Effect of Thermal Acclimation on Change in Cerebral Blood Flow during LPS-pyrogen Fever in Rabbits

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Abstract: Local blood flow in the hypothalamus (BFhy) and the reticular formation in the midbrain (BFrf) in fever induced by lipopolysaccharide (LPS-pyrogen: 3μg/kg i. v.) were measured by the hydrogen clearance method together with respiratory rate (RR), rectal (Tre), hypothalamic (Thy) and ear skin (Tea) temperatures in rabbits exposed to normal (25℃), heat (30℃) and cold (10℃) temperature for 4 weeks. BFhy and BFrf were analyzed till 100 min after LPS-pyrogen injections during the early phase in fever. In normal acclimated rabbits: (1) Mean of Tre, Thy, Tea, RR, BFhy and BFrf just before the injection of LPS-pyrogen were 38.93±0.12℃, 38.55±0.14℃, 30.5±1.1℃, 106±7 min^{-1}, 36.84±2.11 ml/100 g/min and 35.62±3.10 ml/ 100 g/min, respectively. (2) BFhy and BFrf significantly increased with increase in Tre and Thy during fever. (3) There were no significant difference between BFhy and BFrf and between Tre and Thy before and during fever. (4) Correlations among BFhy, BFrf, Tre and Thy were statistically significant. (5) In heat and cold acclimated rabbits, BFhy and BFrf hardly increased during fever. The increase in BFhy and BFrf is considered to have useful effects in the process of fever, because the endogenous pyrogen and the thermal signals of core temperature are speedily transported to the brain by bood circulation.

Key words: LPS-pyrogen fever, Cerebral blood flow, Hypothalamus, Reticular formation in midbrain, Thermal acclimation, Rabbit

INTRODUCTION

It is well-known that heat conservation by peripheral vasoconstriction and heat production of non-shivering thermogenesis and cold shivering are induced by lipopolysaccharide (LPS-pyrogen) administration. Brain temperature, as well as temperatures in other core tissues, are increased by these responses.

There were many reports that the blood flow hardly changes in the brain which has an important role in affecting its own circulating regulation. However an increase of cerebral blood flow in the hypothalamus during fever induced by pyrogen in rabbits was reported by Cranston and Rosendroff (1968) and Rosendroff (1973).

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The brain temperature plays an important role in thermoregulation is also well-known. Factors causing change in brain temperature are considered to be changes in metabolism in the brain, the temperature of circulating blood and cerebral blood flow (Hayward and Baker, 1969).

In this study, relationships among cerebral blood flow in the hypothalamus (BFhy), reticular formation in midbrain (BFrf) and temperatures of the hypothalamus (Thy) and rectum (Tre) during fever induced by LPS-pyrogen administration were analyzed in rabbits exposed at 25℃ for 4 weeks. And we also discussed the reasons in which BFhy and BFrf during LPS-pyrogen fever hardly changed in rabbits exposed at 10℃ and 30℃ for 4 weeks preliminarily in previous experiments (Kosaka *et al.*, 1989).

Materials and Methods

Male albino rabbits, 2.6±0.3 kg in body weight, were used in this study. The rabbits were reared individually for 4 weeks under 25±2℃ and 60±5% rh, and the photo-period was 12:12 hr (Light: 6:00−18:00). Comparison with rabbits in three groups, rabbits of normal, heat and cold groups were exposed for 4 weeks at 25±2℃, 30±2℃ and 10±2℃, respectively. Humidity was 60±5% rh and the photo-period was 12:12 hr (Light: 6:00−18:00) in all groups.

The surgical procedures were carried out under anesthesia with sodium pentobarbital (30 mg/kg i. v.). The head of the rabbit was fixed in a prone position with a stereotaxic instrument, and then three holes were drilled in the exposed skull above the bilateral anterior hypothalamus and left of the midline above the reticular formation in the midbrain according to the atlas of Monnier and Gangloff (1961). The sensitive electrodes for measurement of local blood flow were stereotaxically inserted into regions of the anterior hypothalamus and the reticular formation in midbrain through left side holes, and a copper-constantan thermocouple (1 mm in diameter) was also inserted into hypothalamic region through another hole. These electrodes and thermocouple were anchored rigidly to the skull.

