Journal of Physiology (1991), **432**, *pp.* 99–110
With 7 figures
Printed in Great Britain

DIFFERENTIAL SENSITIVITY IN THE SITES OF FEVER PRODUCTION BY PROSTAGLANDIN E_1 WITHIN THE HYPOTHALAMUS OF THE RAT

BY JOHN T. STITT

From the John B. Pierce Foundation Laboratory, Yale University School of Medicine, New Haven, CT 06519, USA

(*Received 24 May 1990*)

SUMMARY

1. The febrile sensitivity of male Sprague–Dawley rats to microinjections of prostaglandin E_1 (PGE) was investigated at three different locations in the rostromedial hypothalamic region. These were the preoptic anterior hypothalamic area (PO–AH), the organum vasculosum laminae terminalis (OVLT) and the rostral third ventricle (3V).

2. Stainless-steel cannula guide tubes were implanted in the OVLT region of one group of animals, within the PO–AH area of a second group and into the third ventricle of a third group of rats. After their recovery, the febrile response of each group was tested to a variety of doses of PGE, each administered in a volume of 1 μl sterile 0·9% saline, via a sterile cannula inserted into the implanted guide tubes. Metabolic, vasomotor and rectal temperature changes were monitored continuously for the duration of the fevers.

3. Surprisingly, not only did the introduction of PGE into the OVLT region produce fevers, but the sensitivity of this region to PGE in the production of fever greatly exceeded that of the PO–AH area and the third ventricle. Fevers produced by microinjection of PGE into the PO–AH and 3V were identical.

4. Doses of PGE as low as 0·5 ng injected into the OVLT produced fevers of 0·5 °C. The fever dose threshold for the OVLT region was one-fifth those of the PO–AH area and the 3V, and the slope of the OVLT dose–response curve was twice those of the PO–AH and the 3V dose–response curves.

5. This study demonstrates that there is an anatomically distinct, regional sensitivity in the febrile responsiveness to PGE within the hypothalamus. These results are interpreted as evidence that the site of action of PGE in the production of fever is located within or immediately adjacent to the OVLT region, rather than within the medial PO–AH neuropil as has been believed previously.

INTRODUCTION

It is generally believed that febrile responses to pyrogens are mediated by the production and the action of prostaglandins of the E series within the preoptic anterior hypothalamic (PO–AH) neuropil (Milton & Wendlandt, 1970; Veale & Cooper, 1974). Early evidence cited to support this view includes the studies of

Cooper, Cranston & Honour (1967), Feldberg & Saxena (1971) and Stitt (1973). These studies showed that microinjection of both endogenous pyrogen (EP) and prostaglandin E_1 (PGE) directly into the tissues of the PO–AH region elicited fevers of a characteristically short latency and duration. More recent studies devoted to investigating the relationship between EP and PGE_2 production in the brain, while hotly debated at one time, also supported the idea that EP in some manner causes release of PGE_2 within the brain tissue, which in turn causes fever production (Bernheim, Gilbert & Stitt, 1980; Coceani, Bishai, Dinarello & Fitzpatrick, 1982). Evidence has also been produced that EP can induce the production of PGE from brain tissue when incubated *in vitro* (Dinarello & Bernheim, 1981; Fontana, Kristenson, Dubs, Gemsa & Weber, 1982). Because of these studies, it was believed that EP, initially produced within the circulation by monocytes and fixed tissue macrophages in response to a variety of infectious or endotoxic stimuli, travelled via the cerebral circulation into the brain where it acted upon some unknown brain cells to produce PGE, which in turn acted upon the neurons controlling body temperature to produce fever (Stitt, Hardy & Stolwijk, 1974; Veale & Cooper, 1974; Hellon & Townsend, 1983). However, there is no direct evidence that EP, either elaborated within the circulation in response to pyrogenic stimuli or directly injected into the circulation, can cross the blood–brain barrier and enter the brain neuropil (Dinarello, Weiner & Wolff, 1978; Coceani, Lees & Dinarello, 1988; Blatteis, Dinarello, Shibata, Llanos-Q, Quan & Busija, 1989). Recent studies comparing the fever-producing effects of EP, administered by the intravenous and intracerebroventricular (I.C.V.) routes, demonstrated that when EP was administered by the I.C.V. route, it produced fevers in rabbits that had onset latencies, durations to peak fever and overall fever durations that were more than twice those of the fevers that were induced by the same EP when it was administered intravenously (Stitt & Bernheim, 1985). Furthermore, we have demonstrated that the area of the organum vasculosum laminae terminalis (OVLT), one of the circumventricular organs that is devoid of normal blood–brain barrier characteristics, may be involved in the mediation of the febrile event (Stitt, 1985). Small lesions placed within the confines of this periventricular, supraoptic, mid-line region, augmented and enhanced the febrile responses of rabbits and rats to a standard dose of EP. We suggested that the OVLT might be the site of action of EP entering from the cerebral circulation. More recently we have also shown that fevers in rats, in response to intravenously administered EP, can be enhanced by pre-treating the animals 3 days previously with immuno-adjuvants such as lipopolysaccharides, zymosan or muramyl dipeptide via the intravenous route (Stitt & Shimada, 1987, 1989*a*). This febrile enhancement can also be produced if minute quantities of these immuno-adjuvants are microinjected directly into the OVLT region of the brain, but not when they are placed in the PO–AH (Stitt & Shimada, 1989*b*). These results led us to postulate that the site of action of EP in the production of fever is within the OVLT region rather than in the neuropil of the PO–AH area. We suggested that the EP could cause the release of PGE from some cell type located within the confines of the OVLT and consequently the lipophilic PGE molecule could then cross the blood–brain barrier surrounding the OVLT and enter the adjacent PO–AH neuropil to cause fever. However, if PGE were to be elaborated by the action of EP on the *outside* of the blood–brain barrier, but

