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Effect of Intravascular Infusion of Endogenous Pyrogen or Prostaglandin E_2 on Neuronal Activity of Rat's Hypothalamus

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Abstract We investigated the effects of intracarotid infusion of prostaglandin E_2 or intravenous infusion of an endogenous pyrogen on the neuronal activity of the preoptic and anterior hypothalamic (PO/AH) region in rats. The present results suggest that thermore sponsive neurons of the PO/AH region respond well to intravascular application of prostaglandin E_2 or the endogenous pyrogen, compared with thermally insensitive neurons. Intravenous infusion of the endogenous pyrogen attenuated the activities of warm-responsive and facilitated those of cold-responsive neurons. However, the direction of neuronal response induced by intracarotid infusion of prostaglandin E_2 could not be categorized based on the thermoresponsiveness of the individual neuron.

Key Words : Fever, Thermoregulation, Prostaglandin, Endogenous pyrogen, Thermoresponsive neuron

Introduction

According to the current theory, fever is caused by an endogenous pyrogen (EP), an active component of which is now thought to be interleukin-1, and which is released by circulating and reticuloendothelial macrophages in response to a variety of pathogenic stimuli such as bacterial endotoxin[1-3]. It has been generally belived that EP induces fever by its action on the central nervous system (CNS). Furthermore, since Milton and Wendlandt observed the strong pyrogenic action of intracerebroventricularly injected prostaglandin E_1, prostaglandins have been assumed to be possible candidates for the final mediators in the CNS involved in the pathogenesis of fever[4,5].

Since many thermoresponsive neurons have been observed in the preoptic and anterior hypothalamic (PO/AH) region, the roles of the neurons in body temperature regulation have been investigated[6]. Some investiga tors have examined the changes in the neuronal activity of the PO/AH region after intravenous injection of several kinds of pyrogen[7-9] and have reported that the activities of warm-responsive neurons were decreased and those of cold-responsive neurons were increased. It has also been reported that local injection of EP or prostaglandin into the PO/AH region induced significant fever.[5,10,11] These results are consistent with the idea that fever might be processed by the suppression of heat loss closely related to the attenuation of warm-responsive neurons

and/or by the enhancement of heat production related to the activation of cold-responsive neurons[12]. Recently we examined the direct effect of EP and prostaglandin E_2 (PGE_2) on neuronal activity of the PO/AH region *in vitro* using brain slice preparations of rats[13] and guinea pigs[14]. The results showed that EP and PGE_2 changed the neuronal activity of thermoresponsive neurons in the PO/AH region, but the direction of neuronal response induced by these substances could not be generally categorized based on the thermoresponsiveness of individual neurons.

Recently we have shown that prostaglandin related to the fever induction is synthesized both inside and outside the blood-brain barrier (BBB)[15,16]. Furthermore, according to the recent report of Stitt[17,18], the site of release of endogenous prostaglandin in response to circulating EP in rats is the organum vasculosum laminae terminalis (OVLT), and the OVLT, rather than the PO/AH region, is the site most sensitive to prostagladin for producing fever. At present, it still remains unknown whether prostaglandin synthesized outside the BBB affects the receptors of the OVLT neurons which functionally locate outside the BBB and subsequently induce the changes in the activities of thermoresponsive neurons of PO/AH region, or whether prostaglandin passing through the BBB and/or synthesized within the BBB affects the neuronal activities of brain regions other than the PO/AH region[19] and subsequently induces the changes in the activities of thermoresponsive neurons in the PO/AH region. Therefore, we examined in the present study how the activity of thermoresponsive neuron in the PO/AH region changes during fever induced by intracarotid infusion of PGE_2. Moreover, by comparing changes in neuronal activity of the PO/AH region induced by intracarotid infusion of PGE_2 with those induced by intravenous infusion of EP, we tried to discuss the effect of PGE_2 and EP on thermoresponsive neurons in the PO/AH region and a role of these substances for fever development.

