Changes in Hypothalamic Blood Flow Due to Thermal Acclimation

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Abstract: Hypothalamic blood flow as well as thermal inputs from several thermosensores plays a role in temperature regulation at a neural level in the hypothalamus. Thermal acclimation causes some alteration in the mechanisms of temperature regulation. Therefore, the present study was designed to clarify the influence of thermal acclimation on hypothalamic blood flow. Cerebral blood flow (CBF) of preoptic area and anterior hypothalamus (PO/AH) during general heating and cooling in the heat-acclimated, cold-acclimated and thermally non-acclimated (control) rabbit was measured by hydrogen clearance method. The change in CBF was closely associated with ambient temperature as well as other thermoregulatory parameters. Not only the less increase in CBF due to heating but also less decrease due to cooling was observed in the heat-acclimated rabbit compared to those of control rabbit, and similar results were obtained in the cold-acclimated rabbit. The reduction of gain in thermoregulatory responses occurred during general heating and cooling in both heat- and cold-acclimated rabbit. The present finding suggests that the reduced change in CBF of PO/AH in the process of thermal acclimation might cause habituation phenomenon of thermoregulatory responses to heat and cold stimulation.

Key words: Temperature regulation, Hypothalamic blood flow, Hydrogen clearance method, Hypothalamic temparature, Heat- and cold-acclimated rabbit

Introduction

There are several thermoregulatory inputs to the hypothalamus, such as thermosensors in the brain-stem, spinal cord, abdominal viscera, large vessels and skin (Simon, 1974; Kosaka *et al.*, 1985), and, most theories consider that the central and peripheral inputs in thermoregulation interact as a neural level in the hypothalamus (Bligh, 1973). However, the humoral control mechanism of the hypothalamus such as an increase in hypothalamic blood flow during pyrogen-induced fever (Inomoto *et al.*, 1979 a, b; Rosendorff, 1974) as well as during general skin heating (Kosaka *et al.*, 1983, 1988) is not yet precisely deter-

mined. But it is at least theoretically conceivable that changes in CBF constitute a controlling signal for integrating the very disparate varieties of input, and for producing an appropriate input to thermoregulatory effector system. Therefore, the present investigation was performed to clarify whether the changes in CBF seen in general thermal stimulation are closely associated with the central mechanism of thermoregulation and the development of thermal acclimation or not.

MATERIALS AND METHODS

Nineteen male adult albino rabbits (*Oryctolagus cuniculus*) weighing 2.7-3.4kg were used in these experiments. Ambient air temperature (Ta) and relative humidity (rh) of animal room were kept at 25℃ and 60%, respectively. Rabbits were divided into three groups at random and the first group was exposed to hot environment at 30℃ of Ta and 60% of rh for 4 weeks as heat-acclimated rabbits (Heat-acclimated) and the second group was exposed to cold environment at 10℃ of Ta for 4 weeks as cold-acclimated and the third group was kept at 25℃ of Ta and 60% of rh in the thermoneutral condition for the same duration as control (thermally non-acclimated) rabbits. For the measurement of CBF in preoptic
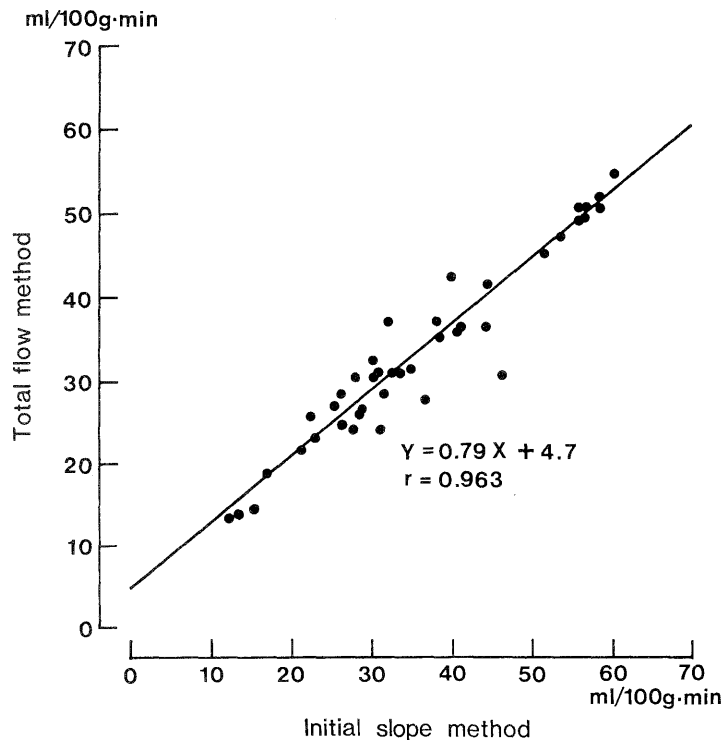