within the OVLT region, then I wondered whether or not the OVLT itself, in addition to the PO–AH neuropil, might be sensitive to the fever-producing effects of PGE. Such information would be useful in establishing the location of the site of action of EP, the site of action of PGE and whether or not these two sites were functionally different. To this end I studied the effects of microinjecting small quantities of PGE directly into the OVLT region and compared the fever sensitivity of this site in the brain to that of the PO–AH area and the rostral portion of the third ventricle. Both these latter sites allow PGE direct access to the hypothalamic neuropil and have been studied extensively in the past (Stitt, 1986).

METHODS

The animals used in this study were male Sprague–Dawley rats weighing between 200 and 300 g. They were trained previously to accept restraint in simple neck stocks. Two weeks prior to the experiments, chronically implanted stainless-steel cannula guide tubes were positioned in the brain, directly over the medially located OVLT region in the septal region or over the medial PO–AH region or directly into the rostral 3V, under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.P.) using sterile stereotaxic techniques. The stereotaxic co-ordinates and atlas of de Groot (1967) were employed. In a group of eight rats, the tips of the cannula guide tubes were located above the OVLT (Fig. 1*A*) at A = +8·5 mm, L = 0·0 mm and V = 0·5 mm. In the second group of six rats, the tips of the cannula guide tubes were located within the PO–AH area (Fig. 1*B*) at A = +7·2 mm, L = 1·5 mm and V = −1·0 mm. In the third group of seven rats, the tips of the cannula guide tubes were located within the rostral third ventricle (Fig. 1*C*) at A = +6·8 mm, L = 0·0 mm and V = +0·5 mm. Each guide tube was affixed to the calvaria of the skull using an acrylic dental cement attached to two self-tapping stainless-steel screws that were inserted into the frontal bones of each animal. The reflected muscle and skin were then sutured around the acrylic mound on the skull that contained the hub of the cannula guide tube.

The prostaglandin E_1 used in the study was supplied as the saline soluble sodium salt, courtesy of the Upjohn Company (Kalamazoo, MI, USA), and it was dissolved in sterile, non-pyrogenic, 0·9% saline in concentrations ranging from 0·5 to 500 μg/ml. PGE_1 was used because it alone was available as a sodium salt and required no vehicles such as alcohol and sodium bicarbonate to put it into solution. Rats are especially sensitive to injectiòns of these vehicles and exhibit gradual hyperthermias in response to intracerebral injections of vehicle solution alone. While it is acknowledged that PGE_2 is likely to be the natural mediator of fever, no evidence to date had been educed that PGE_1 acts in any way different to PGE_2 in the production of fever. Microinjections of PGE_1 were made via a sterile stainless-steel cannula that was inserted into the lumen of the guide tube and projected 1·0 mm beyond the end of the tip of the guide tube. Injections were made in a volume of 1 μl, delivered over a period of 30 s by a hydraulically driven microsyringe attached to the cannula insert by a length of sterile vinyl tubing, except in the series of ventricular injections where two different volumes of injection were compared, when a volume of 10 μl was chosen to study the diffusional dilution of PGE_1 after injection into the ventricle. At the end of the experiments, rats were killed under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.V.) and the brain of each animal was preserved in 10% formalin and sectioned for histological verification of the location of the cannula tip (Fig. 2).

The experiments were conducted between 08.00 and 14.00 h on the conscious rats in a partitional calorimeter at T_a (ambient temperature) = 26 °C. Each animal sat calmly in neck stocks and was enclosed in a flat cylindrical plexiglass chamber (30·5 cm in diameter, 23 cm in height and 8·7 l in volume) through which air was drawn at a rate of 10 l/min to permit the continuous determination of oxygen consumption. Oxygen consumption was measured by an Applied Electrochemistry O_2 cell.