BFhy and BFrf were measured by a hydrogen clearance method. The sensitive electrode is made of Pt/Pt-black and the sensitive area of the tip is 1 mm in length and 300 μm in diameter. For measurement of local blood flow, the rabbit was given a hydrogen-air mixture gas to breathe spontaneously for 1−2 min (Inomoto *et al.*, 1979). A partial pressure of hydrogen in the tissue was measured with PH_2 monitor (PHG−300, M. T. GIKEN) and recorded with a data recorder (RMG−5204, NIHON KODEN Co.). The change in the pressure forms the hydrogen clearance curve and the hydrogen clearance curve for longer than 15 min is necessary for calculation of blood flow. The total flow method and the initial slope method (Olesen *et al.*, 1971) are well known to calculate the blood flow in the hydrogen clearance method. Cerebral blood flow was calculated from the hydrogen clearance curve with a computer program which was created to calculate the cerebral blood flow on the both methods with an analogue computer (ATAC−450, NIHON KODEN Co). In this study, the cerebral blood flows calculated by the initial slope method were shown, because a correlation

between cerebral blood flow calculated by the initial slope method and those calculated by the total flow method was statistically significant (Kosaka *et al.*, 1989).

Experiments were carried out in an environmental chamber controlled at 25℃ and 60% rh. Interval time of measurement in each BFhy and BFrf was 20 min. After BFhy and BFrf were measured twice in stable state on the rabbit, LPS-pyrogen (*E. coli*, B−8, SIGMA) was intravenously injected at 3 μg/kg of dose. Temperatures of Thy, Tre, ear skin (Tea) and ambience (Ta) were recorded with copper-constantan thermocouples every minute. Respiratory rate (RR) was picked up with a strain-gauge around the chest and RR was counted and stored with a computer (ATAC−450, NIHON KODEN Co.).

RESULTS

Mean values of BFhy, BFrf, Tre, Thy, Tea and RR in 6 rabbits of normal group are shown in Table 1. The values at 0 min as control values were measured just before LPS-pyrogen injection.

The control values of BFhy and BFrf were 36.84±2.11 ml/100 g/min and 35.62±3.10 ml/100 g/min, respectively. There was little difference between BFhy and BFrf in control values. BFhy and BFrf increased with increase in Tre and Thy due to LPS-pyrogen administration. Increase in BFhy after 40 min and that in BFrf after 80 min from the LPS-pyrogen injection increased significantly in comparison with the control value. However, differences between BFhy and BFrf at the same time were not significant throughout the experimental period.

Control values of Tre and Thy were 38.93±0.12℃ and 38.55±0.14℃, respectively. Tre was higher than Thy but the differences were not significant throughout the experimental period. Both Tre and Thy slightly fell at 20 min, but increase in Thy was higher than that in Tre at 40 min after LPS-pyrogen administration. Tre as well as Thy after 60 min from the

Table 1. Changes in local blood flows of the hypotalamus (BFhy) and the reticular formation in midbrain (BFrf), temperatures of the hypothalamus (Thy), rectum (Tre) and ear skin (Tea) and respiratory rate (RR) during fever induced by LPS-pyrogen injected intravenously at time zero in 6 rabbits

Time (min)	n	BFhy (ml/100g/min)	BFrf (ml/100g/min)	Tre (℃)	Thy (℃)	Tea (℃)	RR (min^{-1})
−20	6	36.50±2.63	38.49±3.83	38.95±0.11	38.63±0.18	31.1±0.9	109±5
0	6	36.84±2.11	35.62±3.10	38.93±0.12	38.55±0.14	30.5±1.1	106±7
20	6	37.98±1.92	37.42±2.88	38.84±0.09	38.46±0.15	28.5±0.7	85±8
40	6	42.77±1.29*	43.12±3.12	39.03±0.07	38.81±0.14	27.1±0.6*	48±6**
60	6	43.51±1.32*	44.55±3.21	39.36±0.10*	39.15±0.11**	26.8±0.7*	50±5**
80	6	44.98±1.83*	46.33±3.14*	39.51±0.08**	39.23±0.09**	26.4±0.6**	51±5**
100	6	44.24±2.01*	48.47±3.51*	39.54±0.11**	39.49±0.11**	26.8±0.9*	49±6**

Mean±SE. *$p<0.05$ and **$p<0.01$ compared with each value at time 0 min.