Fig. 1. Correlation between CBF calculated from the total flow method of two compartmental analysis of hydrogen clearance method and CBF calculated from the initial slope method. y=0.79x+4.7, r=0.963, n=42 (For details see text).

area (PO/AH) and midbrain reticular formation (RF), Pt/Pt black electrodes were inserted stereotaxically into the PO/AH and RF region according to the atlas of Monnier and Gangloff (1961). The indifferent Ag/AgCl electrode was placed under the incised skin. A polirizing voltage of 10-50mV was applied between the two electrodes, and about 30 minutes were allowed for stabilization of the electrode system before hydrogen clearance was measured. The animal was given a hydrogen-air mixture to breathe spontaneously for 60 seconds. A hydrogen monitor (PHG-300, M. T. GIKEN) and an electronic polyrecorder (EPR-10B, TOA DENPA) was used for amplification and recording, respectively. The first 40 seconds of the clearance curve recorded after inhalation of hydrogen had been stopped was discounted in order to correct for arterial recirculation of hydrogen as reported by Pasztor *et al.* (1973) and Halsey *et al.* (1977). The curves were replotted on semilogarithmic paper, and blood flows were calculated from the two-minute initial slope of the curve, accuracy to Olesen *et al.* (1971). As shown in Fig. 1, total blood flow (33.7±11.0 ml/100g•min, n=44) calculated from the total flow method of two compartmental analysis was correlated with result of the initial slope method (36.5±13.4 ml/100g•min, n=44). In the present study, the initial slope method was used for CBF calculation. For the temperature measurement of the PO/AH and RF, copper-constantan thermocouples were inserted stereotaxically into the PO/AH and RF through another side of craniotomy hole. Temperatures of rectum, ear skin and experimental room were measured with the thermocouples connected to electric thermometer (TYPE TE3, ELLAB). The respiratory rate was recorded in order to monitor evaporative heat loss response due to thermal panting during heating. In an environmental control chamber, to assess the thermoregulatory responses against heat and cold loads, each rabbit from three groups was submitted to the following general thermal stimulation: 25℃ and 60% rh (thermoneutral environment) for 30min → 40℃ and 60% rh (hot environment) for 60min → 15℃ (cold environment) for 60min → 40℃ (hot) or 25℃ (thermoneutral environment) again for 30min. For the statistical estimation, the Student's t-test was applied to the analysis of data obtained from the experiment.