Materials and Methods

Male Wistar strain rats weighing between 270 g and 400 g were used in this study. The animals were anesthetized with Urethane (1.0-1.2 g/kg, i.p.). A polyethylene tubing was surgically inserted into the superior caval vein or the carotid artery. Intravenous or intracarotid infusion was performed through each tubing, at a slow rate (0.02 ml/min) with a syringe pump.

To examine the activity of the thermoresponsive neurons in the PO/AH region, a pair of stainless steel tubes (0.9mm in O.D.) were stereotaxically implanted into the right half of the brain according to the rat brain coordinates[20] : AP ; 2.0mm anterior and 1.5mm posterior to the bregma, L ; 2.0mm lateral to the midline, V ; to the bottom. The anterior tube was used as the thermode and the posterior one was used to measure the brain temperature by a copper-constantan thermocouple. Local heating and cooling of the PO/AH region was accomplished by means of a water-perfused thermode system. A piece of skull bone and lining dura at the left side were removed to insert a recording electrode. The skull opening was filled with liquid paraffin. Using a glass microelectrode filled with Pontamine sky blue (2%) dissolved in 0.5 M sodium acetate, single unit discharges were extracellularly recorded at coordinates : AP ; 1.0 -2.0 mm, L ; 0.5-1.2 mm V ; 6.5-10.0 mm. During the experiment, rectal temperature and tail skin temperature were measured by copper-constantan thermocouples, and a water-perfusion hot mat (33-35 ℃) was placed beneath the abdomen. PGE_2 was dissolved at a concentration of 0.25 mg/ml in sterile saline.

The endogenous pyrogen (EP) used in this experiment was prepared from white blood cells of male rabbits (New Zealand white strain). The white blood cells were stimulated by lipopolysaccharide of *Salmonella typhosa* endotoxin (Difco). The general procedures have been described in detail elsewhere[21]. Partial purification was achieved by ultrafiltration using two types of membranes (10YM10 and 50XM10, Amicon), which removed all substances of the molecular weight outside the range of 10000-50000. Consequently, 1.0 ml of the partially purified EP solution was derived from approximately 1.5×10^7 white blood cells. The intravenous injection of 0.2 ml/kg of the EP preparation to a rabbit produced monophasic fever, whereas the injection of the same amount of EP pretreated at 60 ℃ for 30 min

did not cause fever. Thus the EP preparation was not contaminated with endotoxin.

After the end of the experiment, the animal was sacrificed by an overdose of Nembutal, and the brain was fixed by 10% formalin solution. The recording site marked with Pontamine sky blue, electrophoretically applied, was histologically examined.

Results

First, we examined the effect of intracarotid injection (bolus) of PGE_2 on the rectal temperature in rats. As shown in Figure 1, the intracarotid injection of PGE_2 over the range of 50-200 μg/kg produced dose-dependent fever in 4 different rats even under general anesthesia. The febrile pattern was typically monophasic, and the peak in the rectal temperature attained between 20-30 min after injection.

Figure 2 shows the responses of a warm-responsive neuron recorded at the indicated site in the PO/AH region after intracarotid infusion of PGE_2. Since the neuron increased its firing rate with a rise in hypothalamic temperature, it was a so called warm-responsive neuron. The activity of the neuron was not affected by the infusion of saline, but was affected by the infusion of PGE_2 (total 200 μg/kg). Ten min after the beginning of the infusion, the firing rate markedly increased, and then returned to the initial level 20 min after the end of the infusion. The maximal increase in rectal temperature was 0.3 ℃. Figure 3 shows the response of a cold-responsive neuron recorded at the indicated site in the PO/AH region after intracarotid infusion of PGE_2. The neuron increased its firing rate with a decrease in hypothalamic temperature. The firing rate of the neuron markedly increased 6 min after the beginning of the intracarotid infusion (total 90 μg/kg) of PGE_2 and returned to the initial level 10 min after the end of the infusion. The increase in rectal temperature was not seen, which was different from that (0.4 ℃) in the similar dose (100 μg/kg) in Figure 1. The infusion of saline did not affect the activity of the neuron.