LPS-pyrogen injection increased significantly in comparison with the control value. Tea and RR decreased significantly at 40 min and the beginning of decreases in Tea and RR were earlier than increases in Tre and Thy.

Changes in BFhy, BFrf, Thy, Tre, Tea and RR calculated from Table 1 were shown in Fig. 1. In this figure, LPS-pyrogen was injected intravenously at 0 min, and star marks on zero lines were control values in Table 1. Percents of differences from each control value in BFhy and BFrf, and differences from each control values in Thy, Tre, Tea and RR were shown. Heat conservative responses in Tea and RR were induced by LPS-pyrogen administration. Tre rose rapidly from 40 min to 60 min after LPS-pyrogen administration. On the other hand, the rapid increase in Thy were observed twice from 20 min to 40 min and from 80 min to 100 min after LPS-pyrogen administration.

The correlation coefficients and regression lines among Tre, Thy, BFhy and BFrf in all data of normal group were calculated. The correlational diagrams and regression lines of Thy on Tre in Fig. 2, of BFhy on BFrf in Fig. 3, of BFhy on Thy in Fig. 4 and of BFhy on Tre in Fig. 5 were shown, and there were significant positive correlations in all relations.

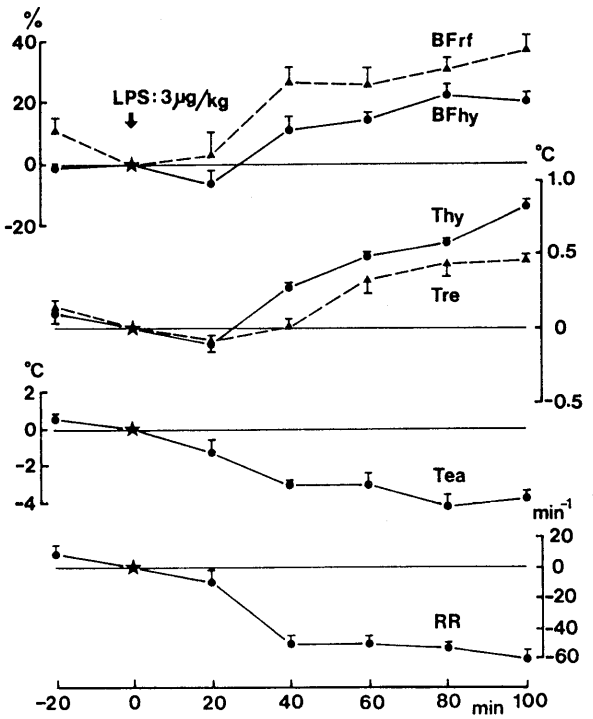

Fig. 1. Changes of cerebral blood flow in the reticular formation in midbrain (BFrf) and the hypothalamus (BFhy), temperatures of hypothalamus (Thy), rectum (Tre) and ear skin (Tea) and respiratory rate (RR) during LPS-pyrogen fever. Differences from values (star marks) just before LPS-pyrogen injected intravenously in each curve were shown.