RESULTS

Repeated measurements of cerebral blood flow (PO/AH and RF) were carried out to determined stress-induced changes in blood flow and thermoregulatory parameters due to the physical restraint and placement of the electrodes and thermocouples during over two-hour experimental period (Fig. 2). CBF in PO/AH and RF as well as thermoregulatory parameters such as rectal and ear skin temperature and respiratory rate were relatively constant over a period of the experiment. Generally, restraint causes skin pressure reflex that is non-specific inhibition of vegetable and extrapyramidal motor regulatory processes concerning thermoregulation (Takagi *et al.*, 1950; Kosaka, 1969a), however, there were little changes in the data shown in Fig. 2. So that, it is considered that reasonable restraint which does not cause skin pressure reflex was given in this study.
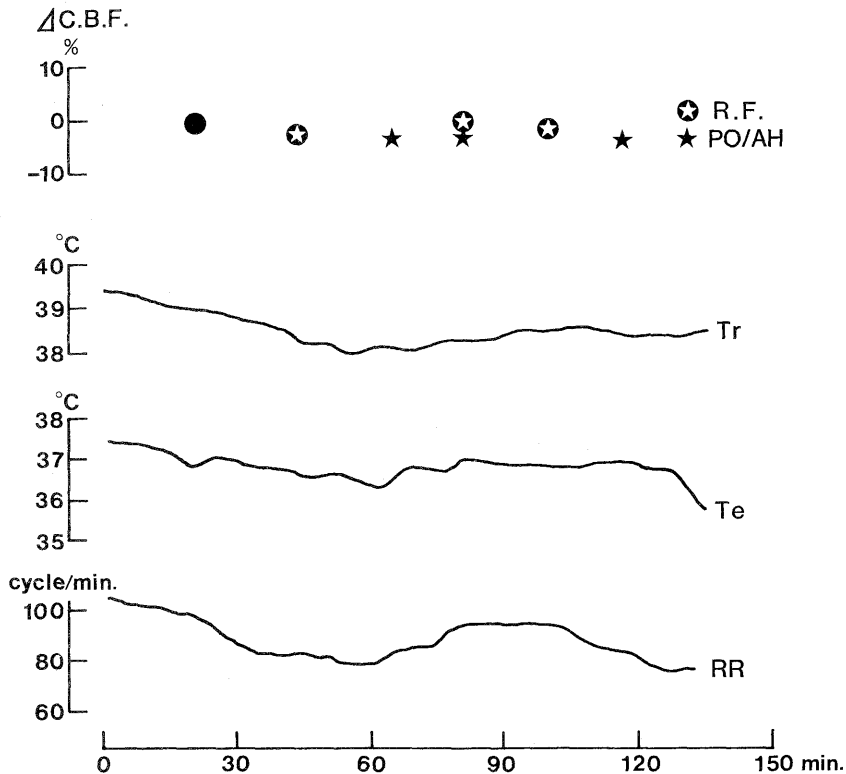
Fig. 2. Time course of a control experiment in an unanesthetized and lightly restrained rabbit. PO/AH: pre-optic Area and anterior hypothalamus, RF: midbrain reticular formation, △CBF: changes in cerebral blood flow of PO/AH and RF, Tr: rectal temperature, Te: ear skin temperature, RR: respiratory rate.