A total of 35 neurons of the PO/AH region were examined to study the effect of the intracarotid infusion of PGE_2. Among them, 10 were warm-responsive, 6 were cold-responsive, and 19 were thermally insensitive neurons. These neurons were classified by the temperature quotient (Q10) ; warm-responsive, cold-responsive, and thermally insensitive neurons had $Q10 > 2.0$, $Q10 < 1.0$, $1.0 < Q10 < 2.0$, respectively. A change in firing rate over ± 50% as compared with the control level was judged as a neuronal response. Among the warm-responsive neurons, the activities of 4 neurons were facilitated, those of 3 neurons were inhibited, and those of 3 neurons were not affected by the intracatotid infusion. Among the cold-responsive neurons, activities of 2 neurons were facilitated and 4 remaining neurons were not affected by infusion of PGE_2. In contrast to the thermally sensitive neurons, thermally insensitive neurons were less affected by the intracarotid infusion of PGE_2. Thus, one was facilitated, 3 were inhibited, and 15 remaining neurons were not affected.

Next, we examined the effect of intravenous injection of EP through the superior caval vein on the rectal temperature in rats.

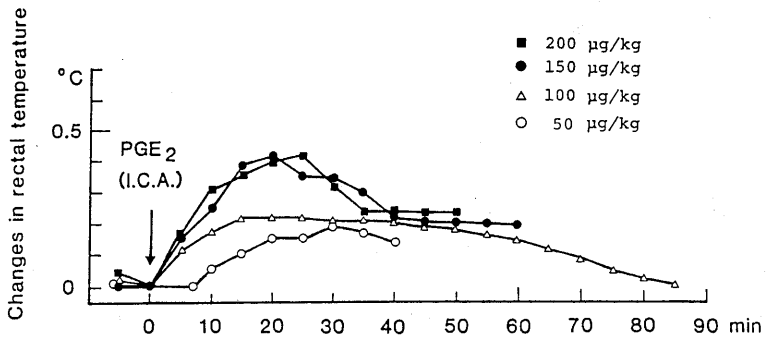

Fig. 1. Changes in rectal temperature of anesthetized rats after intracarotid injection of PGE_2.

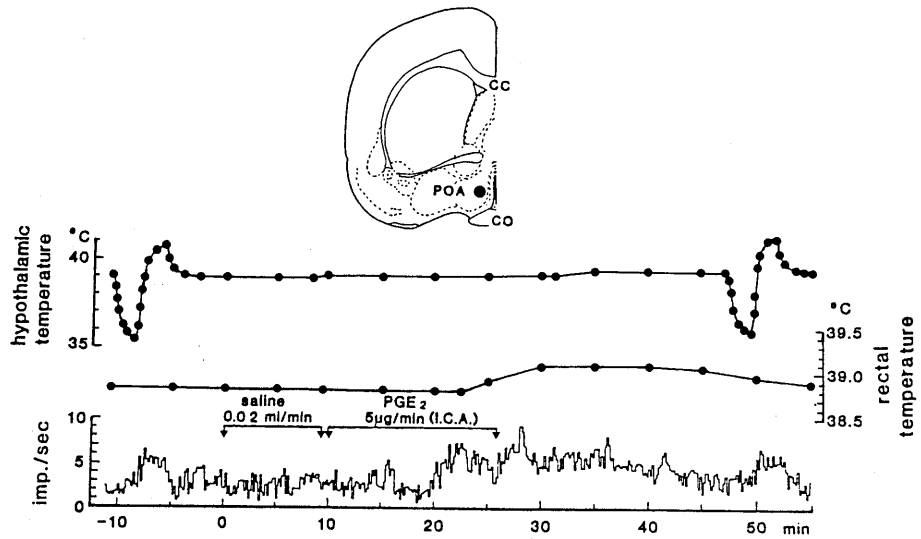

Fig. 2. Response of a warm-responsive neuron of PO/AH region in rat after intracarotid infusion of PGE_2 and recording site of this neuron.
CC : corpus callosum, POA : preoptic area
CO : chiasma opticum

