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THERMAL AND PGE_2 SENSITIVITY OF THE ORGANUM VASCULOSUM LAMINA TERMINALIS REGION AND PREOPTIC AREA IN RAT BRAIN SLICES

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SUMMARY

1. The effects of local applications of prostaglandin E_2 (PGE_2) on the unit activity of fifty-one neurones in the organum vasculosum lamina terminalis (OVLT) region and fifty-eight neurones in the preoptic area (POA) were investigated in small tissue slices from the rat hypothalamus containing the OVLT and POA isolated from each other.

2. Of these, thirty OVLT and twenty-eight POA neurones were warm sensitive and increased their discharge rate in response to a rise in tissue temperature. One OVLT neurone and one POA neurone were cold sensitive and showed the opposite type of responses to changes in temperature. The thermosensitivity of these neurones was still observed in a Ca^{2+} free–high Mg^{2+} solution.

3. Perfusion with PGE_2 in doses between 1 and 250 nM changed the discharge rate in forty-two of fifty-one OVLT neurones and in thirty-two of fifty-eight POA neurones in a dose-dependent manner. The responses to PGE_2 were not lost during synaptic blockade. The threshold dose of PGE_2 to alter the discharge rate of the OVLT neurones ($4{\cdot}8 \pm 1{\cdot}1$ (S.E.M.) nM, $n = 16$) was significantly lower than that of the POA neurones ($40{\cdot}9 \pm 12{\cdot}2$ nM, $n = 16$).

4. Fifteen of forty-two OVLT neurones exhibited the responses with a slower onset (latency 5–13 min) and a longer duration (20 min to 3 h), but such responses were observed in only one of thirty-two POA neurones.

5. The responses of OVLT and POA neurones to PGE_2 (50–250 nM) were reversibly blocked by a concurrent application of AH6809, a prostanoid EP_1 and/or a DP receptor antagonist.

6. While there was no clear correlation between the type of thermosensitivity and the type of response to PGE_2 among the POA neurones, a significantly higher incidence of inhibitory response to PGE_2 was found among the warm-sensitive neurones in the OVLT region.

7. The lower threshold responses to PGE_2 and the higher incidence of PGE_2 responsiveness among OVLT neurones are consistent with previous findings which showed that the highest density of PGE_2 receptor binding and the highest pyrogenic sensitivity to microinjected PGE_2 were observed in the OVLT region. The results

provide further evidence for the critical involvement of the OVLT region in mediating the febrile responses to blood-borne endogenous pyrogen through the local release of PGE_2.

INTRODUCTION

It has not yet been fully determined how blood-borne endogenous pyrogens (EPs), such as interleukin-1 (IL-1), eventually reach or otherwise signal their receptors in the brain to produce fever. There is good evidence for the view that prostaglandin, most probably the E_2 type (PGE_2), mediates the febrile actions of EPs/IL-1. The major findings to support this are (1) the increased release of PGE_2 in the hypothalamus and into the cerebrospinal fluid (CSF) during EPs/IL-1 induced fever (Feldberg, Gupta, Milton & Wendlandt, 1973; Bernheim, Gilbert & Stitt, 1980), (2) the antipyretic actions of cyclo-oxygenase inhibitors associated with the decreased release of PGE_2 in the brain (Feldberg *et al.* 1973) and (3) the ability to produce fever promptly by minute amounts of PGE_2 microinjected into the rostral hypothalamus (see review, Milton, 1982). However, the brain site which senses blood-borne EPs/IL-1 and the type of cells which are responsible for the release of PGE_2 in response to EPs/IL-1 have not yet been determined. The hypothalamus, particularly the preoptic area (POA), has long been proposed as the primary site of action of EPs/IL-1 (Hori, Shibata, Nakashima, Yamasaki, Asami, Asami & Koga, 1988). However, the lack of evidence showing the actual entry of blood-borne EPs/IL-1 into the brain (Dinarello, Weiner & Wolff, 1978) and the lack of a consistent relationship between the thermosensitivity and the PGE_2 responsiveness of POA neurones (Stitt & Hardy, 1975; Boulant & Scott, 1986) do not support this, although EPs/IL-1, which is synthesized in the brain, might contribute, at least partly, to the development of fever (Fontana, Weber & Dayer, 1984; Hori *et al.* 1988; Nakashima, Hori, Mori, Kuriyama & Mizuno, 1989).

Recently, it was shown that the placement of lesions in the organum vasculosum lamina terminalis (OVLT) and its adjacent region altered the febrile response to intravenous injection of EPs/IL-1 (Blatteis, Bealer, Hunter, Llanos-Q, Ahokas & Mashburn, 1983; Stitt, 1985). A microinjection study revealed that the OVLT region was more sensitive to PGE_2 to produce fever than the POA (Stitt, 1986). It has been suggested that PGE_2, which is released from as yet unidentified cells in the OVLT region, either diffuses into the adjacent POA neurones to cause fever or act on the neurones in the OVLT region which sends the signals synaptically to the POA neurones thereby producing the fever (Stitt, 1986). These findings raise questions whether neurones in the OVLT region and POA possess a sensitivity to PGE_2 and, if so, how such PGE_2 sensitivity of neurones is correlated with their thermosensitivity.

In the present study, we investigated the responses to PGE_2 of thermosensitive and thermally insensitive neurones recorded in rat OVLT and POA tissue slices *in vitro* which were isolated from each other.

