmented in others (27) the response to intracerebroventricular PGE1. As pointed out by Sehic and Blatteis (26), it is possible that, in previous studies, the lesions impinged on the nearby VMPO, an area proposed to be a critical locus for PGE-induced fever generation (11). Such a lesion could therefore have disrupted central thermogenesis involving the prostaglandin end mediators. In our studies, the lesions appeared to spare this area (albeit this area is not defined by well-demarcated landmarks in our Nissl-stained sections). It should be pointed out that these data do not eliminate the OVLT as a potential access point for circulating pyrogens to influence body temperature; in the lesioned animal other routes of access (e.g., the subdiaphragmatic vagus, other primary afferent fibers, other CVOs) may provide adequate access to sustain the febrile response, although an intact OVLT may normally contribute to the overall response.

A similar argument cautions against eliminating the AP as a potential responsive site for circulating pyrogens. Despite our findings of fevers identical in control and lesioned rats, some aspects of the CNS response to circulating pyrogens may reside in this structure; for example, the AP has recently been identified as a site involved in central cardiovascular control (6, 8), and the cardiovascular perturbations associated with LPS exposure, particularly at higher doses (14, 28), may be a result of actions of circulating pyrogens acting at the AP.

The SFO has not been previously implicated in central thermoregulatory control, yet SFO-lesioned rats developed significantly lower fevers than did their peri-SFO-lesioned or control counterparts. As the hypothermic response to intracerebroventricular PGE1 was not affected, we conclude that this represents a reduced response to the circulating pyrogenic signal. One possibility is that the SFO is a structure where circulating pyrogens can activate cells, which, in turn, project to sites such as the VMPO and the PVN to activate PGE synthesis and cause fever. The central connections of the SFO would certainly support such a possibility (21). It is also possible that the SFO lesions unmask a strong antipyretic response to LPS. Finally, we cannot ignore the possibility that animals with lesioned SFO somehow respond differently to the intravenously injected LPS, such that the distribution of the pyrogen is altered so that access to other responsive tissues (CVOs, vagus) is impaired.

Perspectives

It is apparent that the means by which the signal elaborated by a circulating pyrogen can gain access to the brain may be more complex than originally envisioned. Under different circumstances the organism may use markedly different means to transduce such a signal. A local, peripheral inflammation may activate a peripheral nerve, thereby obviating the need for pyrogens to escape from the inflammatory site to reach the CNS. In contrast, systemic infections wherein pyrogens...
may be present in the cerebral circulation, may make use of the responsive cells in the circumventricular organs to transduce the signal to a neural one. In light of the presumed survival value of fever (13), it is not surprising that evolution has provided more than one route of access for signaling the brain concerning peripheral immune state. In this respect, the autonomic response involved in fever is markedly similar to that of many other homeostatic processes (i.e., energy balance, water balance, etc.) that employ multiple mechanisms and many different structures to provide information concerning the body environment to the brain.

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