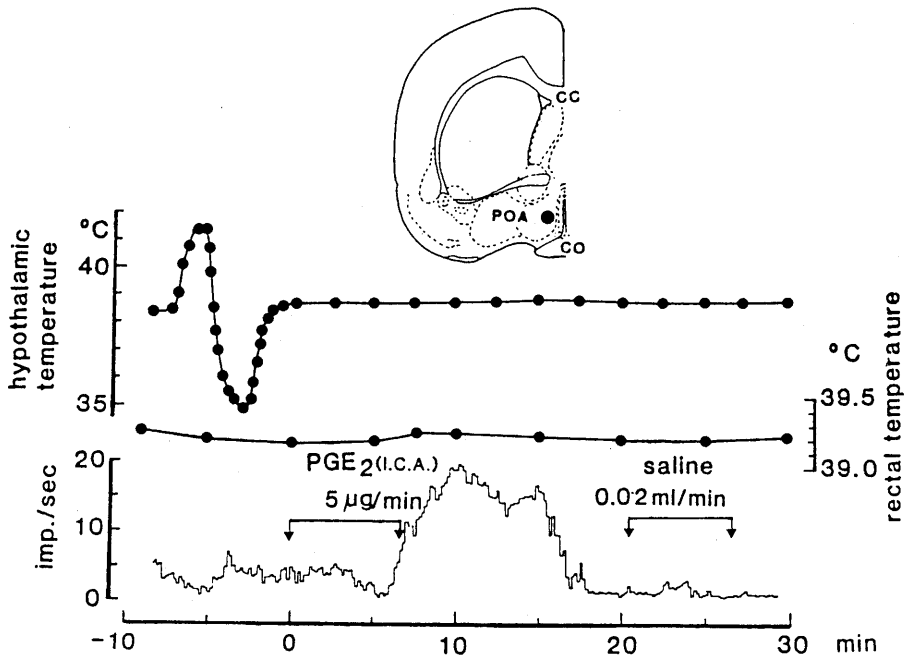

Fig. 3. Response of a cold-responsive neuron of PO/AH region in rat after intracarotid infusion of PGE_2 and recording site of this neuron.
CC : corpus callosum, POA : preoptic area
CO : chiasma opticum

As shown in Figure 4, the intravenous injection (bolus) of EP over a range of 0.6-1.6 ml/kg produced dose-dependent fever in 5 different rats even under qeneral anesthesia. The febrile patterns were monophasic or biphasic. The initial phase in fever was induced between 20-60 min after the injection, and the second phase was induced from 60 min after the injection. A half of rats did not produce fever after EP injection under anesthesia (data not shown).

Figure 5 shows the response of a warm-responsive neuron recorded at the indicated site in the PO/AH region after intravenous infusion (within 1 min) of EP. The activity of the neuron began to decrease 5 min after the beginning of the infusion of EP and returned to the initial level 25 min after the infusion, while the increase in rectal temperature was small, as compared with that in the same dose (Fig. 4). In contrast, as shown in Figure 6, a cold-responsive neuron was markedly excited 3 min after the intravenous infusion (within 1 min) of the EP. This excitatory response returned to the control level after 30 min. The increase in rectal temperature after the infusion of EP was not seen.

A total of 38 PO/AH neurons were tested for intravenous infusion of EP. Sixteen were warm-responsive neurons, 11 were cold-responsive neurons and 11 were thermally insensitive neurons. The activity of 10 warm-responsive neurons was inhibited and one neuron was excited after intravenous infusion of EP. Five neurons were not affected. The activity in 6 cold-responsive neurons was excited and one neuron was inhibited after intravenous infusion of EP. The activity of 10 thermally insensitive neurons was not affected by intravenous infusion of EP, while that of one neuron was facilitated.

Discussion

Fever has been believed to be caused by the action of EP on the CNS, and the PO/AH region has been thought to play an important role in the development of fever because it was specifically sensitive to the microinjection of EP[10,22]. At present it is generally known, however, that circulating EP acts on structures inside and outside the BBB to synthesize and release prostaglandins[16], which in turn act on the CNS to produce fever. As for the CNS sites involved in fever production in response to prostaglandin, the PO/AH region is postulated because it is specifically sensitive to the mediator[5]. Recently, however, sites more sensitive than the PO/AH region, such as the OVLT[17] or the ventromedial hypothalamus[19], have been found, and multiple and complex neuronal networks are suggested for fever production[19,22,23].