Rectal and tail skin temperatures measured by copper–constantin thermocouples and metabolic rate calculated from oxygen consumption were recorded once per minute by an on-line data acquisition system and an index of tail skin blood flow was calculated in order to monitor peripheral vasomotor tone (Stitt, 1976; Stitt & Shimada, 1985). Animals were permitted a period of 60 min to attain a thermal steady state before their febrile responses to injections of PGE were

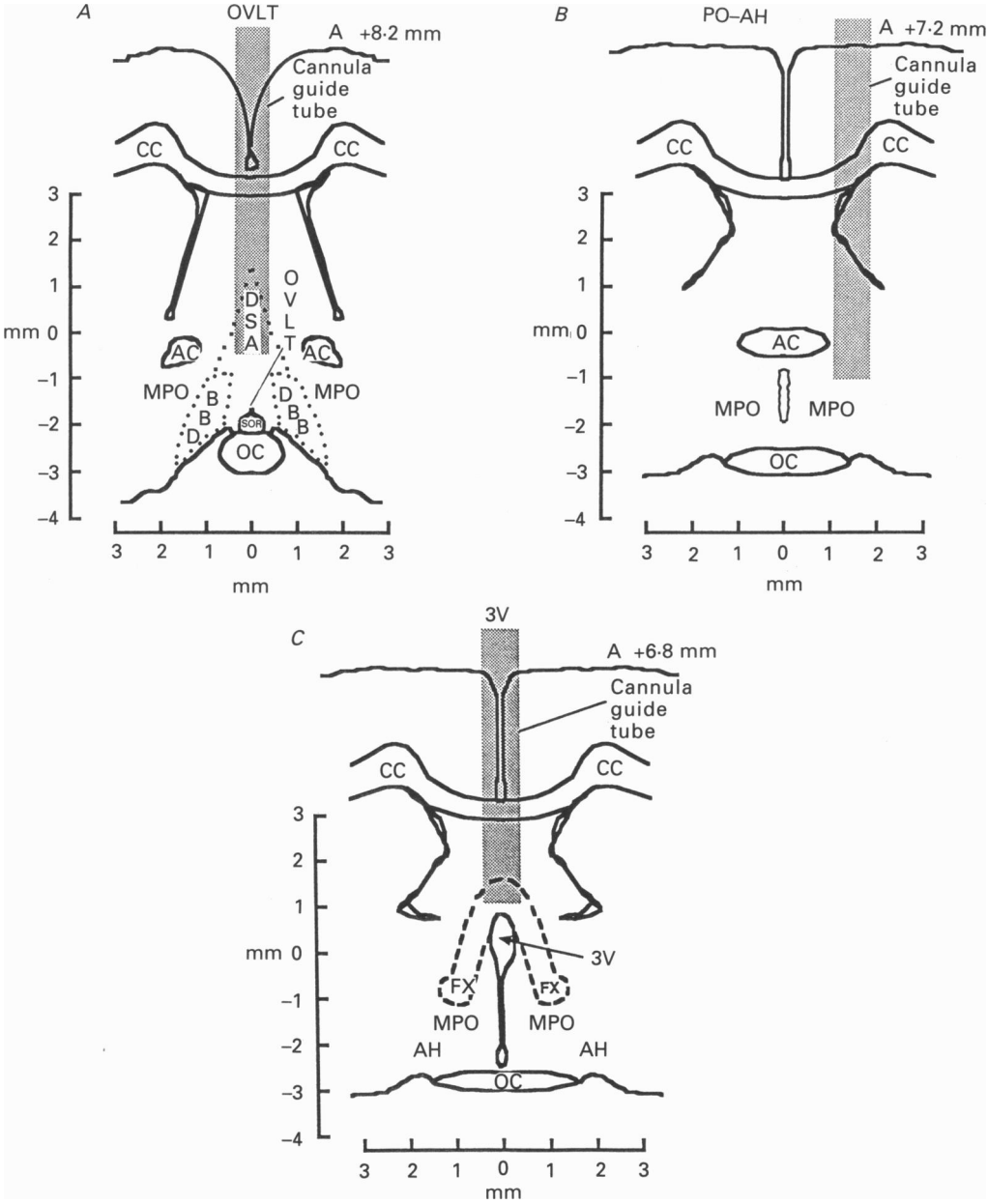

Fig. 1. Stereotaxic representations of coronal sections of the rat brain illustrating the location of the placement of the cannula guide tubes within the hypothalamus of the rat. Panel *A* shows the location of the OVLT cannula on coronal section A = +8·2 mm. Panel *B* shows the location of the PO–AH cannula on coronal section A = +7·2 mm. Panel *C* shows the location of the 3V cannula on coronal section A = +6·8 mm. AC = anterior commissure, MPO = medial preoptic area, DBB = diagonal bands of Broca, OC = optic chiasma, OVLT = organum vasculosum lamina terminalis, FX = fornix, SOR = supra-optic recess of the 3V, 3V = third ventricle. (After de Groot, 1967.)

determined. In every case the rectal temperature of the animal under study was between 38 and 39 °C before its febrile response was determined. After each injection of PGE, the animal's body temperature and thermoregulatory responses were monitored for a further period of at least 1 h.