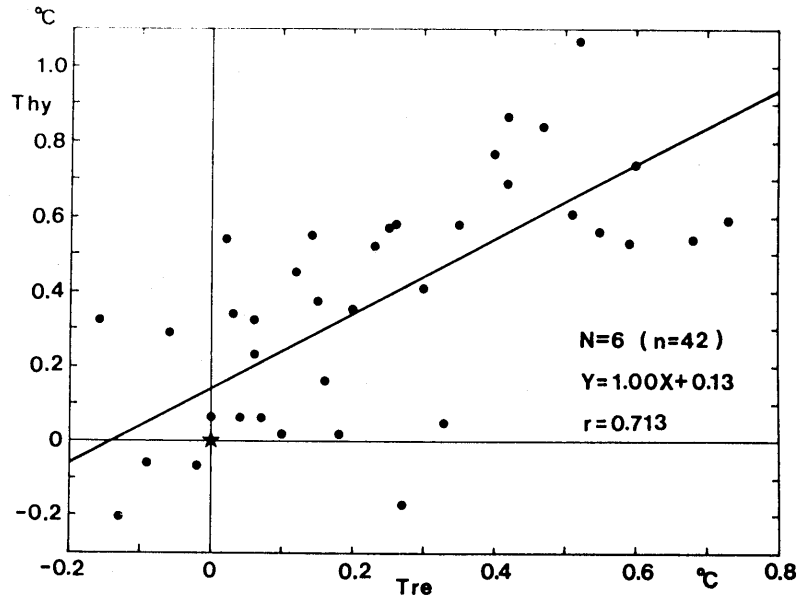

Fig. 2. The correlational diagram and the regression line of hypothalamic temperature (Thy) on rectal temperature (Tre) during LPS-pyrogen fever. Differences from values just before LPS-pyrogen injected intravenously were shown. The zero levels were 38.93±0.12℃ for Tre and 38.55±0.14℃ for Thy. The correlation between Thy and Tre was statistically significant.

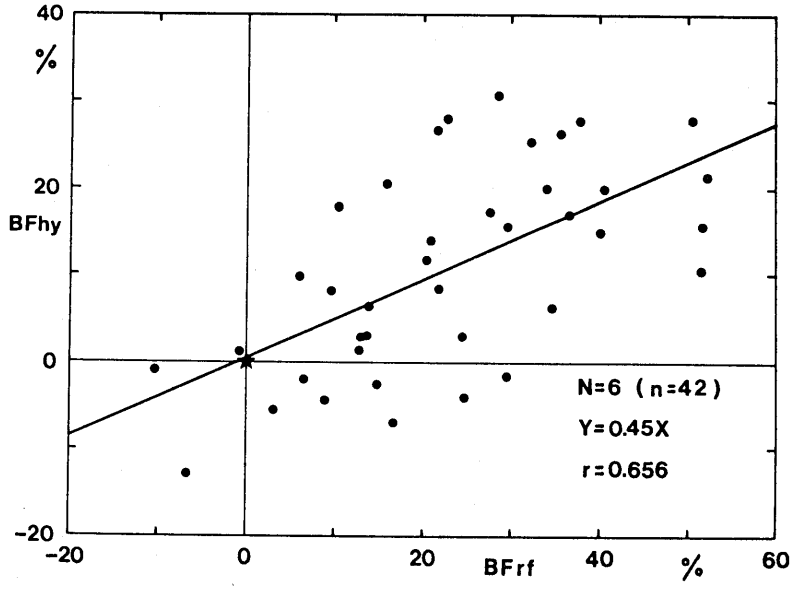

Fig. 3. The correlational diagram and the regression line of cerebral blood flow in hypothalamus (BFhy) on cerebral blood flow in reticular formation of midbrain (BFrf) during LPS-pyrogen fever. Differences from values just before LPS-pyrogen injected intravenously were shown. The zero levels were 36.84±2.11 ml/100 g/min for BFhy and 35.62±3.10 ml/100 g/min for BFrf. The correlation between BFhy and BFrf was statistically significant.