Changes in CBF (PO/AH), temperature of PO/AH, RF, rectum and ear skin and respiratory rate during general thermal stimulation ($25°C \rightarrow 40°C \rightarrow 15°C \rightarrow 25°C$), at constant 60% relative humidity were shown in Fig. 3 in thermally non-acclimated (control) rabbit. Temperatures in the brain (PO/AH and RF), rectum and ear skin and respiratory rate showed considerable changes closely associated with changes in ambient temperature. Temperatures in PO/AH and RF were almost same, and the change in rectal temperature was less and dull compared to brain temperature (PO/AH and RF). It may be considered that PO/AH and RF have high sensitivity to temperature because of rich blood flow per tissue volume. CBF in PO/AH and RF were observed to be almost same in the present study and closely associated change could be seen between ambient temperature and thermoregulatory parameters as shown in Fig. 3 (CBF increases at heating period and then decreases at cooling). Generally, respiratory rate in rabbit at neutral condition is 80-100 cycle/min (Crosfill and Widdicombe, 1961; Kosaka et al., 1969b), however, higher rate of about 150 cycle/min was obtained at 25°C in the non-acclimated rabbit. It may be not neutral, considerably high temperature for rabbits at 25°C, although it is neutral for human beings.
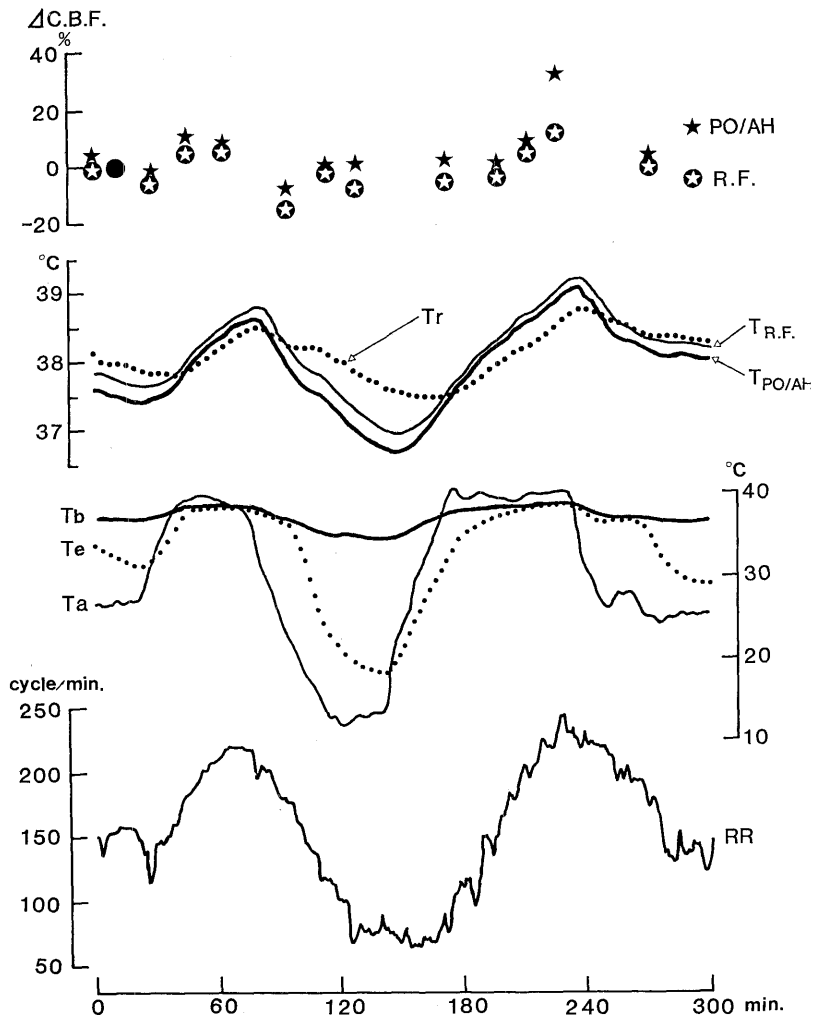
Fig. 3. Effect of external heating and cooling on changes in CBF as well as thermoregulatory responses. Time course of a typical experiment in a thermally non-acclimated (control) rabbit. Tb: back skin temperature, Ta: ambient temperature. (For details see text).