Fever dose–response curves for PGE in the OVLT-cannulated, PO–AH-cannulated and the 3V-cannulated rats were determined using at least five different doses of PGE. The doses were

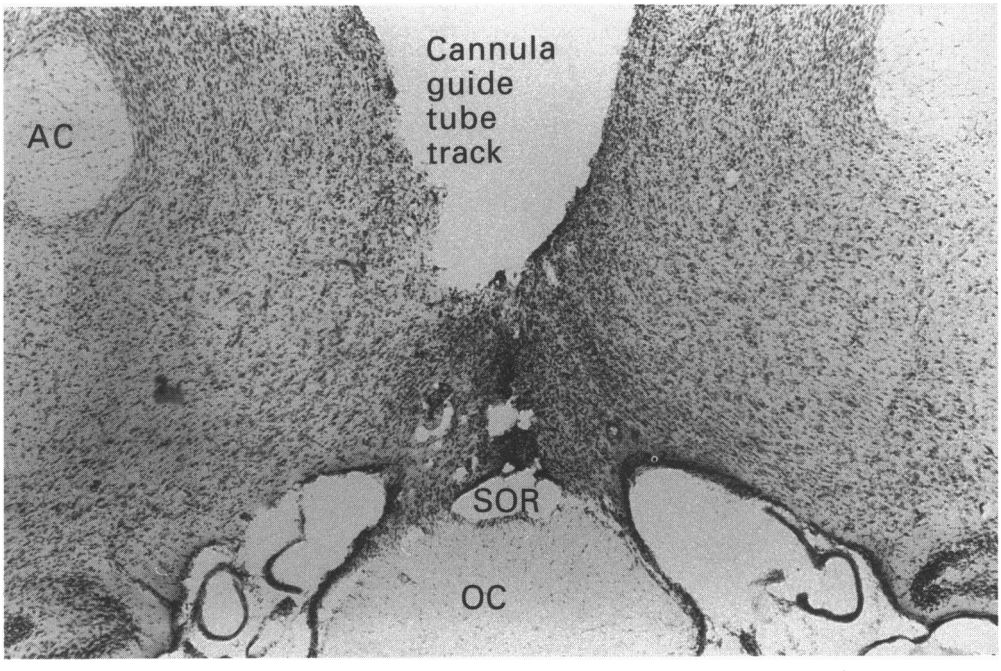

Fig. 2. A photomicrograph of a coronal section of a rat's brain showing the actual location of the cannula guide tube track above the OVLT region. This section approximates the stereotaxic atlas section illustrated in Fig. 1*A* (legend as shown in Fig. 1).

administered using a quasi-random assignment of dose and no animal received more than two microinjections during any one session. Each animal was permitted a recovery period of at least 3 days between each experiment. The mean peak rectal temperature increases produced by each of three groups of animals in response to injections of the several different doses of PGE were then plotted on a semilogarithmic scale and the slopes and intercepts of these curves were then determined by regression analysis, using the method of least squares (Stitt, Shimada & Bernheim, 1985).

RESULTS

Individual febrile responses of two animals to microinjection of 20 ng PGE into the OVLT region and the PO–AH area are compared in Fig. 3. Panel *A* illustrates the response produced when the PGE_1 is delivered into the OVLT region; panel *B* illustrates the response produced when the PGE is delivered into the PO–AH region. In both cases a fever resulted; however, it was noted that the onset of the metabolic rate increase and the rise in rectal temperature (T_{re}) were more rapid and steeper in the case of the OVLT injections that in the PO–AH injections of PGE. The results obtained when 20 ng PGE were injected into the 3V were identical to those obtained in the PO–AH and therefore are not illustrated.

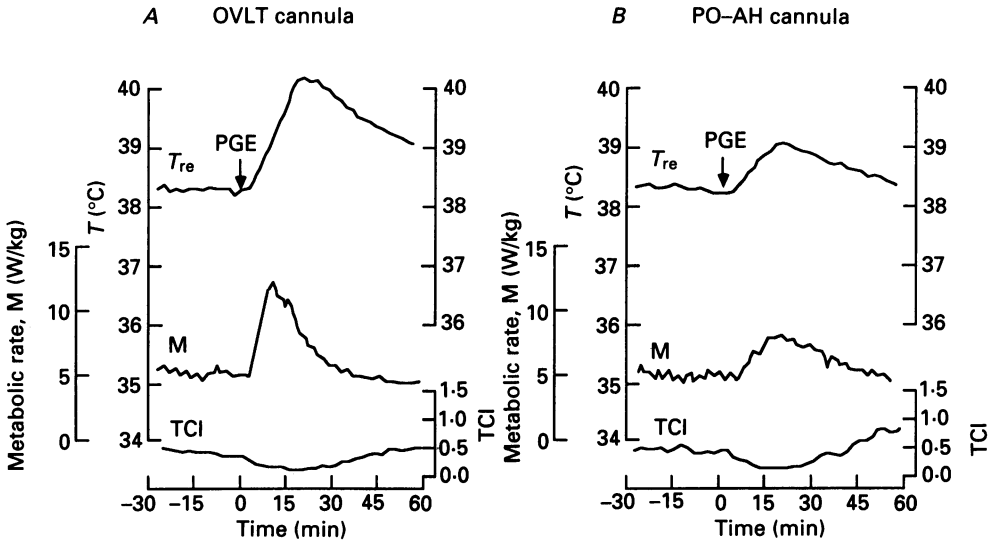

Fig. 3. A comparison of the individual metabolic (M), tail vasomotor (TCI, tail circulatory index) and rectal temperature (T_{re}) responses of two rats after microinjection of 20 ng PGE into the OVLT region (*A*) and the PO–AH area (*B*).

