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Responses of Temperature-Responsive Neurons in the Medulla Oblongata of the Rabbit to Micro-Electrophoretic Application of Endogenous Pyrogen

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Abstract Although the temperature-responsive neurons in the preoptic/anterior hypothalamic region, midbrain and the medulla oblongata show responsiveness to endogenous pyrogen (EP) injected systemically and locally, the question as to whether EP acts directly on these neurons per se still remains unknown. To answer this question the experiments were attempted to administer EP, partially purified by ultrafiltration, onto the medullary temperature-responsive neurons in the rabbits, using method of micro-electrophoretical application. Before the application, it was proved that this partially purified EP could be transported through the tip of a five-barrelled electrode with micro-electrophoretical application. A total of 69 neurons was examined for the action of EP. Twenty-two out of 35 temperature-responsive neurons examined changed their activities in response to the EP applied with a positive current over 50 nA. None of the 23 temperature-insensitive neurons was affected by the application of the EP. All of these 58 neurons did not respond to heated EP or saline with the same current but excited to sodium glutamate. Ten of 69 neurons were affected by the sodium ion. From these results it is concluded that the EP acts very closely or directly onto the limited temperatrue-responsive neurons in the medulla.

It has generally been recognized that fever is induced by endogenous pyrogen (EP) released from phagocytic leucocytes and that action of the pyrogen is not to inactivate or disorganize the control of body temperature, but to raise the level at which body temperature is controlled; Fever is regarded as a regulated upward adjustment of thermoregulatory set-point in the temperature control system of the CNS. Such a theory has been based on observations of the effects of pyrogen upon the activity/temperature characteristics of temperature-responsive neurons in the preoptic/anterior hypothalamic region (PO/AH); the activity of warm-responsive neurons is inhibited and that of cold-responsive neurons is facilitated by systemic or local application of pyrogen.[1-4] It has also been reported that the medullary temperature-responsive neurons has responsiveness to the EP administered systemically or locally.[5,6] More recently, several substances other than EP were proposed to mediate the development of

fever.[7,8] However, none of these experiments can be taken as evidence for direct action of the substances on the temperature-responsive neuron per se. Thus, the question as to whether the EP acts directly on the temperature-responsive neurons per se still remains unknown. We attempted to answer this question by means of micro-electrophoresis of the EP onto the medullary temperature-responsive neurons.

Methods

Twenty-five rabbits of New Zealand White,weighing 2.5–3.5 kg were used. Under anesthesia with Urethane (1.1–1.3 g/kg, i.p.), the animal was positioned on a stereotaxic instrument. The animal's head was bent ventrally as much as possible and decerebellated to expose the dorsal surface of the medulla oblongata. Two stainless-tubes sealed at one end were implanted unilaterally in the same parasagital line 0.5 mm to the right from the midline of the medulla. One was used as a thermode, at a point 1–4 mm rostral from the obex and at a depth of 6 mm from the dorsal surface, and the other for the measurement of the medullary temperature. A thermocouple was introduced in the second tube placed 2.5 mm rostrally from the thermode. A tip of glass-microelectrode filled with 3M NaCl which was attached to the five-barrelled electrode by adhesive (Arone Alpha 202, TOA-GOSEI) in protruding 20–30 μm from the top of the five-barrelled electrode, was used for the extracellular recording of the single unit activity of the medullary neurons. This multi-barrelled electrode was used for a micro-electrophoretical application of the drugs onto the medullary temperature-responsive neurons, and inserted into the left hand side of the medulla at a point 2.5 mm lateral to the thermode. The method used for changing the medullary temperature was the same as described previously[5]. The action potential of the neuron was amplified by differential amplifier, displayed on occiloscope (VC-9, NIHON-KODEN) and counted the rate of impulses by a digital counter-printer every 10 sec. The micro-electrophoretical application of the drugs was performed by Microiontophoresis Unit (DPI-310, DIAMEDICAL), which can pass the direct current through the multi-pipette with the maximal current up to 1 μA under an electric resistance less than one thousand mega ohm.