In the heat-acclimated rabbit, changes in CBF, respiratory rate and the temperatures of PO/AH, rectum and ear skin were closely associated with the change in the ambient temperature, too (Fig. 4). There was 15min delay between the changes of ear skin and ambient temperature during general cooling. This delay indicates the latent time of physiological response and reasonable measurement of ear skin temperature. The change ratio of CBF in the heat-acclimated rabbit was less compared to thermally non-acclimated rabbit. The respiratory rate in heat-acclimated rabbit was higher (170 cycle/min) than control rabbit at 25℃. It is considered that increase in the respiratory rate occurred in the process of acclimation to heat (30℃, 60% rh) for four weeks.
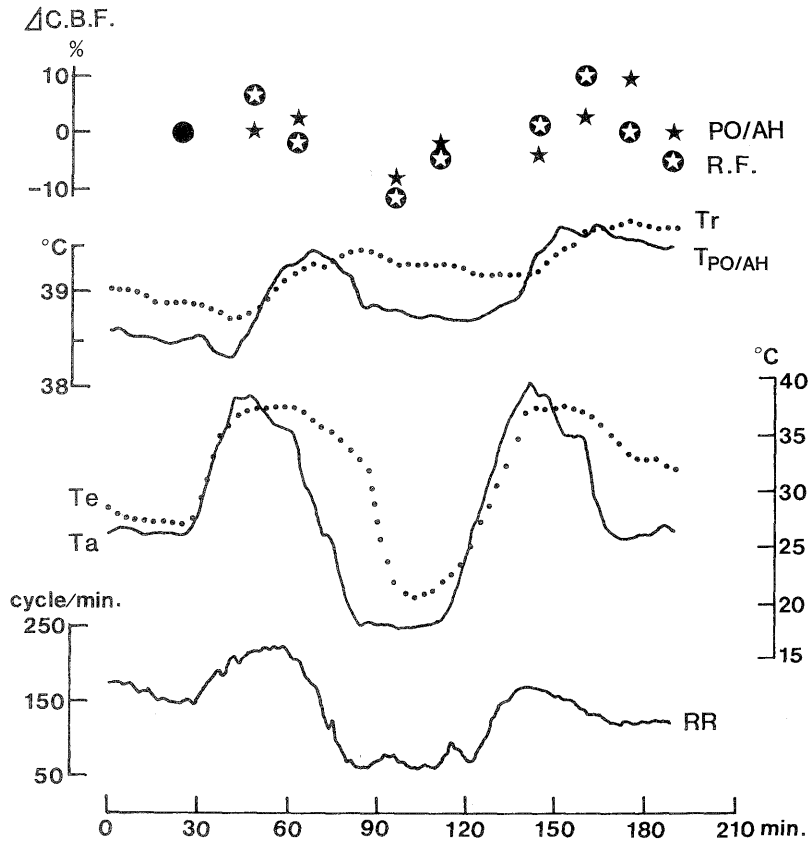
Fig. 4. Effect of external heating and cooling on changes in CBF as well as thermoregulatory responses. Time course of a typical experiment in a heat-acclimated rabbit. (For details see text).

Fig. 5 shows that respiratory rate at 25℃ (neutral temperature) in the cold-acclimated rabbit was markedly less compared to control rabbit shown in Fig. 3, and was suppressed below 150 cycle/min even at 40℃ ambient temperature. It is considered that reduction of respiratory rate occurred in the process of cold-acclimation to 10℃. The changes could be seen in CBF (PO/AH and RF), the temperatures of PO/AH, rectum and ear skin as well as in ambient temperature. The ratio in changes of CBF in the cold-acclimated rabbit was less than non-acclimated rabbit. The results obtained in the cold-acclimated rabbit represent the feature of tentative thermal acclimation to cold.