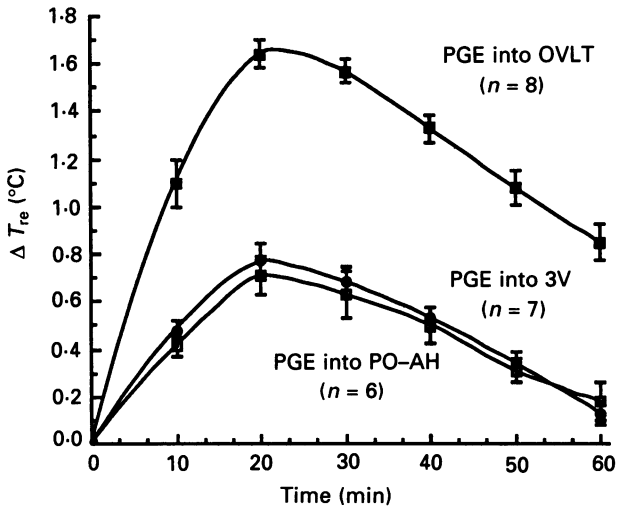

Fig. 4. A comparison of the changes in rectal temperature (ΔT_{re}) in each group of rats, measured at 10 min intervals after the microinjection of 20 ng of PGE into the OVLT region (■), the 3V (●) and the PO–AH area (□). Values are mean $\Delta T_{re} \pm$ S.E.M.

The mean increases in T_{re} at 10 min intervals in response to the 20 ng PGE dose level at all three sites are illustrated in Fig. 4, along with the standard errors of the mean. It will be noted that while all groups of rats appear to show short latencies and similar durations of fever, the peak increases in T_{re} at 20 min after injection in the

OVLT-injected animals are more than twice those produced in the PO–AH- and 3V-injected animals. This was a consistent finding at all dose levels of PGE_1 investigated in the study.

Figure 5 compares the fever dose–response curves obtained from the three groups of rats over a wide range of PGE doses. PGE dose is expressed in logarithmic co-

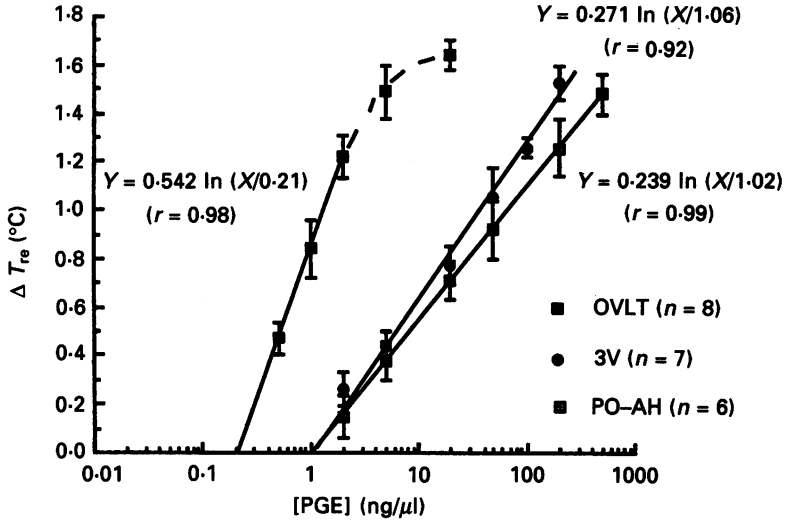

Fig. 5. Fever dose–response curves of rats to PGE microinjected into the OVLT, the PO–AH and the 3V. ΔT_{re} = peak increase in rectal temperature. All doses were made in a volume of 1 μl at the concentration indicated. The equation constants were determined from the linear portions of each relationship by the method of least squares.

ordinates on the abscissa and the peak increase in body temperature is expressed linearly on the ordinate. This construction yields a relationship that is linear over the major portion of its range, and the line that is fitted to these data points is derived by constraining them to a linear regression analysis. In this manner, the slope of the steep portion of each curve could be quantified for purposes of comparison. Using this method, the equation relating the peak increase in body temperature to PGE dose has the form:

$$Y = b \ln [X/a], \tag{1}$$

where X is the PGE dose, Y is the peak increase in T_{re} produced by that dose, a is the intercept on the abscissa (or threshold dose for a febrile response), and b is the slope (or the sensitivity of the febrile response to increasingly larger doses of PGE).