METHODS

Materials and preparation

Male Wistar rats weighing 150–200 g were deeply anaesthetized with ether and quickly decapitated. Thin (300–400 μm), coronally oriented tissue slices containing the organum

vasculosum lamina terminalis (OVLT) or the preoptic area (POA) were prepared with a brain slicer. One or two slices containing the OVLT and two or three slices of POA were usually obtained from each brain. The OVLT slices were trimmed to sizes of approximately 2 × 2 mm so that they contained the OVLT and the adjacent area, but not the nucleus medianus, the preoptic

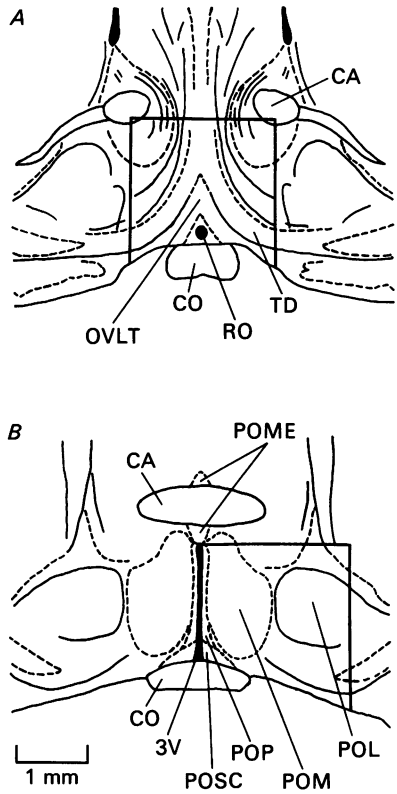

Fig. 1. Schematic brain slice preparations cut coronally. *A* and *B* show representative slices containing the OVLT and POA regions, respectively. Areas confined by thick straight lines show the parts trimmed from these coronally sectioned slices. CA, corpus callosum; CO, optic chiasma; OVLT, organum vasculosum lamina terminalis; POL, lateral preoptic nucleus; POM, medial preoptic nucleus; POME, median preoptic nucleus; POP, periventricular preoptic nucleus; POSC, suprachiasmatic part of preoptic nucleus; RO, optic recess; 3V, third ventricle; TD, diagonal tract of Broca.

periventricular nucleus and the preoptic suprachiasmatic nucleus (Fig. 1*A*). The POA slices were also trimmed to sizes of about 2 × 2 mm and consisted mainly of the medial and lateral preoptic nuclei (Fig. 1*B*). After having been incubated for at least 1 h in oxygenated (95% O_2 and 5% CO_2) Krebs–Ringer solution (pH 7·4) at 37 °C, each slice was transferred to a recording chamber and submerged in the Krebs–Ringer solution perfusing the chamber at a rate of 2·0 ml/min. The composition of the Krebs–Ringer solution was (mM): NaCl, 124; KCl, 5; KH_2PO_4, 1·24; $MgSO_4$, 1·3; $CaCl_2$, 2·6; $NaHCO_3$, 26 and glucose, 10. The temperature of the chamber was controlled by a water-perfused mantle. The slice temperature could be maintained at, or changed to, any temperature within the range of 32–41 °C by changing the temperature of the mantle and was continuously measured with a fine copper-constantan thermocouple placed on the slices.

Recording

Extracellular single-unit activities were recorded with a glass microelectrode filled with Pontamine sky blue acetate (impedance, 10–60 MΩ) from the OVLT region and the medial preoptic nucleus of the POA. To prevent any possible bias in comparing the responsiveness of neurones

recorded in the OVLT and the POA, the order of single neurone studies in daily alternating experiments on the OVLT slices and the POA slices was randomized. Spontaneous neural activity was amplified, displayed on an oscilloscope and stored as digital signals on floppy disks in a microcomputer.

Experimental protocol

When the discharge of a single neurone had been isolated and stable recording established at 36–37 °C for at least 5 min, thermal responsiveness of neurones were studied by changing the slice temperature slowly over the range between 34 and 41 °C. Neurones having thermal coefficients of greater than 0·7 impulses/(s °C) or Q_{10} over 2 were considered warm sensitive and neurones having negative thermal coefficients of −0·7 impulses/(s °C) or less were taken to be cold sensitive (Hori & Shinohara, 1979; Asami, Asami, Hori, Kiyohara & Nakashima, 1988).

Subsequently, the activities of neurones were observed at a constant temperature (36–37 °C) during perfusion of prostaglandin E_2 (PGE_2) (1–900 nM, Sigma) and a prostaglandin antagonist, AH6809, (0·3–30 μM, kindly provided by Dr B. M. Bain, Glaxo Group Research Limited) with or without PGE_2. The drugs were dissolved in the Krebs–Ringer solution and injected into the main perfusing line using infusing pumps. It took 30 s after the start of drug injection to perfuse into the dead space. The final concentrations of each drug in the recording chamber was calculated on the basis of the injection rate, the flow rate of main perfusion system and the concentration of injected solution (Nakashima, Hori, Kuriyama & Matsuda, 1988).

At each concentration PGE_2 was applied for 2 min and then followed by at least a 15 min period of perfusion with the control solution. This interval was usually sufficient for the neurone to return to its control activity and also to be completely free from the tachyphylactic effect of PGE_2, as described later in the Results. About one third of OVLT neurones showed a response of distinctly different time courses, i.e., long latency (5–13 min) and long duration (20–177 min) (see Results). In these neurones, a second application of PGE_2 was performed after the neurone returned to about 80% of its original activity. Since none of the neurones which responded to PGE_2 showed a response with a latency of longer than 15 min, the interval of 15 min, as stated above, as a rule, was thus considered to be sufficient for studying the neurones responding to PGE_2 with different time courses.

In some experiments, after the units were examined for their responsiveness to changes in temperature and PGE_2 in normal solution, the effects of temperature and PGE_2 were again observed in a solution which contained no $CaCl_2$ but 6·5 mM-$MgCl_2$, where synaptic transmission was effectively blocked (Nakashima, Pierau, Simon & Hori, 1987). The pH and osmolality of perfusing medium was not affected by changing the electrolyte concentration (Nakashima *et al.* 1987) or by adding the drugs at the concentration used in the present study.