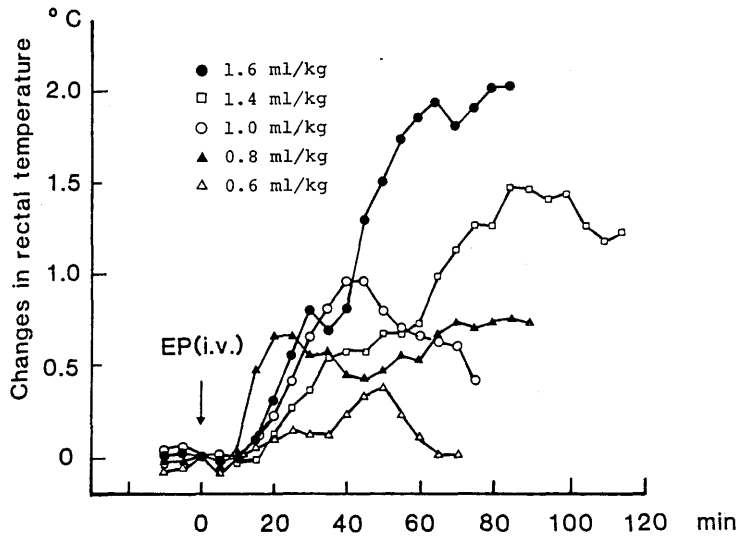

Fig. 4. Changes in rectal temperature of anesthetized rats after intravenous injection of EP.

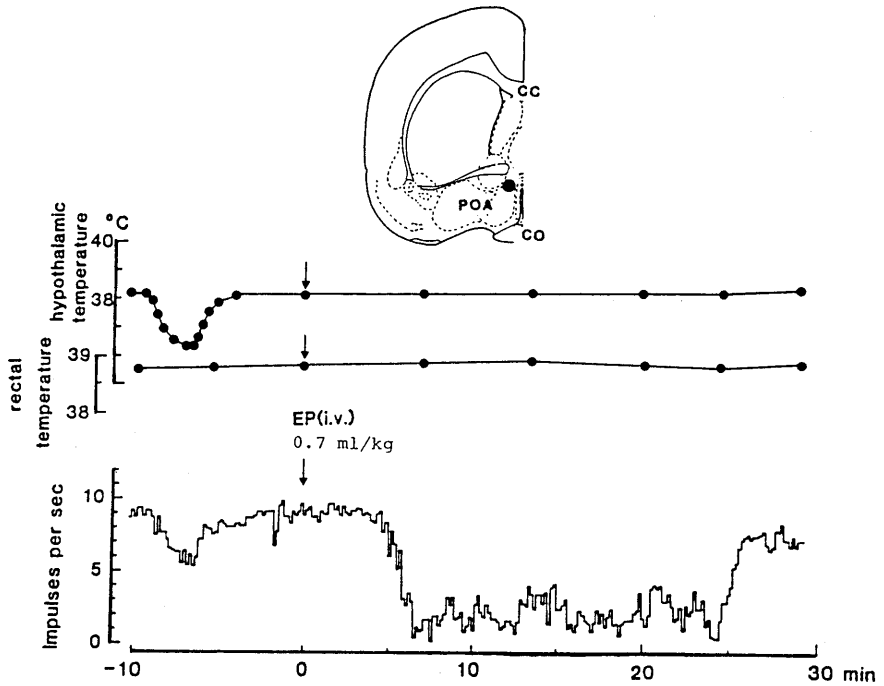

Fig. 5. Response of a warm-responsive neuron in PO/AH region of rat to intravenous infusion of EP and recording site of this neuron.
CC : corpus callosum, POA : preoptic area
CO : chiasma opticum