In the case of the febrile responses of the rats to PGE microinjected into the OVLT region, eqn (1) is evaluated as:

$$Y = 0{\cdot}542 \ln [X/0{\cdot}21]. \tag{2}$$

In the case of the febrile responses of the rats to PGE microinjected into the PO–AH area, eqn (1) is evaluated as:

$$Y = 0{\cdot}239 \ln [X/1{\cdot}02]. \tag{3}$$

In the case of the febrile responses of the rats to PGE microinjected into the third ventricle, eqn (1) is evaluated as:

$$Y = 0{\cdot}271 \ln [X/1{\cdot}06]. \quad (4)$$

Clearly, the OVLT region was much more sensitive to the fever producing effects of PGE than either the PO–AH area or the 3V. Not only is the absolute dose

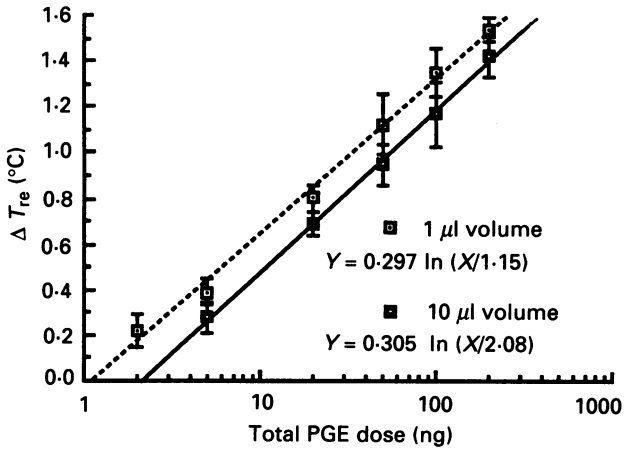

Fig. 6. Fever dose–response curves of rats to different amounts of PGE microinjected into the 3V in volumes of 1 and 10 μl. ΔT_{re} = peak increase in rectal temperature. The regression lines and equation constants were determined by the method of least squares.

threshold for a febrile response in the OVLT lower than those of the PO–AH and the 3V (0·2 ng *versus* 1·0 ng PGE), but slope of the OVLT dose–response curve is more than twice that of the PO–AH or the 3V dose–response curves. This could mean that the actual receptors for PGE were located in the region of the OVLT and that the fever dose–response curves obtained from the 3V and the PO–AH area were less sensitive than that obtained at the OVLT because PGE deposited at the latter sites was diluted as it diffused toward the active site at the OVLT. This dilutional effect would be constant and result in dose–response curves for the PO–AH and 3V sites that had response thresholds that were higher than that of the OVLT site.

The hypothesis was tested using another group of six animals that had cannulae implanted in the 3V. Due to the distensibility of the ventricular space, PGE could be injected in two different volumes (10 and 1 μl) and, therefore, an estimate of the volume of this dilution could be calculated from the differing intercepts in the resulting fever dose–response curves obtained using two different volumes of injection. The fever dose–response curve for amounts of PGE contained in a volume of 10 μl would be further displaced to the right of the 1 μl fever dose–response curve by a factor equal to (the total volume of dilution)/10 μl. Several different amounts of PGE were injected into the 3V in volumes of 1 and 10 μl and the respective dose–response curves for each volume of injection were plotted. These are illustrated in Fig. 6, where the febrile responses are plotted as functions of the total amount of

PGE injected into the 3V. It can be seen that the 10 μl volume PGE dose–response curve intercepted the x-axis at 2·08 ng/μl, whereas the 1 μl volume PGE dose–response curve intercepted at 1·15 ng/μl, yielding a ratio of 1·81. Hence, the total volume of dilution was 18·1 μl. This represents the volume contained by a

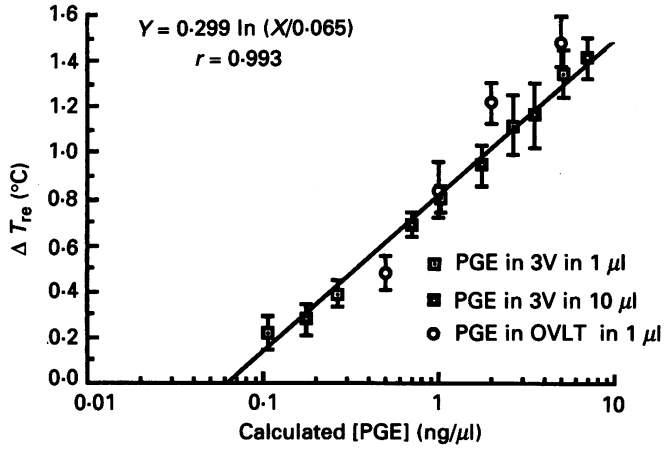

Fig. 7. Fever dose–response curve of rats for PGE microinjected into the 3V in a 10 or a 1 μl volume, calculated by assuming an extra diffusional volume of distribution of 18 μl, compared with actual data obtained when PGE is injected in 1 μl into the OVLT. ΔT_{re} = peak increase in rectal temperature. The regression line and equation constants were determined by the method of least squares.

sphere of radius 1·6 mm, which in turn is the distance between the location of the tip of the cannula tip in the 3V to the location of the OVLT in the rostral wall of the third ventricle (see Fig. 1). If the OVLT were the actual site of action of PGE, and if one recalculated the PGE concentrations of the two ventricular PGE dose–response curves (assuming a volume of dilution of 18·1 μl of CSF), the resulting composite ventricular PGE dose–response curve should then coincide with the previously obtained OVLT dose–response curve. The results of these calculations are illustrated in Fig. 7 and it can be seen that the composite ventricular PGE concentration dose–response curve, calculated by assuming a volume of distribution of 18 μl, coincides well with the fever dose–response curve that was obtained by injecting PGE in a volume of 1 μl directly into the OVLT.