Data analysis

For evaluating the responsiveness to PGE_2, the average discharge rate of a single neurone was measured for the 5 min period prior to drug application and for the 1 min period of the apparent maximum response. The neurones were considered to be responsive to PGE_2 if the average discharge rate changed by more than 20% of the pre-application discharge rate when PGE_2 in doses of 250 nM or less was perfused through the medium. These doses of PGE_2 are comparable or even lower than the concentration of PGE_2 in the rat brain (Berchtold-Kanz, Anhut, Heldt, Neufang & Hertting, 1981; Scott, Fertel & Boulant, 1987).

The latency of a response was expressed as the interval between the change from control solution to the drug-containing solution and the onset of the neuronal response, and included the period of 30 s which was necessary for the new solution to pass through the dead space.

Statistics

All the data were presented as means ± S.E.M. Statistical analyses were performed using χ^2 test or one-way analysis of variance, and a P value of less than 0·05 was considered to be statistically significant.

RESULTS

Thermosensitivity of OVLT and POA neurones

A total of fifty-one neurones recorded in OVLT slices and fifty-eight neurones in POA slices were investigated for their responsiveness to changes in slice temperature

and to perfusion of PGE_2. Only one neurone was studied in each brain slice. They showed spontaneous and stable activities for periods long enough to allow studies of both thermal and pharmacological stimulations.

Out of fifty-one OVLT neurones, thirty (58·8%) units were warm-sensitive neurones which increased the discharge rate in response to a rise in slice temperature

TABLE 1. Effects of changes in local temperature and local application of PGE_2 on the activity of OVLT and POA neurones

	Responses to PGE_2			
	Excitation	Inhibition	No effect	Total
	OVLT neurones			
Warm-sensitive neurones	7	21*	2	30
Cold-sensitive neurones	0	1	0	1
Thermally insensitive neurones	7	6	7	20
Total	14	28	9	51
	POA neurones			
Warm-sensitive neurones	11	9	8	28
Cold-sensitive neurones	0	0	1	1
Thermally insensitive neurones	6	6	17	29
Total	17	15	26	58

* $\chi^2 = 6{\cdot}71$, $P < 0{\cdot}01$.

with positive thermal coefficients between 0·7 and 4·1 impulses/(s °C) ($1{\cdot}55 \pm 0{\cdot}25$ (mean ± S.E.M.)) (Table 1). Examples of responses of warm-sensitive neurones are shown in Fig. 4*A*. One (2·0%) unit was a cold-sensitive neurone which showed the opposite type of response to temperatures with a thermal coefficient of −1·1 impulses/(s °C) (Fig. 6*A*) and the remaining twenty (39·2%) units did not respond to changes in slice temperatures between 34 and 41 °C.

In the POA, on the other hand, twenty-eight (48·3%) of fifty-eight neurones were warm sensitive having positive thermal coefficients between 0·7 and 3·5 impulses/(s °C) ($1{\cdot}67 \pm 0{\cdot}17$, mean ± S.E.M.) (Figs 2 and 3*C*), one (1·7%) was cold sensitive with a thermal coefficient of −3·7 impulses/(s °C) and twenty-nine (50·0%) were thermally insensitive. No statistical difference of thermosensitivity was found between OVLT neurones and POA neurones in terms of the populations of thermosensitive neurones (χ^2 test, n.s.) and the degree of warm sensitivity of individual neurones (Student's *t* test, n.s.).

Some OVLT neurones (eight warm units and five thermally insensitive units) and POA neurones (nine warm units, one cold unit and six thermally insensitive units) were further examined for their responsiveness to changes in slice temperature in a Ca^{2+}-free–high Mg^{2+} (6·5 mM) solution (Fig. 3*C*). As in the previous observations

(Hori, Nakashima, Kiyohara, Shibata & Hori, 1980), none of the neurones tested in this way changed their type of responses to temperature, indicating that the thermal responsiveness was an inherent property of the neurones.

Tachyphylactic actions of PGE_2

Since it has been demonstrated that repeated applications of PGD_2 on the neurones with short intervals resulted in tachyphylaxis (Inokuchi & Oomura, 1986),

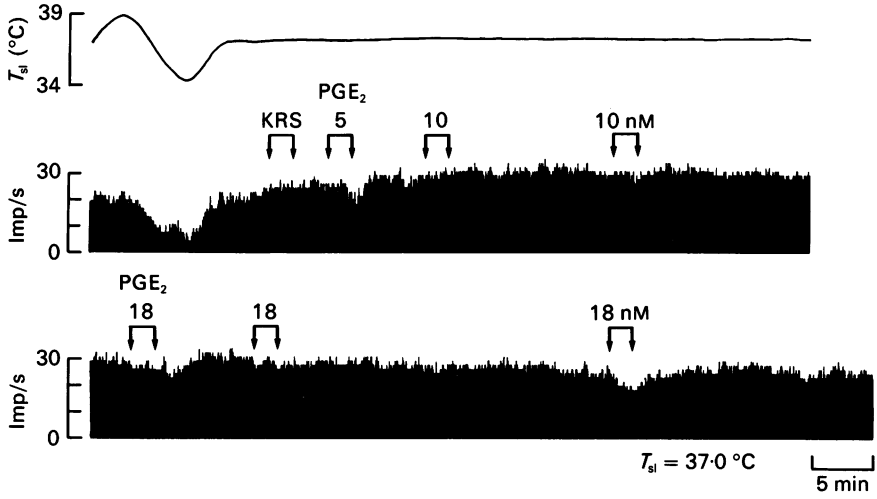

Fig. 2. Firing rate responses of a warm-sensitive POA neurone to changes in slice temperature (T_{s1}) and repeated applications of PGE_2 which resulted in a tachyphylaxis. KRS, Krebs–Ringer solution.

we investigated how long it took for neurones to recover their responsiveness to PGE_2 after a prior application of PGE_2. As shown in Fig. 2, a POA neurone exhibiting warm sensitivity responded to an application of 5 nM-PGE_2 with a decreased rate of discharge, but 10 nM-PGE_2, when applied after 6·5 min, did not affect the neuronal activity. Application the same amount of PGE_2 12 min thereafter was also ineffective, showing tachyphylaxis. However, when a larger amount (18 nM) of PGE_2 was applied about 15 min after the previous application, this neurone again exhibited an inhibitory response. An application of the same dose of PGE_2 after 8·5 min again resulted in tachyphylaxis. When tested with PGE_2 at 18 nM 26 min thereafter, a complete recovery of PGE_2 responsiveness was observed. Based on these findings, repeated applications of PGE_2 in the present study were performed at intervals of longer than 15 min.