Crude EP was prepared by the method of Cranston et al[9]. with slight modification. The whole blood collected from the carotid artery of a rabbit was stimulated by mixing Lipopolysaccharide (Salmonella typhosa, Difco Lab.) at a concentration of 5 μg/ml and incubated at 37°C for 2 hr. After centrifuging the blood at 2000 rpm for 10 min, the buffy coat layer was collected, repeatedly washed with 0.9% saline containing 100 unit/ml penicillin G, 100 μg/ml streptomycine and 0.1% glucose, resuspended in 0.9% saline at a concentration of $5–8\times10^3$ leucocytes/μl, and incubated at 37°C for 18 hr. After centrifuging the suspension at 1500 rpm for 10 min, the supernatant contained EP was used as crude EP. The crude EP solution contains many kinds of proteins other than the EP. And not all proteins have the pyrogenic action on animals. Hence, the crude EP, many protein other than the EP, may possibly interfere with the electrophoretical transportation of the EP through the multi-barrelled pipette. For electrophoretical application the curde EP solution should be purified to contain only EP. Recently, it has been demonstrated that there are two kinds of pyrogens with molecular weight of 15,000 and 45,000[10]. By using two types of membrane filter (10YM10, 10XM50 Amicon) substances with molecular weight less than 10,000 or more than 50,000 M.W. was removed from this crude EP solution and then, the solution was concentrated under nitrogen gas pressure of 3 kg/cm^2 in 4°C. Intravenous injections of this partially purified solution could produce monophasic fever at a short latency within 15 min in rabbits, while this solution did not show any pyrogenicity when heated at 93°C for 40 minutes.

Next, it should be verified that the partially purified EP is actually transported through the multi-barrelled pipette during the micro-electrophoresis. This was evidenced by following experiment. Only the tip of multi-barrelled electrode filled with the EP solution, was lowered into a small quantity (150 μl) of sterile saline. The electrophoretical application of the EP with 80 or 50 nA for 60 sec was repeated 1000 times with intervals of 100 sec. Then, this saline solution containing transported EP was administered into the PO/AH region of rabbits with a diffusion through guide cannulae

implanted chronically, according to the coordinate [11].

Fig. 1 represents changes in the rectal temperature following an administration of this solution of 30 μl into the PO/AH region. The mean of the rectal temperature increased approximately 1.0 or 1.5°C by the 1000 times application of 50 and 80 nA, respectively, as shown with solid lines. However, no significant changes in the rectal temperature were observed when the heated solution was administered in the same way, as indicated by the dotted line. This result revealed the EP had been transported through the tip of the pipette during the micro-electrophoresis. The electrophoretical application of the ultrafiltrated EP onto temperature-responsive neurons became now possible.

Results

A total of 69 medullary neurons was examined for the micro-electrophoresis of the EP, heated EP, saline and sodium glutamate ; 18 wa-rmresponsive neurons, 22 cold-responsive neurons and 29 temperature-insensitive neurons.

Fig. 2 shows the activity changes of the representative neuron displaying the effect of EP applied micro-electrophoretically onto the medullary warm-responsive neurons. This neuron whose activity decreased with the medullary cooling was identified as warm-responsive neuron. The micro-electrophoretical injection of the EP with currents of 80 and 60nA caused the remarkable inhibition in the activity ,which was evoked by the application of the EP with a current 60 nA for a longer time. While no changes was obtained with an application of 50 nA. However, no significant change in the activity was caused by micro-electrophoretical application of the heated EP with positive current of 80 nA, through which the application of EP was enough to inhibit the activity. Application of sodium glutamate with a negative current of 40 nA could induce the facilitatory response, implying the tip of the multi- barrelled electrode was in close proximity to this neuron.