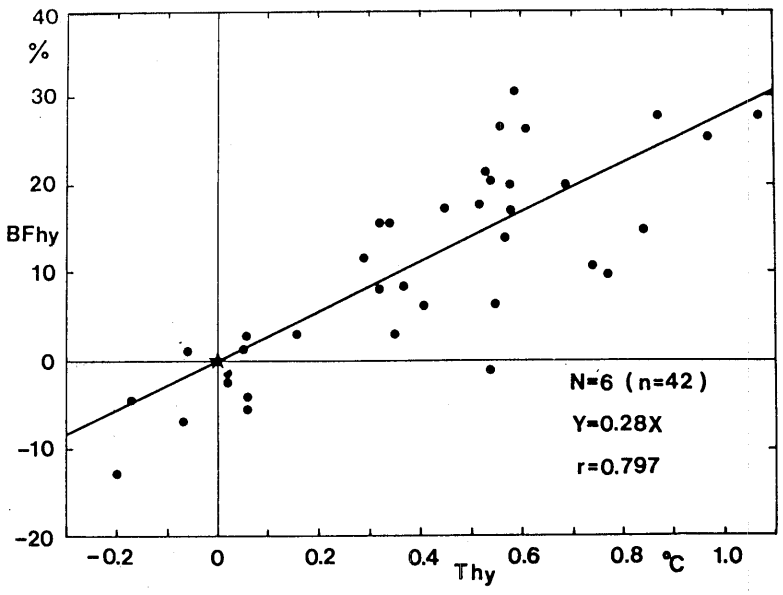

Fig. 4. The correlational diagram and the regression line of cerebral blood flow in hypothalamus (BFhy) on hypothalamic temperature (Thy) during LPS-pyrogen fever. Differences from values just before LPS-pyrogen injected intravenously were shown. The zero levels were 36.84±2.11 ml/100 g/min for BFhy and 38.55 ±0.14℃ for Thy. The correlation between BFhy and Thy was statistically significant.

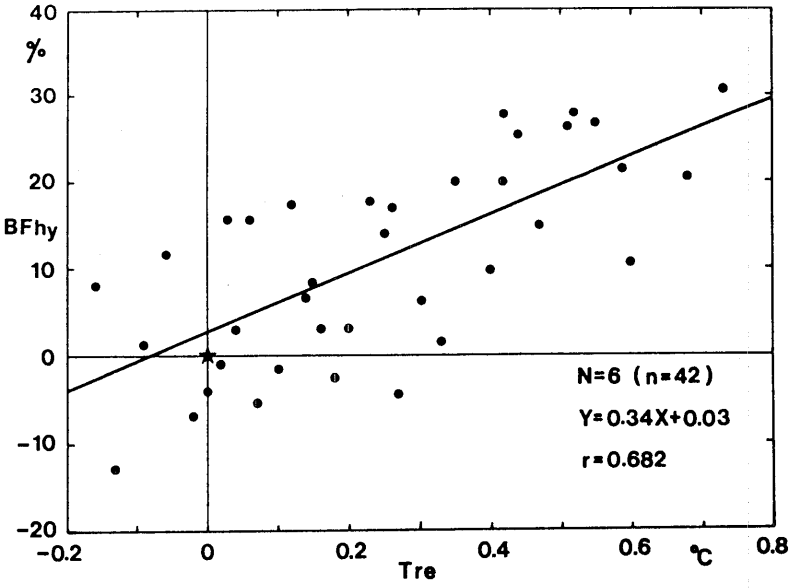

Fig. 5. The correlational diagram and the regression line of cerebral blood flow in hypothalamus (BFhy) on rectal temperature (Tre) during LPS-pyrogen fever. Differences from values just before LPS-pyrogen injected intravenously were shown. The zero levels were 36.84±2.11 ml/100 g/min for BFhy and 38.93± 0.12℃ for Tre. The correlation between BFhy and Tre was a statistically significant.

Changes in BFhy and BFrf before and after LPS-pyrogen administration in each group were shown in Fig. 6. BFhy and BFrf measured at 80 min or 100 min were selected as values after LPS-pyrogen administration in fever. BFhy and BFrf of rabbits in the heat and cold groups hardly increased in comparison with the values before LPS-pyrogen administration. BFhy of control values were 37.6±13.6 ml/100 g/min for the normal group, 38.8±7.5 ml/100 g/min for heat group and 31.7±5.5 ml/100 g/min for the cold group. Only difference of BFhy between heat and cold groups was statistically significant. Increase in BFhy by LPS-pyrogen administration was statistically significant in the normal group, and the percent of increase in BFhy was 21.1±8.2%. On the other hand, BFrf of control values were 37.2±7.5 ml/100 g/min for normal group, 39.9±6.8 ml/100 g/min for heat group and 28.9±7.4 ml/100 g/min for cold group. Only difference of BFrf between heat and cold groups was statistically significant. An increase in BFrf by LPS-pyrogen administration was also statistically significant in normal group, and the percent of an increase in BFrf was 18.6±10.4%.