Postsynaptic responsiveness of OVLT and POA neurones to application of PGE_2

Application of PGE_2 in doses of less than 250 nM affected the discharge rate of 42/51 (82·4%) OVLT neurones and 32/58 (55·2%) POA neurones (Table 1). Statistical analysis revealed that there is a higher incidence of the responsiveness to PGE_2 in OVLT neurones when compared with POA neurones ($\chi^2 = 9{\cdot}20$ with d.f. = 1, $P < 0{\cdot}01$).

Twelve OVLT neurones and five POA neurones, which had responded to PGE_2 in the normal Krebs–Ringer solution, were tested again for their PGE_2 responsiveness in a Ca^{2+}-free–high Mg^{2+} (6·5 mM) solution. Shown in Fig. 3 are typical examples of such responses. In *A* and *B*, two OVLT neurones responded to an application of

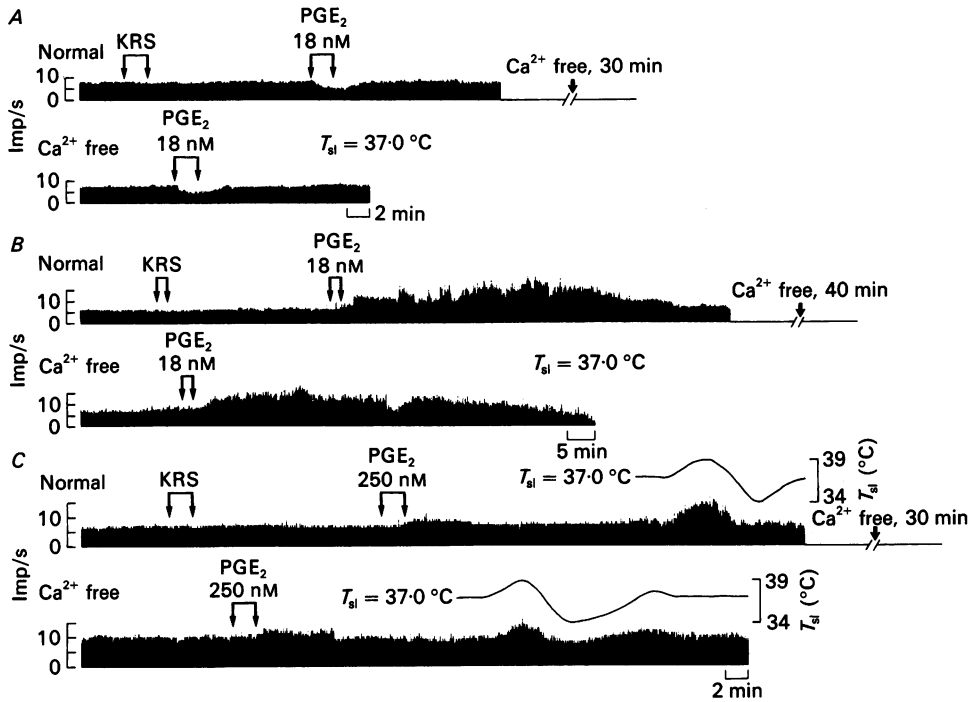

Fig. 3. Firing rate responses of two OVLT neurones (*A* and *B*) and one POA neurone (*C*) to local application of PGE_2 both in the normal Krebs–Ringer solution and a Ca^{2+}-free–high Mg^{2+} (6·5 mM) solution. The warm sensitivity of a POA neurone shown in *C* was preserved even in a Ca^{2+}-deficient solution. Same abbreviations as in Fig. 2.

18 nM-PGE_2 with a decrease and an increase in the rate of discharge, respectively, in the normal Krebs–Ringer solution. The neurones still responded to the same dose of PGE_2 in a Ca^{2+}-deficient solution, making it unlikely that these effects are due to synaptic mediation. Similar findings were observed in the remaining ten OVLT neurones as well as in the five POA neurones tested (Fig. 3*C*).

The dose–response relationship of PGE_2 responsiveness of OVLT and POA neurones

To investigate the dose–response relationship, sixteen OVLT neurones and sixteen POA neurones which responded to PGE_2 at less than 250 nM were tested at different PGE_2 concentrations, ranging from 1–900 nM. Figure 4 shows representative examples of the dose-dependent effects of PGE_2 which either increased or decreased the discharge rates of two OVLT and two POA neurones. These neurones exhibited responsiveness to minimum doses of PGE_2. While the smallest doses of PGE_2 to evoke a decrease and an increase in the discharge rate of the OVLT neurones were found to be 2 nM and 5 nM, respectively (Fig. 4*A* and *B*), those of the POA neurones