On the contrary, Fig. 3 represents the facilitatory effect of the EP applied micro-electrophoretically onto cold-responsive neuron. This neuron's activity was inversely related to the changes in the medullary temperature. Micro-electrophoretical application of EP with currents of 50 and 80 nA caused augumentation of the activity in the cold-responsive neuron, while an application of EP with 20 nA induced no significant changes. The magnitude of the facilitatory response was augmented with increasing of the current intensity. Micre-electrophoresis of the heated EP with a current of 80 nA had no effect on the activity and sodium glutamate (40 nA) always had the excitatory response. Fig. 4 indicates no responsiveness of temperature-insensitive neuron to the application of the EP, heated EP and saline with a current of 80 nA, while the excitatory response to sodium glutamate (40 nA) was observed as well as the temperature-responsive neuron.

Based on the effects of the EP, heated EP and saline applied micro-electrophoretically with currents of 50–100 nA onto the medullary neurons, all of which responded in the excitatory manner to sodium glutamate, the result was summarized in Table 1. The activity in 16 out of 18 warm-responsive neurons did not significantly change by the heated EP and saline (8 neurons were not tested). The 10 out of these 16 neurons showed the inhibitory response to the ultrafiltrated EP. Six out of these 16 warm-responsive neurons did not show any responses to the EP. However, 2 out of the 18 warm-responsive neurons respresented the excitatory response to the heated EP, saline and the EP (one neuron is not affected by the EP). This response is not due to the action of EP but rather sodium ion. The activity in 19 out of the 22 cold-responsive neurons showed no response to heated EP and salne (7 neurons were not tested). Twelve out of these 19 neurons were excited by the EP.

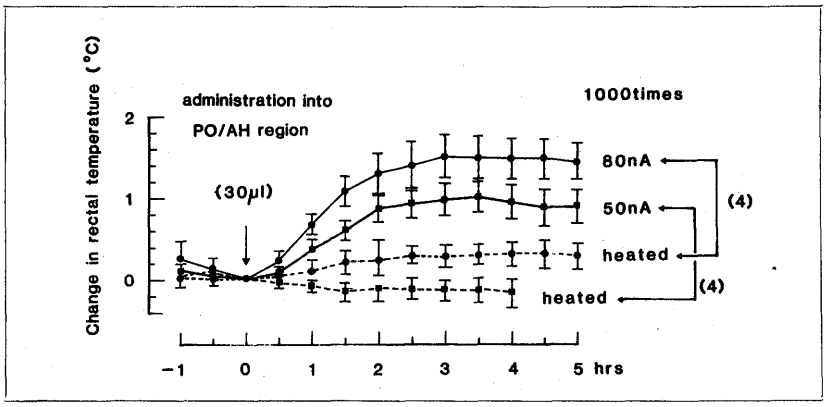

Fig. 1 Changes in rectal temperature (solid line) when the solution in which the ultrafiltrated EP was transported through the multi-barrelled pipette during the micro-iontophoresis with 80 nA (circles) or 50 nA (squares) during 60 sec which was repeated 1000 times with interval of 100 sec, was administered into the PO/AH of the four rabbits. No changes in rectal temperature (dotted lines) when the heated solution was administered into the same region. Vertical bars indicate standard error.