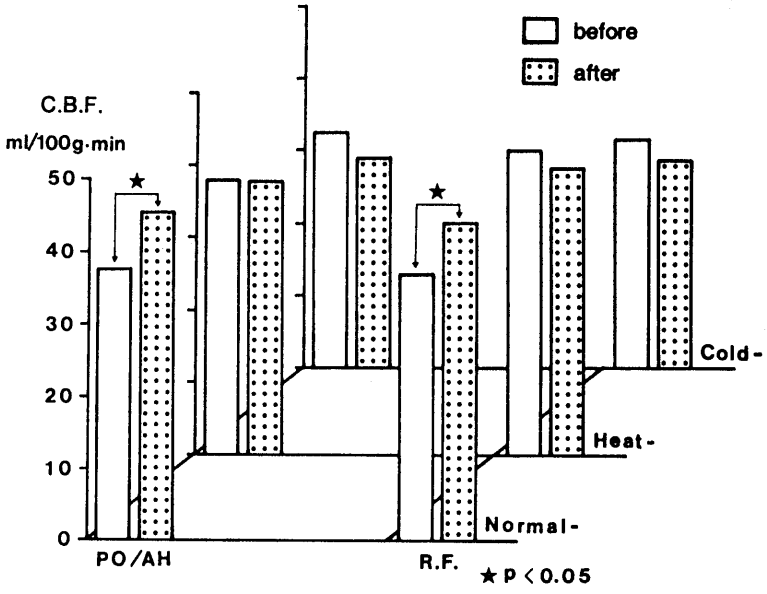

Fig. 6. Comparison of cerebral blood flows before and after LPS-pyrogen injection in normal, heat and cold groups.
PO/AH: Preoptic area and anterior hypothalamus, R. F.: Reticular formation in midbrain, Normal−: Rabbits exposed at 25℃ for 4 weeks, Heat−: Rabbits exposed at 30℃ for 4 weeks, Cold−: Rabbits exposed at 10℃ for 4 weeks.

DISCUSSION

The methods of measuring blood flow in a tissue are mainly two types. One is the microsphere method, the other is the clearance method. Hydrogen clearance method is an application of the clearance method and the method using ^{133}Xe is well known. In the clearance method, it is unknown whether a change in blood flow is due to change in a cardiac output or not, and the number of measuring tissues are limited in one measurement. However the clearance method can measure in real time, can measure an absolute value and can repeatedly measure without limit in comparison with the microsphere method.

The fever analyzed in this study is considered to be an early phase fever because data is measured up to 100 min after LPS-pyrogen administration (Iriki, 1988).

The decrease in Tea by the peripheral vasoconstriction for inhibition of dry heat loss preceded the increase in Tre and Thy was shown in Fig. 1. The decrease in RR for an inhibition of respiratory evaporative heat loss also preceded it. Although these heat conservation responses are not positive responses to increase of core temperature, these are economical responses without energy loss. The peripheral vasoconstriction and the change in RR are fast responses because these responses are induced by neurogenic control (Saigusa *et al.*, 1989). and the surface per volume in an ear of a rabbit is wide

The non-shivering and cold shivering thermogenesis are positive responses to increase the core temperature. The heat conservation and the increase of metabolism in thermogenesis are certainly induced in a process of increase in the core temperature during a fever induced by LPS-pyrogen (Nakayama, 1978).

The peripheral vasoconstriction relatively incraese a blood flow in central area, and the increase of blood inflow is needed in the tissue which the metabolism for thermo-genesis increases. Therefore these responses increase the blood flow in core tissues.