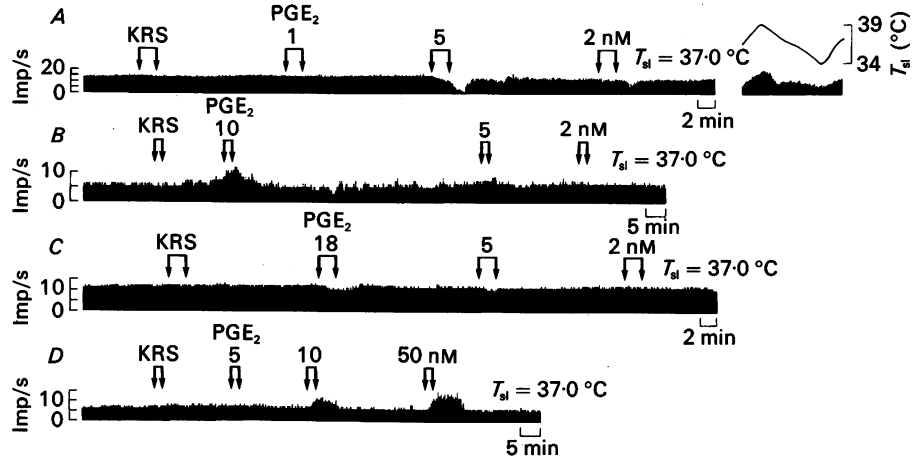

Fig. 4. Firing rate responses of two OVLT neurones (*A* and *B*) and two POA neurones (*C* and *D*) to local application of different doses of PGE_2. Same abbreviations as in Fig. 2.

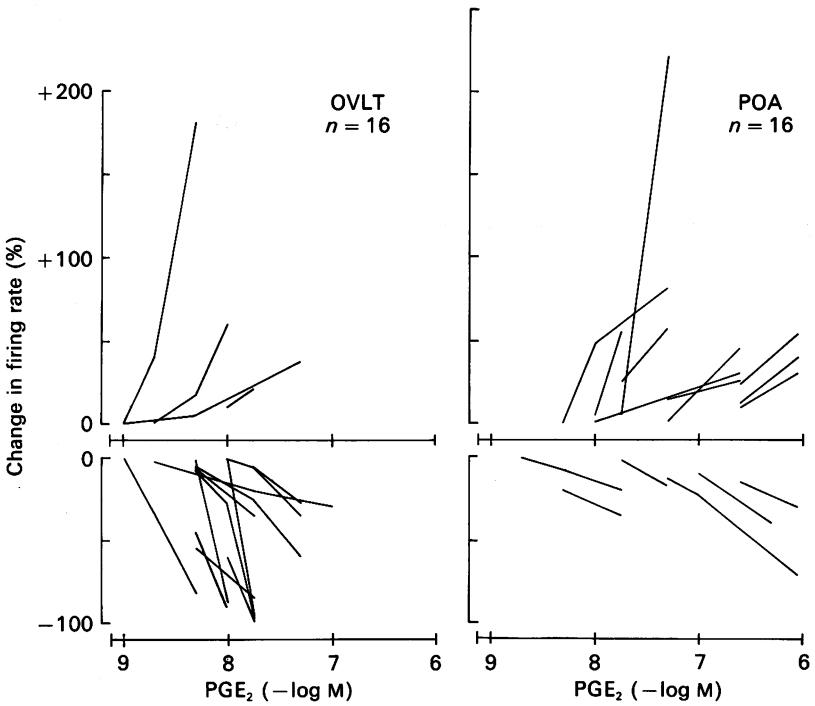

Fig. 5. The dose–response relationship between the changes in activity of OVLT and POA neurones and the concentration of PGE_2. The percentage values of each change in firing rate from the firing rate at the preapplication period was plotted.

were 5 nM and 10 nM (Fig. 4*C* and *D*). As the PGE_2 concentration increased, the magnitude of changes in the discharge rate generally increased but neurones of both regions exhibited a considerable scattering in the dose–response relationship (Fig. 5). This may be because both OVLT and POA neurones are not homogeneous

populations. While variability was observed in the minimum effective doses of PGE_2 to affect individual neurones, the OVLT neurones in general were more sensitive to PGE_2 than the POA neurones. Eight (50%) of the sixteen OVLT neurones were affected by PGE_2 of lower than 10 nM, but such PGE_2 responsiveness was found only

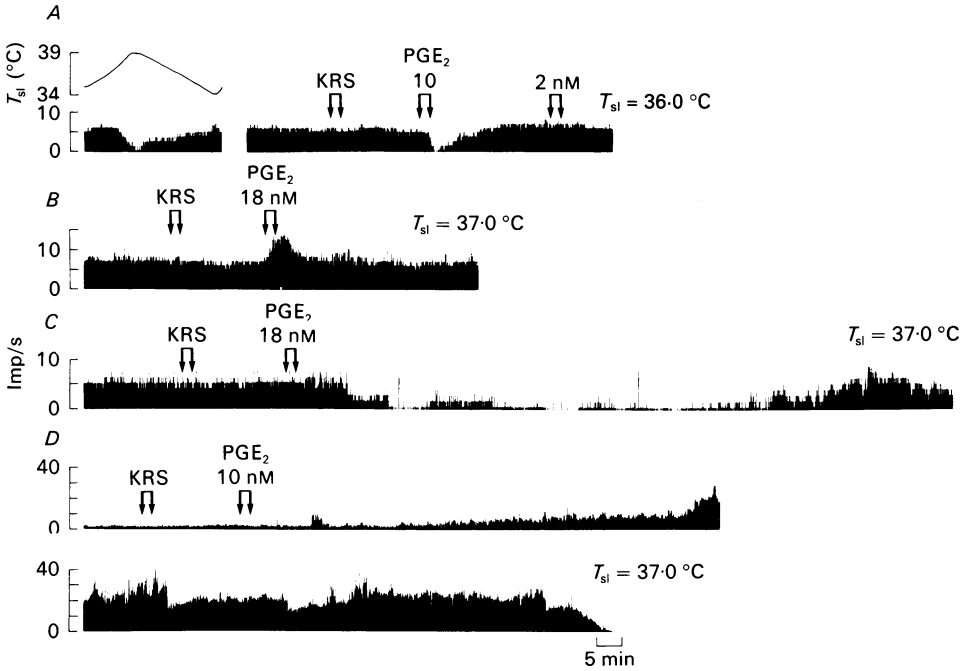

Fig. 6. Firing rate responses of four OVLT neurones to local application of PGE_2 which showed responses with short onset latency and duration (*A* and *B*) and responses with long latency and duration (*C* and *D*).

in two (12·5%) of the sixteen POA neurones, which was a significantly lower rate than that of OVLT neurones ($\chi^2 = 5{\cdot}24$ with d.f. = 1, $P < 0{\cdot}05$). Moreover, the mean minimum effective dose to alter the discharge rate of the sixteen OVLT neurones ($4{\cdot}8 \pm 1{\cdot}1$ (S.E.M.) nM) was significantly smaller than that of sixteen POA neurones ($40{\cdot}9 \pm 12{\cdot}2$ nM) ($t = 3{\cdot}01$, $P < 0{\cdot}01$).