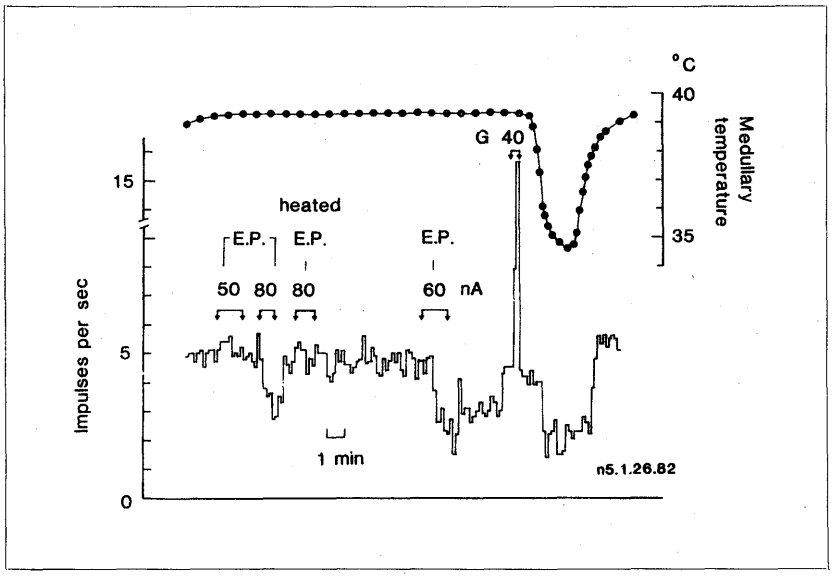

Fig. 2 The responses of the medullary warm-responsive neurons to the micro-iontophoresis of EP, heated EP and sodium glutamate (G).

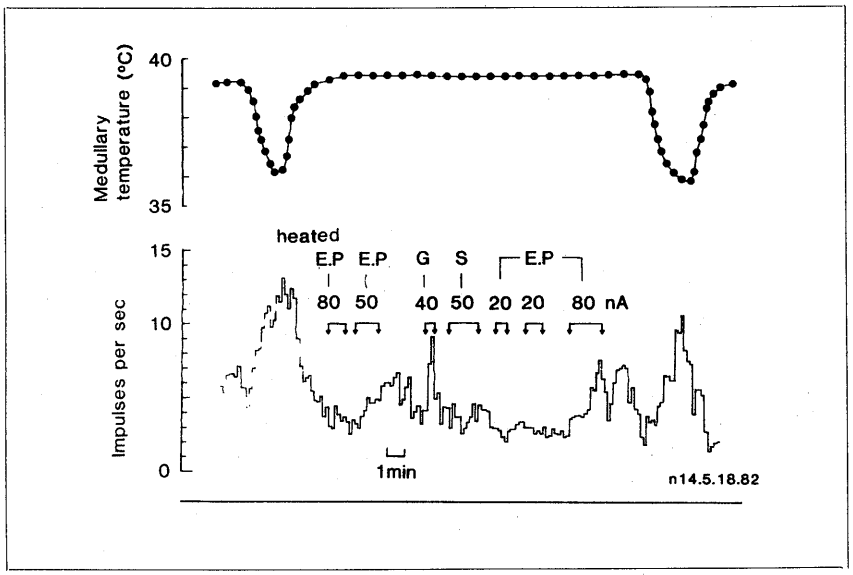

Fig. 3 The responses of the medullary cold-responsive neuron to the micro-iontophoresis of EP, heated EP, saline (S) and sodium glutamate (G).

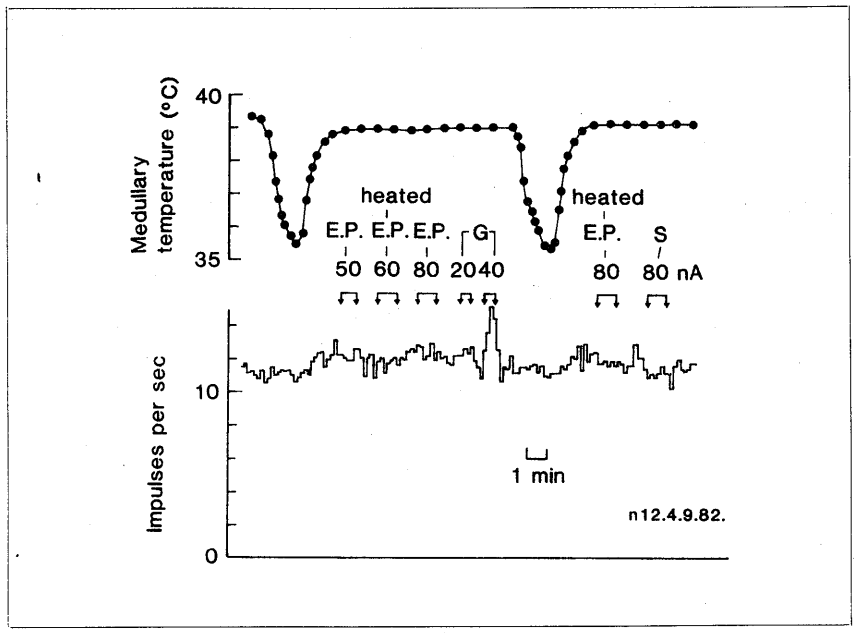

Fig. 4 The responses of the medullary temperature-insensitive neuron to the micro-iontophoresis of EP, heated EP, saline (S) and sodium glutamate (G).