The results of CBF (PO/AH and RF) at 25℃ (thermally neutral temperature) were summarized in Table 1. In heat-acclimated rabbit, CBF in PO/AH was higher than normal rabbit (non-acclimated). CBF of PO/AH in cold-acclimated rabbit was less than normal rabbit. The simillar results in RF were obtained as in PO/AH.
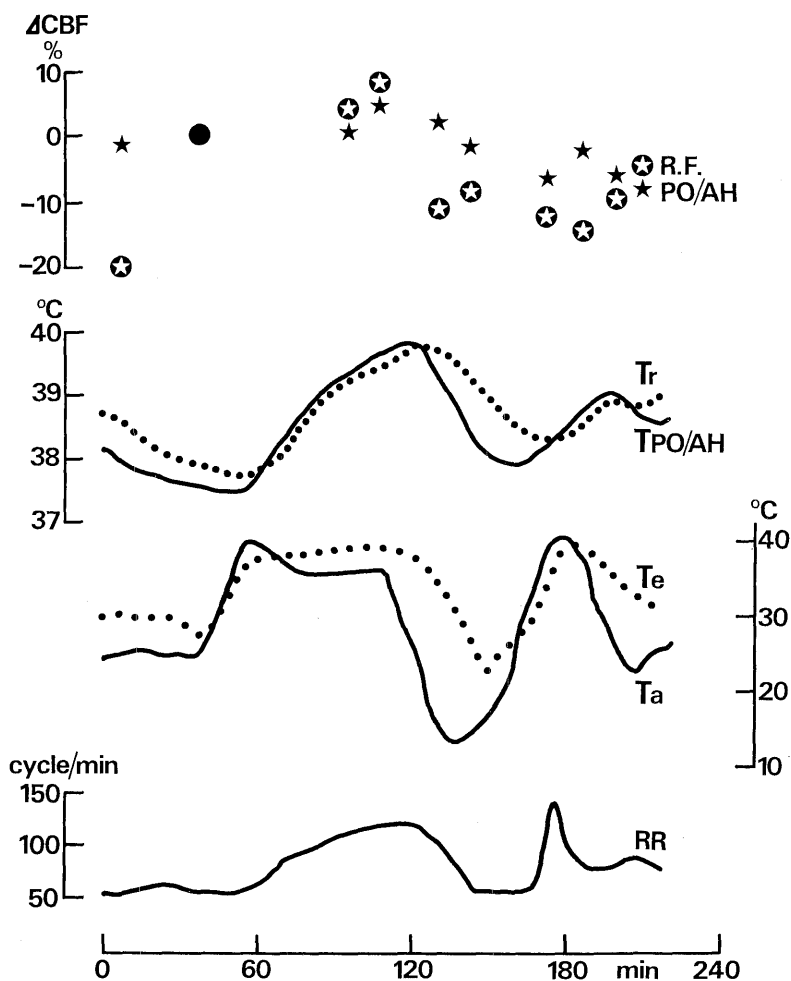
Fig. 5. Effect of external heating and cooling on changes in CBF as well as thermoregulatory responses. Time course of a typical experiment in a cold-acclimated rabbit. (For details see text).

Cerebral blood flow changes (PO/AH) in the non-acclimated (Ta=25℃), cold-acclimated (Ta=10℃) and heat-acclimated (Ta=30℃) rabbit due to general thermal stimulation (heat and cold) were shown in Fig. 6 as a percentage of basal values. Less increase in CBF due to general heating (40℃) in the heat-acclimated rabbit and less decrease due to general cooling (15℃) in the cold-acclimated rabbit were observed. Furthermore, less decrease in CBF due to general cooling in the heat-acclimated rabbit and less increase due to general heating in the cold-acclimated rabbit were found. In both of heat- and cold-acclimated rabbits, less response in CBF due to general thermal stimulation (both heating and cooling) was observed.

Table 1. Cerebral blood flow of thermally acclimated rabbits (Initial slope method)

	No. of animal (Exp. period)	C. B. F. (ml/100g/min)	
		PO/AH	R F
Normal (control) rabbit	n=5 (16)	37.6±13.6	37.2± 7.5
Heat acclimated rabbit	n=7 (21)	38.0± 6.9 ⌉ *	42.2±11.9 ⌉ **
Cold acclimated rabbit	n=7 (23)	32.7± 7.9 ⌋	31.9±10.8 ⌋

*p$<$0.025 **p$<$0.005

CBF was calculated flom the initial slope method of hgdrogen clearance method in an environmental control room (25℃, 60% rh).

DISCUSSION

It is well-known that LPS-pyrogen causes profound circulatory changes (Cranston, 1959) which include not only cutaneous vasoconstriction but also a rise in renal and hepatic blood flow, in the face of a fall of a mean arterial blood pressure (Rosendorff, 1974). However, Heyman et al. (1950) reported experiments in man in which total cerebral blood flow (CBF) was unchanged during pyrogen induced fever.