Time courses of neuronal responsiveness to PGE_2

Application of PGE_2 changed the discharge rate of neurones in each region with variable onset latencies (10 s–13 min) and durations (1–177 min). Thirteen (32%) of forty-two OVLT neurones and nineteen (59%) of thirty-two POA neurones changed the activity within 1 min after the start of application of PGE_2 of less than 250 nM. The responses of such neurones recovered within 15 min. However, as shown in Fig. 6*C* and *D*, some neurones showed responses which were characterized with an unusually longer onset latency (> 5 min) and duration (> 20 min). This type of response appeared to be due to the property of the individual neurones. Changing the amount of PGE_2 applied did not essentially alter the response characteristics with

long time courses, although increasing the PGE_2 dose tended to prolong the duration of responses but with a shorter latency. The viability of tissue slices was not related to the time course of responses, since the incidence to record neurones responding with short time courses was not related to the time elapsed after the tissue slices were

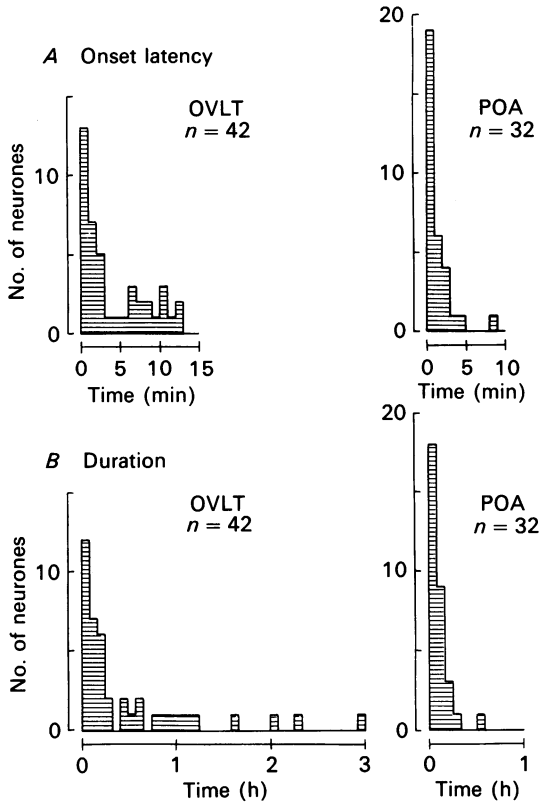

Fig. 7. Histograms of onset latency (*A*) and duration (*B*) of responses of OVLT neurones (left) and POA neurones (right) to local application of PGE_2.

prepared. The responses with short and long time courses were observed in different neurones recorded in the same slice preparation. The responses with long time courses were found more frequently in OVLT neurones than POA neurones (Fig. 7). While fifteen of forty-two OVLT neurones showed the responses of slow onset (latency 5–13 min) and slow recovery (duration longer than 20 min), similar types of responses were observed in only one of thirty-two POA neurones which responded to PGE_2 ($\chi^2 = 11{\cdot}38$ with d.f. = 1, $P < 0{\cdot}01$).

The effects of the simultaneous administration of AH6809 and PGE_2

In order to investigate whether the neuronal responsiveness to PGE_2 is caused by a receptor-mediated process, we examined the effect of AH6809, a prostanoid EP_1 and/or a DP receptor antagonist (Coleman, Kennedy & Sheldrick, 1985; Keery & Lumley, 1988), on the responses to PGE_2. As shown in Fig. 8*A*, the inhibitory effect

on a OVLT neurone induced by 50 nM of PGE_2 was reversibly blocked by the concurrent application of 6 μM of AH6809 which alone did not affect the neuronal discharges. The blocking effects of AH6809 on responses to PGE_2 were also observed in the other two OVLT neurones and four POA neurones (Fig. 8*B*).