Three out of the 22 cold-responsive neurons were affected by all of the drugs (2 neurons were excited and one neuron was inhibited) .Twenty-three out of the 29 temperature-insensitive neurons did not exhibit any significant changes in their activity to all of the drugs (7 neurons are not tested for saline). But, 6 out of the 29 temperature-insensitve neurons were affected by these drugs (2 neurons did not respond to the EP). Considered together, thus, 10 out of the 69 medullary neurons examined have the responsiveness to sodium ion. However, 22 out of the 35 temperature-responsive neurons responded to the EP injected microelectrophoretically. Fig. 5 represents the relation of changes in the discharge activity of the temperature-responsive neurons to the EP doses which are expressed as the electric charge (μC) passed through the piptette onto the neurons, all of which were responsive to EP and sodium glutamate but unresponsive to the heated or saline. Changes in the discharge activity of the neurons relative to the maximal response are plotted as percentage on the ordinate. The %-response of each temperature-responsive neuron was dependent on the dose of EP. Four of the 7 warm-responsive neurons had the threshold (0.3-1.0 μC) of their responses to the EP lower than that (1.2 μC) of the 6 cold-responsive neurons.

Discussion

The results in the present study show that the EP acts directly on the limited temperature-responsive neurons but does not on the temperature-insensitive neurons in the medulla oblongata of rabbits. The responsiveness of the medullary temperature-responsive neurons to EP is different from that of the temperature-responsive neurons in the PO/AH to pyroge injected systemically[1-3]; all of temperature-responsive neurons tested in the PO/AH were affected by the pyrogen.

The microinjection of EP into the brainstem (including the medulla oblongata) of rabbits could produce the weak fever[12]. Therefore, the medullary temperature-responsive neurons responded to the EP may be concerned with developing fever. Also, the medullary temperature-responsive neurons

Table 1 Summarization on the responses of the medullary neurons to ultrafiltrated EP, heated EP, saline and sodium glutamate during the micro-iontophoresis.

	Heated EP		Ultrafiltrated EP			Saline				Sodium glutamate		
			↑	—	↓	↑	—	↓	notest	↑	—	↓
Warm-responsive neuron	↑	2	1	1	0	2	0	0	0	2	0	0
	—	16	0	6	10	0	8	0	8	16	0	0
	↓	0	0	0	0	0	0	0	0	0	0	0
Cold-responsive neuron	↑	2	2	0	0	2	0	0	0	2	0	0
	—	19	12	7	0	0	12	0	7	16	0	0
	↓	1	0	0	1	0	0	0	1	1	0	0
temperature-insensitive neuron	↑	6	4	2	0	6	0	0	0	6	0	0
	—	23	0	23	0	0	16	0	7	23	0	0
	↓	0	0	0	0	0	0	0	0	0	0	0
Total		69										