In this study, BFhy and BFrf increased during LPS-pyrogen fever. The positive correlations between BFhy and BFrf and that between Tre and Thy were statistically significant. The increase in BFhy and BFrf has two useful effects in the process of fever. On the first, the endogenous pyrogen produced from neutrophils, monocytes and macrophages by inducement of LPS-pyrogen in peripheral area (Morimoto et *al.*, 1989) may be speedily transported to the brain in large quantities by the blood circulation as a result of increases in BFhy and BFrf. On the second, the thermo-signal in core temperature except the brain is quickly brought to the thermoregulatory center, because the difference between Tre and Thy becomes smaller as time passes after LPS-pyrogen administration.

BFhy and BFrf in heat and cold groups hardly increased in comparison with the normal group during a fever induced by LPS-pyrogen was shown in Fig. 6. Fig. 7 is a figure to explain our hypothesis about it. In this figure, Tre or Thy is the core temperature (Tc) in the horizontal axis. The vertical axis shows the strength of physiological responses in the thermo-regulation. Heat production (HP) is non-shivering and cold shivering thermogenesis, and evaporative heat loss (EHL) is a thermal panting in rabbits. Neutral zone (NZ) in Tc is the range regulated by peripheral vasoconstriction or peripheral vasodilation. This figure

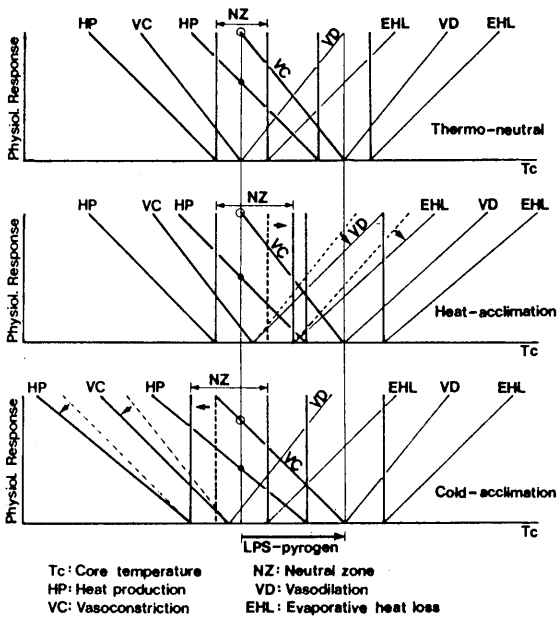

Fig. 7. Influence of LPS-pyrogen injection on thermal responses in thermoregulation (modified Bligh, 1973. for details see discussion).

consists of three parts namely thermo-neutral in control group, heat-acclimation in heat group and cold-acclimation in cold group. Two vertical thin lines are Tc before LPS-pyrogen administration and Tc increased by LPS-pyrogen in fever. It is supposed in this figure that Tc before LPS-pyrogen injection is the same in each group as well as Tc in fever. This result is considered in the early phase in fever because BFhy and BFrf were measured within 100 min after LPS-pyrogen administration in this study. Tc in fever was controlled by the thermoregulator functions, and the gain of thermoregulatory response didn't change. Similar findings were reported by Iriki (1988).

In the normal group, the strength of peripheral VC (open circle) and the strength of HP (closed circle) are requested before beginning of the increase in Tc after LPS-pyrogen injection.

In the heat group, the range of NZ in Tc may be extended to higher temperature by preliminary exposure at 30℃ for 4 weeks, and Tc may be controlled by lower gains of VD and EHL. In consequence, the strength of HP (closed circle) decreased in comparison with the normal group. This suggests that the increase in blood flow by the metabolism in core tissues is small. Therefore, the small increases in BFhy and BFrf is caused by the influence of that.

In the cold group, the range of NZ in Tc is extended to a lower temperature by preliminary exposure at 10℃ for 4 weeks, and Tc can be controlled by lower gains of VC and HP (Bligh, 1973). As the result of these, the strength of peripheral VC (open circle) and HP (closed circle) is at a low level in comparison with the normal group. In addition to the

case of heat group on metabolism, the weak peripheral VC influences on a small increase in blood flow into core tissues. So a small increase in BFhy and BFrf is caused by that influence.

The increase in cerebral blood flow is considered to be useful for the process of fever. Although there are many indistinct points in thermal acclimation, the other effective functions on thermoregulation in a fever may be enhanced by thermal acclimation.

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