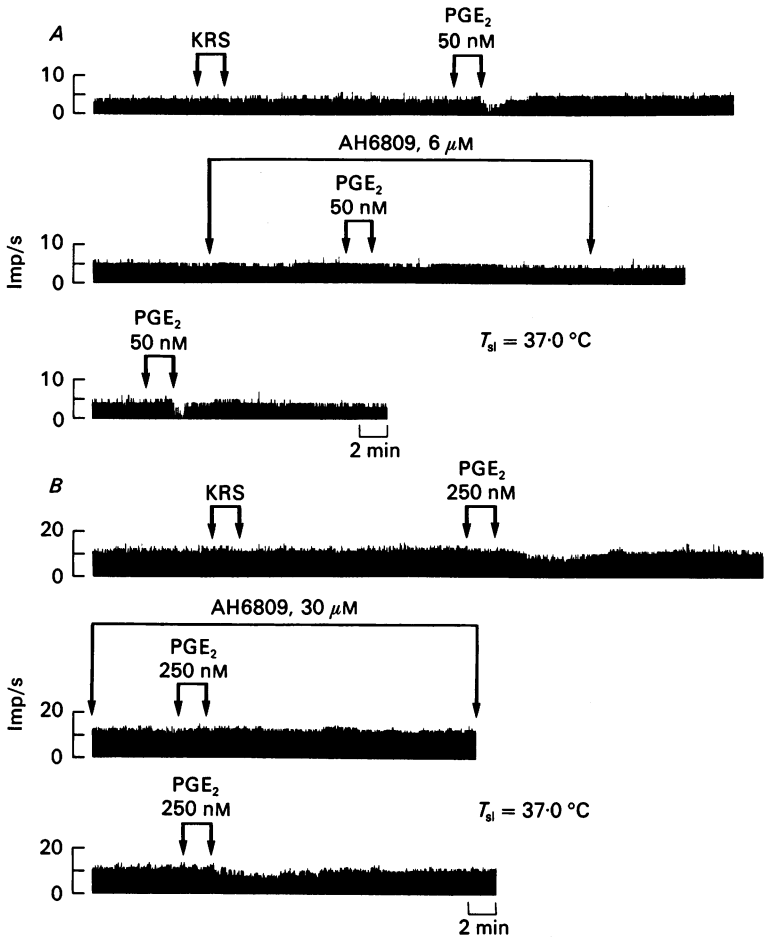

Fig. 8. Firing rate responses of a OVLT neurone (*A*) and a POA neurone (*B*) to local application of PGE_2 with and without AH6809, a prostanoid EP_1 and/or a DP receptor antagonist.

The relationship between thermal responsiveness and PGE_2 responsiveness in OVLT and POA neurones

While 29/31 OVLT thermosensitive neurones and 20/29 POA thermosensitive neurones altered the activity to application of PGE_2 of 250 nM or less, the PGE_2 responding neurones were found in 13/20 OVLT neurones and 12/29 POA neurones which were thermally insensitive. Thermosensitive neurones were more readily affected by PGE_2 than thermally insensitive neurones in both the OVLT ($\chi^2 = 6{\cdot}82$ with d.f. = 1, $P < 0{\cdot}01$) and the POA ($\chi^2 = 4{\cdot}46$ with d.f. = 1, $P < 0{\cdot}05$).

Out of thirty warm-sensitive neurones in the OVLT region, twenty-one neurones decreased the discharge rate to PGE_2 application (Fig. 4*A*), whereas seven neurones increased the activity and two neurones showed no response. The only OVLT cold-sensitive neurone which was tested in the present study was inhibited by PGE_2 (Fig. 6*A*). A statistical analysis revealed that there was a higher incidence of inhibitory responses to PGE_2 among the warm-sensitive neurones in the OVLT region ($\chi^2 = 6{\cdot}71$ with d.f. = 1, $P < 0{\cdot}01$, Table 1). On the other hand, there was no correlation between the type of thermal responsiveness and the type of responses to PGE_2 for POA neurones (Table 1). Out of twenty-eight POA warm-sensitive neurones examined, eleven were excited, nine were inhibited and eight were not affected by PGE_2 (Figs 2 and 3*C*). One cold-sensitive neurone in the POA studied did not change its activity during the perfusion of 250 nM-PGE_2.

DISCUSSION

The present study revealed (1) that 42/51 (82%) OVLT neurones and 32/58 (55%) POA neurones changed the activity in response to PGE_2 less than 250 nM through a receptor-mediated process, (2) that OVLT neurones were more sensitive to PGE_2 than POA neurones, and (3) that PGE_2 decreased the activity in the majority of warm-sensitive neurones in the OVLT, while there was no correlation in the POA neurones between the thermosensitivity and the responsiveness to PGE_2.

Responsiveness of OVLT and POA neurones to PGE_2

Although the responsiveness of OVLT neurones to PGE_2 in the present study is the first demonstration, it has been repeatedly shown that the direct application of PGE_2 by perfusion or by microelectrophoresis may alter the activity of hypothalamic thermosensitive neurones both in *in vivo* (Stitt *et al.* 1975) and *in vitro* studies (Boulant & Scott, 1986; Watanabe, Morimoto & Murakami, 1987; Morimoto, Murakami & Watanabe, 1988). The present results on POA neurones are somewhat different from these previous ones, in which predominantly facilitatory responses of POA neurones *in vitro* (Watanabe *et al.* 1987) or the low sensitivity to PGE_2 in POA neurones *in vivo* (Stitt *et al.* 1975) were demonstrated. However, the application of PGE_2 in unphysiologically high concentrations (e.g. 30 μM-PGE_2, Watanabe *et al.* 1987) or of unknown amounts by microelectrophoresis without paying attention to the tachyphylactic action of prostanoids in these previous studies cannot warrant the conclusions. To overcome these defects, we examined the responsiveness of OVLT and POA neurones to PGE_2 in doses between 1 and 250 nM which were applied at least 15 min after the previous application. These doses of PGE_2 used in the present study were considered to be within the physiological range. One study using a radioimmunoassay revealed that CSF PGE_2 levels in the third ventricle rose from a normal 2·5 ng/ml (about 7·5 nM) to a level of 11·5 ng/ml (34·5 nM) after intravenous injections of EPs/IL-1 in rabbits (Bernheim *et al.* 1980). The PGE_2 level in the rat brain reportedly increases to about 6–10 and 7–17 times the basal level, respectively, after application of EPs/IL-1 (Scott *et al.* 1987) and seizures by electroconvulsive shock or administration of pentetrazol (Berchtold-Kanz *et al.* 1981). A biochemical study also revealed that PGE receptors are found in most tissues in the body with

K_d values between 1 and 100 nM (Robertson, 1986). These figures conform well with the results of the present study in which the minimum effective doses to produce changes in discharge rate of OVLT neurones and POA neurones were 4·8 and 40·9 nM on average, respectively.