↑ : facilitatory response ↓ : inhibitory response
— : no response

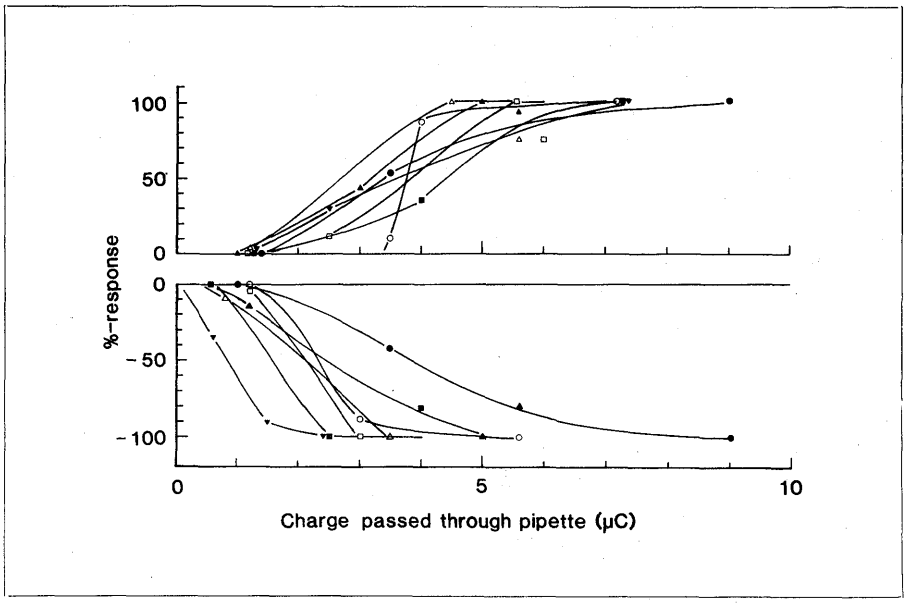

Fig. 5 The relationship between the %-response of each of temperature-responsive neurons to EP and the charge passed through pippete.
Upper curves; cold-responsive neurons
Lower curves; warm-responsive neurons

unresponded to the EP may be not related to the development of fever, but to limiting of the maximal fever[13].

The responsiveness of the medullary neurons to the direct application of EP has shown certain similarity with that of hypothalamic neurons examined in vitro study; The majority of warm- and cold-responsive neurons in the PO/AH tissue slices showed the inhibitory and excitatory response, respectively, to the EP purfused in the tissue chamber[14]. Further, the warm- and cold-responsive neurons showed the characteristic responses directly opposite to each other (Fig. 5 and Table 1). The warm-responsive neurons tend to be more sensitive than the cold-responsive neurons to the EP. The EP sensitivity of both types of the medullary neurons may be inherent. This seems to be different from their temperature sensitivity. That is, Kelso and Boulant reported that after tissue purfusion with a synaptic blocking medium, containing high magnecium and low calcium concentrations, thermosensitivity was retained in nearly all of the warm-responsive neurons in hypothalamic tissue-slices, but thermosensitivity of all cold-responsive neurons was lost[15]. They suggested that the PO/AH cold-responsive neurons depend on synapses from nearly warm-responsive neurons for their temperature sensitivity.

However, we can not exclude a possibility that the EP acts on presynaptic membrane onto the temperature-responsive neurons and modifies the neuronal transmission, because that presynaptic membrane is extremely contiguous to the postsynaptic membrane. Therefore, it is natural to conclude from the present study that EP acts very closely or directly on the limited temperature-responsive neurons.

Recently, it has been suggested that the activity of phospholipase A2, by which arachidonic acid is released from phospholipids in the plasma membrane and a synthesis of arachidonic acid derivatives (Leukotriene, Thromboxane, Prostaglandin and Prostacyclin) is initiated, is important for the mediation of EP fever, and the arachidonic acid derivatives may act to sensitize neurons to the action of EP. Then, it may be possible that in the plasma membrane of the temperature-responsive neuron arachidonic acid cascade is involved in sensitizing or mediating the action of EP.[16]

A unresolved problem is remained as yet whether EP released from leucocytes get into the CNS across blood brain barrier during peripheral infection. But an arival of EP into the CNS may become possible with emigration of leucocytes through vascular system in the brain in inflammatory condition[17]. It also has been reported that Interleukin-1 like factor was produced from murine glia cells of the brain which is cultured in the medium containing Lipopolysaccharide, has characteristics identical to EP, and is related to an immune response in the brain[18,19]. Therefore such Interleukin-1 like factor may be possible to be released from glia cells during inflammation in the brain parenchyma, and interact the temperature-responsive neurons in the brainstem to induce fever.

Further work is necessary to get knowledge as to whether EP reaches directly into the brain parenchyma or not.