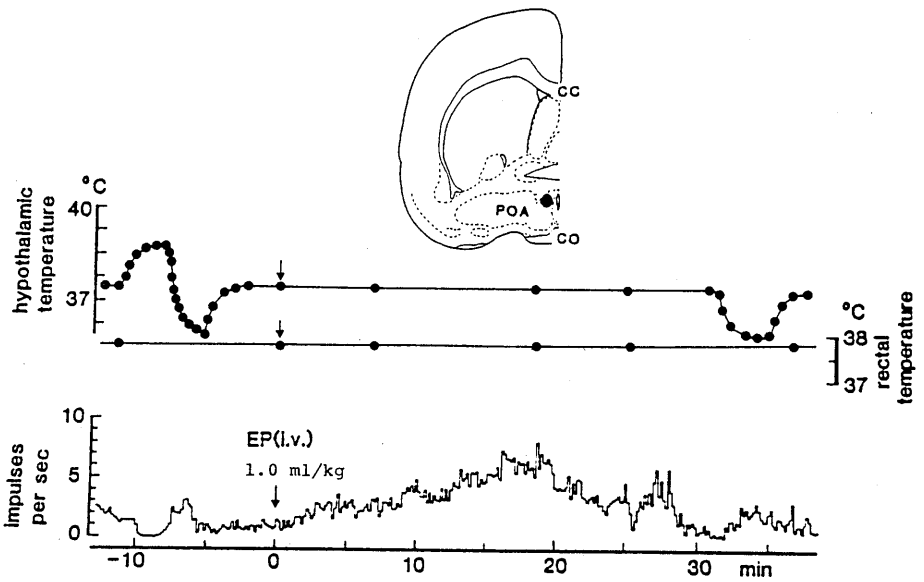

Fig. 6. Response of a cold-responsive neuron in PO/AH region to intravenous infusion of EP and recording site of this neuron.
CC : corpus callosum, POA : preoptic area
CO : chiasma opticum

In the present results, rats produced the monophasic fever when PGE_2 was injected through the carotid artery. This indicates that PGE_2 stimulates the receptors of the OVLT neurons, which functionally locate outside the BBB and/or PGE_2, passing through the BBB, acts on the CNS, and subsequently induces fever. Furthermore, the present results showed that neuronal activities of the PO/AH region were affected by the intracarotid infusion of PGE_2, and thermoresponsive neurons appeared to be more affected than thermally insensitive neurons. However, the direction of neuronal response induced by intracarotid infusion of PGE_2 varied and could not be categorized based on the thermoresponsiveness of individual neurons, although the number of neurons examined in the present study seems to be too small to obtain a definite conclusion. The present results are in accordance with our in vitro results with rat[13] and quinea pig[14] hypothalamic slice preparations, suggesting that thermoresponsive neurons of the PO/AH region tend to respond well to the direct application of PGE_2, compared with thermally insensitive neurons. This suggests that thermoresponsive neurons of the PO/AH region have multiple synaptic with peripheral and/or central PGE receptors involved in fever production, or it is inferred that these neurons are more sensitively affected to maintain the body homeostasis in response to external- and internal-temperature changes.

Intravenous injection of EP produced fever under general anesthesia. The febrile patterns were varied, monophasic or biphasic, depending on injection doses. This indicates that circulating EP acts on structures inside and outside the BBB which subsequently synthesize and release endogenous prostaglandins, which in turn cause fever. The thermoresponsive neurons in the PO/AH region responded well to intravenous infusion of EP, compared with thermally insensitive neurons. However, in contrast to the results obtained from intracarotid infusion of PGE_2 and those obtained from brain slice preparations[13,14], the percentage of warm-responsive neurons whose activities were suppressed and that of cold-responsive neurons whose activities were facilitated seem to be greater. These results are in part similar to the previous results of cats[9,24] and rabbits[7,8], although there were more thermoresponsive neurons which did not respond to intravenous infusion of EP in the present results. It is considered that the amount of endogenous prostaglandins synthesized and released in response to circulating EP might be very small but suffi cient for appropriate stimulation of prostaglandinreceptors involved in fever production. Consequently the activity of thermoresponsive neurons of the PO/AH might be variable depending on the neuronal inputs generated from neurons with these receptors. However, when a large dose of PGE_2 was infused into intracarotid artery or applied directly to hypothalamic slices, PGE_2 may stimulate many receptors, existing in both peripheral and central, which involve not only thermoregulation but also other functions.

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