Sensitivity of OVLT and POA neurones to PGE_2 and the OVLT hypothesis

The obligatory role of PGE_2 in the central mediation of fever has been demonstrated by many workers in the past two decades (reviewed by Milton, 1982). Although the active site of PGE_2 in the brain in fibrogenesis has been considered to be in the POA, recent studies have proposed the critical role of the OVLT region as a possible site of entry and action of blood-borne EPs/IL-1 for the production of fever (Stitt, 1985, 1986) and adrenocorticotrophic hormone (ACTH) release (Katuura, Arimura, Koves & Gottschall, 1990). They suggest a PGE_2-mediated process in the OVLT region which translates the blood-borne signals conveyed by EPs/IL-1 into the brain signals. Lesioning the OVLT region greatly attenuates (Blatteis *et al.* 1983) or enhances (Stitt, 1985) the febrile responses to I.V. injection of pyrogen in guinea-pigs, rats and rabbits. IL-1β-induced release of ACTH increases after electrical and chemical lesions in the OVLT region (Katuura *et al.* 1990). A microinjection of indomethacin, a cyclo-oxygenase inhibitor, into the OVLT region suppresses the ACTH release induced by I.V. injection of IL-1β (Katuura *et al.* 1990). The hyperthermic action of PGE_2 is more potent when microinjected into the OVLT region than when injected into the POA (Stitt, 1986). Furthermore, the highest density of [^{3}H]PGE_2 binding has been observed in the regions surrounding the OVLT by *in vitro* quantitative autoradiography (Matsumura, Watanabe, Onoe, Watanabe & Hayaishi, 1990). The present finding of a higher sensitivity of neurones in the OVLT region to PGE_2 as compared to POA neurones may be consistent with the latter two findings, and strongly support the OVLT hypothesis.

Correlation between the responsiveness of neurones to PGE_2 and temperature

It has been demonstrated that the systemic injection of pyrogens decreases the activity of warm-sensitive neurones and increases the activity of cold-sensitive neurones (Cabanac, Stolwijk & Hardy, 1968; Wit & Wang, 1968) and that acetyl salicylate inhibits the pyrogen-induced neuronal activity (Wit & Wang, 1968). Since it has been proposed that warm-sensitive and cold-sensitive neurones facilitate heat defence responses and cold defence responses of thermoregulation, respectively (Boulant, Curras & Dean, 1989; Hori, 1991), these changes in POA thermosensitive neurones are appropriate in explaining the changes in thermoregulatory responses during the rising phase of fever, i.e., inhibition of heat defence responses and facilitation of cold defence responses. However, no studies on the direct application of PGE_2 on POA neurones have revealed a consistent relationship between the responsiveness of POA neurones to PGE_2 and temperature (Stitt *et al.* 1975: Boulant & Scott, 1986; Watanabe *et al.* 1987; Morimoto *et al.* 1988). In confirmation with these findings, no correlation between the responsiveness of POA neurone to PGE_2 and temperature was found in the present study which was undertaken in a more carefully controlled experimental condition than in the previous ones. Therefore,

none of these neurophysiological studies support the concept that the active site of PGE_2 responsible for fibrogenesis is the POA thermosensitive neurones.

On the other hand, it was found in the present study that the OVLT region contained thermosensitive neurones and the majority of OVLT warm-sensitive neurones decreased the activity after PGE_2 application. The neuronal signals arising from the OVLT region may be transmitted synaptically to the POA on abundant neural connections from OVLT to hypothalamus (Camacho & Phillips, 1981). Electrophysiological studies on thermosensitive neurones in the brain have suggested that warm-sensitive neurones and cold-sensitive neurones at one site are connected mostly to warm-sensitive neurones and cold-sensitive neurones in other sites, respectively, but rarely to thermosensitive neurones having different types of thermosensitivity and to thermally insensitive neurones (see review, Hori, 1991). In view of the thermoregulatory functions ascribed to thermosensitive neurones in the brain, one may speculate that PGE_2, which is released at the OVLT region in response to blood-borne EPs/IL-1 decreased the activity of warm-sensitive neurones locally; these neurones presumably have a high density of PGE_2 receptor, and the changes in the activity of OVLT warm-sensitive neurones, in turn, may decrease the activity of warm-sensitive neurones in the POA transsynaptically which will lead to a decrease in heat defence responses, as was observed during the rising phase of fever.

As for the responsiveness of cold-sensitive neurones to PGE_2, the present study cannot offer any definite conclusions. Only one cold-sensitive neurone each was studied in the OVLT and the POA. While the activity of the OVLT cold-sensitive neurone was inhibited by PGE_2, the POA cold-sensitive neurone did not respond to PGE_2 at less than 250 nM. Although about 5–10% of rat POA neurones corresponded to cold-sensitive ones when studied in *in vivo* preparations (Hori & Shinohara, 1979; Boulant *et al.* 1989; Hori, 1991), there is a consensus that a very few cold-sensitive neurones are recorded in the POA *in vitro* (Hori *et al.* 1980; Nakashima *et al.* 1987). This seems to be partly due to the low rate of spontaneous discharges in cold-sensitive neurones.

OVLT neurones exhibiting responses with long time courses

Another new finding here is that about one third of the neurones in the OVLT region, but not in the POA, showed excitatory and inhibitory responses to PGE_2 which are characterized with a slower onset (5–13 min) and slow recovery (longer than 20 min). We did not observe that the neurones exhibiting responses with short time courses showed any responses with long time courses by changing the amount of PGE_2 applied, or vice versa. The viability of brain slice preparations did not affect the time courses of responses. The responses with different time courses, therefore, are the properties of the individual neurones. Furthermore, this response is not a synaptically mediated one, because it was preserved in a Ca^{2+}-deficient solution. However, it is unknown whether the response of long time courses are prostanoid receptor-mediated ones, since the study of receptor blocking was not made on this type of response in the present study. Further studies will be required to elucidate whether the response with long time courses was produced by the release of other neurotransmitters induced by PGE_2, or by the changes in intracellular signal processing systems.

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