DISCUSSION

It is widely believed that EP produces fever in mammals by entering the PO–AH region through the cerebral circulation and acting on cells within the neuropil to produce PGE_2 which consequently mediates the febrile response by altering the activity of neurons controlling thermoregulation. However, there are a number of factors that militate against such a series of events. Firstly, there is no good evidence that the EP molecule can cross the blood–brain barrier to enter the neuropil of the

PO–AH (Dinarello *et al.* 1978; Coceani *et al.* 1988; Blatteis *et al.* 1989). While it is true that several studies have demonstrated that EP and endotoxins can elicit the production of PGE from neural tissue when they are incubated *in vitro* (Dinarello & Bernheim, 1981; Fontana *et al.* 1982), this evidence *per se* is not particularly compelling, since PGE is the product of cellular insult or damage in a wide variety of tissues (Vane, 1971). Secondly, we have demonstrated that when EP is introduced into the brain via the I.C.V. route, although fever ensues, the latency to onset of such a fever is more than twice that obtained when EP is introduced intravenously (Stitt & Bernheim, 1985). It is likely that these I.C.V. fevers are due to a slow, non-specific release of PGE at multiple sites within the brain by the mechanism alluded to in the *in vitro* studies of PGE mentioned above. For these reasons we postulated that the site of action of EP was closer to the cerebral circulation than to the brain neuropil (Stitt & Bernheim, 1985; Rotondo, Abdul, Milton & Davidson, 1988). Finally, we have demonstrated that a circumventricular organ, the organum vasculosum laminae terminalis, which is bounded on its lateral sides by the PO–AH area, abuts the third ventricle medially and is devoid of the normal blood–brain barrier characteristics, may be involved in the mediation of the febrile event (Stitt, 1985). Small lesions placed within the confines of this area, located within the rostro-anterior wall of the third ventricle in the hypothalamus, augmented and enhanced the febrile responses of rabbits and rats to a standard intravenous dose of EP. We suggested that the OVLT might be the site of action of EP after it leaves the cerebral circulation. This possibility was reinforced by our recent observations that the febrile responses in rats to intravenously administered EP were enhanced by prior administration of any one of a variety of immuno-adjuvant substances intravenously, and that this fever enhancement phenomenon could also be produced if minute quantities of these immuno-adjuvants were microinjected directly into the OVLT region of the brain, but not when they are injected into the PO–AH (Stitt & Shimada, 1987, 1989*a*, *b*). If EP were to act within the OVLT to produce fever, then the most likely event would be that it stimulates some target cell within the OVLT to release PGE. Since it is lipophilic, PGE could easily cross the tight-junctioned ependymal cells separating the OVLT from the adjacent PO–AH neuropil, and thus produce fever. I wondered whether the site of action of EP, which we had postulated to be within the OVLT, could be shown to be distinct from the previously proposed site of action of PGE, i.e. within the tissue of the PO–AH or from the 3V.

Surprisingly, I found that not only did the introduction of PGE into the OVLT region produce fevers, but that the sensitivity of this region to PGE, in the production of fever, was considerably greater than that of the PO–AH area or the rostral third ventricle. While the fever dose–response curves that were determined for the PO–AH area and the 3V are typical for those reported by other investigators for PGE microinjected into the PO–AH area or the cerebral ventricles of many mammalian species (Stitt, 1986), to my knowledge the extreme sensitivity of the OVLT region of the rat to PGE for fever production reported here has no parallel in the literature. If PGE were to act *within* the PO–AH neuropil to produce fever, then one might expect that the febrile sensitivity to injections of PGE, made directly into the PO–AH region, would be greater than that observed anywhere else within the hypothalamus or by injection directly into the rostral 3V. This does not appear

to be the case, since the OVLT region has a PGE dose threshold that is one-fifth lower, and a PGE fever sensitivity that is more than twice that found either within the PO–AH area or the third ventricle.

The data obtained by injecting PGE into the 3V in two different volumes of injection indicate that the volume of dilution of these injections is a sphere of radius 1·6 mm, which coincides with the direct distance between the tip of the cannula in the rostral 3V and the location of the OVLT. Furthermore, this is also the distance between location of the tip of the PO–AH cannula and the OVLT (see Fig. 1). Assuming that the small lipophilic PGE molecule is equally diffusible through CSF or the brain interstital space, this could explain the similarity between the dose–response curves obtained in the PO–AH and 3V injection sites as illustrated in Fig. 5. Taken as a whole, I interpret these results as evidence that the site of action of PGE in the production of fever is located within or immediately adjacent to the OVLT region, rather than within the deeper reaches of the medial PO–AH neuropil, as had been believed previously.