References

1) Cabanac, M., Stolwijk, J.A.J. and Hardy, J.D.: Effect of temperature and pyrogens on single unit activity in rabbit's brain stem. *J. Appl. physiol.*, 24 : 653-654, 1968.

2) Wit, A. and Wang, S.C.: Temperature-sensitive neurons in preoptic/anterior hypothalamic region: action of pyrogen and acetylsalicylate. *Am. J. Physiol.*, 215 : 1160-1169, 1968.

3) Eisenman, J.S.: Pyrogen-induced changes in the thermosensitivity of septal and preoptic neurons. *Am. J. Physiol.*, 216 : 330-334, 1969.

4) Schoener, E.P. and Wang, S.C.: Leukocytic pyrogen and sodium acetylsalicylate on hypothalamic neurons in the cat.

5) Sakata, Y.: Effects of pyrogen on the medullary temperature-responsive neurons of rabbits. *Jpn. J. Physiol.*, 29 : 585-596, 1979.

6) Sakata, Y., Morimoto, A., Takase, Y. and Murakami, N.: Direct effects of endogenous pyrogen on medullary temperature-responsive neurons in rabbits. *Jpn. J. Physiol.*, 31 : 247-257, 1981.

7) Hellon, R.F., Cranston, W.I., Townsent, Y., Mitchell, D., Dawson, N. and Duff, G.W.: Some tests of the prostaglandin hypothesis of fever. *In:* J.M. Lipton (eds), Fever, Raven press, New York, 1980, p. 159-164.

8) Milton, A.S.: Evidence for the involvement of prostaglandins in pyrogen fever. *In:* J.M. Lipton (eds), Fever, Raven press, New York, 1980 p. 141-148.

9) Cranston, W.I., Luff, R.H. and Rosendorff, C.: The effects of salicylate on temperature regulation in rabbits. *J. Physiol.*, 208 : 251-259, 1970.

10) Dinarello, C.A., Goldin, N.P. and Wolff, S.M.: demonstration and characterization of two distinct human leukocytic pyrogens. *J. Exp. Med*., 139 : 1369-1381, 1974.

11) Sawyer, C.H., Everett, J.W. and Green, J.D.: The rabbit diencephalon in stereotaxic coordinate. *J. Com. Neurol.*, 101 : 801-824, 1954.

12) Rosendorff, C. and Mooney, J.J.: Central nervous system sites of action of a purified leucocytes pyrogen. *Am. J. Physiol.*, 220 : 597-603, 1971.

13) DuBois, E.F.: Why are fever temperature over 106 F rare? *Am. J. Med. Sci.*, 217 : 361-368, 1949.

14) Boulant, J.A. and Scott, I.M.: Effects of leukocytic pyrogen on hypothalamic neurons in tissue slices. In environment, drug and thermoreguration. Karger press, Basel, 1983, p. 125-127.

15) Kelso, S.R. and Boulant, J.A.: Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. *Am. J. Physiol.*, 243 : R480-490, 1983.

16) Cranston, W.I., Hellon, R.H., Mitchell, D and

Townsent, Y.: intraventricular injections of drugs which inhibit phospholipase A2 suppress fever in rabbits. *J. Physiol.*, 339 : 97–105, 1983

17) Ogata, T.: The role of inflammatory chemotaxic factor (Leukoegresin) and permeability factor (Vasoexin) in acute inflammation: an electron microscopic observation of biologic action of these natural mediators. *Kumamoto Med. J.*, 24 : 103–123, 1971.

18) Dinarello, C.A., Maronoy, S.O. and Rosenwasser, L.J.: Role of arachidonate metabolism in the inflammatory function of human leukocytic pyrogen/lymphocyte-activating factor/Interleukin 1. *J. Immunol.*, 130 : 890–895, 1983.

19) Fontana, A., Kristensen, F., Dubs, R., Gemsa, D. and Weber, E.: Production of prostaglandin E and an interleukin-1 like factor by cultured astrocytes and C6 glioma cells. *J. Immunol.*, 129 : 2413–2419, 1982.