This work was supported by NIH Grant NS 11487. The author is indebted to Mr David Barboza for his invaluable technical assistance in all phases of the study.

REFERENCES

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

BLATTEIS, C. M., DINARELLO, C. A., SHIBATA, M., LLANOS-Q, J., QUAN, N. & BUSIJA, D. (1989). Does circulating interleukin-1 enter the brain? In *Thermal Physiology*, ed. MERCER, J., pp. 385–390. Elsevier, Amsterdam.

COCEANI, F., BISHAI, I., DINARELLO, C. A. & FITZPATRICK, F. A. (1982). Prostaglandin E_2 and Thromboxane B_2 in cerebrospinal fluid of the febrile and afebrile cat. *American Journal of Physiology* **224**, R785–793.

COCEANI, F., LEES, J. & DINARELLO, C. A. (1988). Occurrence of Interleukin-1 in cerebrospinal fluid of the conscious cat. *Brain Research* **446**, 245–250.

COOPER, K. E., CRANSTON, W. I. & HONOUR, A. J. (1967). Observations on the site and mode of action of pyrogens in the rabbit brain. *Journal of Physiology* **191**, 325–337.

DE GROOT, J. (1967). *The Rat Forebrain in Stereotaxic Coordinates*. North-Holland, Amsterdam.

DINARELLO, C. A. & BERNHEIM, H. A. (1981). Ability of human leukocytic pyrogen to stimulate brain prostaglandin synthesis *in vitro*. *Journal of Neurochemistry* **37**, 702–708.

DINARELLO, C. A., WEINER, P. & WOLFF, S. M. (1978). Radiolabeling and disposition in rabbits of purified human leukocyte pyrogen. *Clinical Research* **26**, 522A.

FELDBERG, W. & SAXENA, P. N. (1971). Further studies on prostaglandin E_1 fever in cats. *Journal of Physiology* **219**, 739–745.

FONTANA, A., KRISTENSON, F., DUBS, R., GEMSA, D. & WEBER, E. (1982). Production of Prostaglandin E and Interleukin-1 like factor by cultured astrocytes and C_6 glioma cells. *Journal of Immunology* **129**, 2147–2152.

HELLON, R. R. & TOWNSEND, Y. (1983). Mechanisms of fever. *Pharmacology and Therapeutics* **19**, 211–244.

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

ROTONDON, D., ABDUL, H. T., MILTON, A. S. & DAVIDSON, J. (1988). Pyrogenic immunomodulators increase the level of prostaglandin E_2 in the blood simultaneously with the onset of fever. *European Journal of Pharmacology* **154**, 145–152

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

STITT, J. T. (1976). The regulation of respiratory evaporative heat loss in the rabbit. *Journal of Physiology* **258**, 157–171.

STITT, J. T. (1985). Evidence for the involvement of the organum vasculosum laminae terminalis in the febrile response of rabbits and rats. *Journal of Physiology* **368**, 501–511.

STITT, J. T. (1986). Prostaglandin E as the neural mediator of the febrile response. *Yale Journal of Biology and Medicine* **59**, 137–149.

STITT, J. T. & BERNHEIM, H. A. (1985). Fundamental differences between endogenous pyrogen fevers produced by intravenous and intracerebroventricular routes in the rabbit. *Journal of Applied Physiology* **59**, 342–347.

STITT, J. T., HARDY, J. D. & STOLWIJK, J. A. J. (1974). PGE_1 fever: its effects on temperature regulation at different low ambient temperatures. *American Journal of Physiology* **227**, 622–629.

STITT, J. T. & SHIMADA, S. G. (1985). The effect of low ambient temperature on the febrile responses of rats to semi-purified human endogenous pyrogen. *Yale Journal of Biology and Medicine* **58**, 189–194.

STITT, J. T. & SHIMADA, S. G. (1987). A comparison of the febrile responses of the Brattleboro and Sprague Dawley strains of rats to endotoxin and endogenous pyrogens. *Canadian Journal of Physiology and Pharmacology* **65**, 1377–1381.

STITT, J. T. & SHIMADA, S. G. (1989*a*). Immuno-adjuvants enhance the febrile responses of rats to endogenous pyrogen. *Journal of Applied Physiology* **67**, 1734–1739.

STITT, J. T. & SHIMADA, S. G. (1989*b*). Enhancement of the febrile responses of rats to endogenous pyrogen occurs within the OVLT region. *Journal of Applied Physiology* **67**, 1740–1746.

STITT, J. T., SHIMADA, S. G. & BERNHEIM, H. A. (1985). Comparison of the febrile responses of rabbits and rats to endogenous pyrogen. *Journal of Applied Physiology* **59**, 1721–1725.

VANE, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* **231**, 232–235.

VEALE, W. L. & COOPER, K. E. (1974). Evidence for the involvement of prostaglandins in fever. In *Recent Studies of Hypothalamic Function*, ed. LEDERIS, K. & VEALE, W., pp. 359–370. Karger, Basel.