The mechanism of the hypothalamic vasodilatation during purified leukocyte pyrogen induced fever (Rosendorff, 1974) as well as LPS-pyrogen induced fever (Inomoto et al., 1979a, b) is of interest. As shown in the present results, external heating of the rabbit by raising ambient temperature also results in increased hypothalamic blood flow. Whether the increase in hypothalamic blood flow induced following intravenous pyrogen is due directly to the LPS-pyrogen or is a non-specific change due to a rise of body temperature (Tre) was not clear, up to the present study, though there was some evidence to suggest that the rise in body temperature induced by external heating may cause a fall in renal blood flow (Cooper et al., 1960). No such dissociation of the effects of pyrogen-induced fever and temperature rise due to external heating was observed in the present study.

As shown in Fig. 2, 3 and 4, temperature in hypothalamus (PO/AH) and midbrain reticular formation (RF) responded promptly and consistently to external heating and cooling, while the change in rectal temperature (Tre) was less and dull compared to those of PO/AH and RF. This indicates that cerebral tissues such as PO/AH and RF are constructed to be well-circulated and highly sensitive to the temperature displacement of the circulating blood through the brain. Therefore, change in temperature and blood flow in PO/AH and extrahypothalamic thermosensitive tissues (RF-midbrain-medulla-spinal cord) play a primary role in the process of thermoregulation as well as thermal acclimation.

In regard to the relations between heat- and cold-acclimation, heat and cold acclimation are not mutually exclusive, and both heat and cold acclimation can co-exist. Namely, man can be acclimated simultaneously to both heat and cold (Glaser and Shepherd, 1963; Bligh, 1973). In a report on habituation, which is defined as the central nervous suppres-
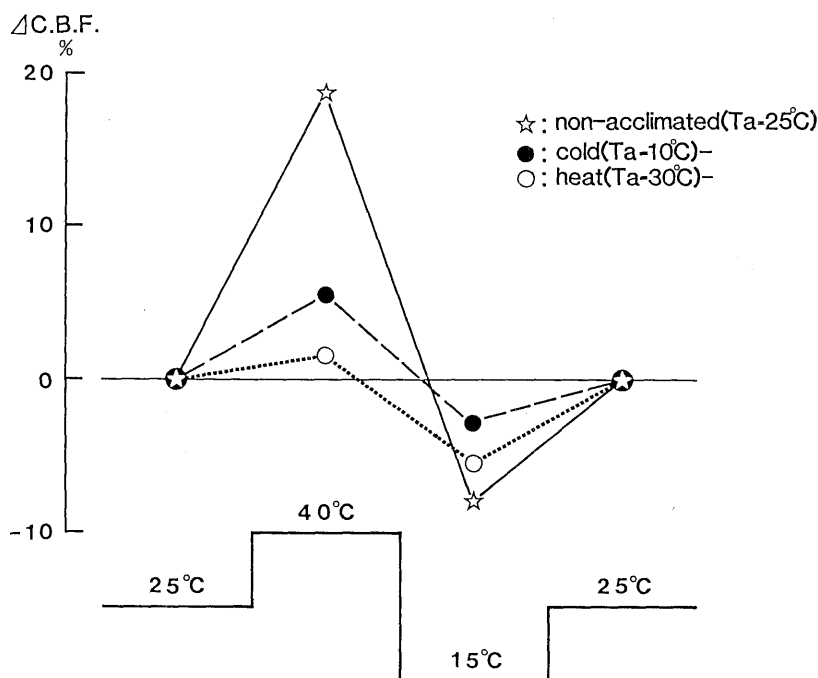
Fig. 6. Changes in hypothalamic blood flow due to external heating and cooling in thermally non-acclimated (control), heat-acclimated and cold-acclimated rabbits. (For details see text).

sion of the effects of thermal stimulation when this is reported frequently, Glaser (1966) suggested that acclimation to heat and cold involves the relations between temperature sensors and thermoregulatory effectors. It is considerd that the reduced changes of CBF in heat- and cold-acclimated rabbit in the present study may act not only as an afferent signal to temperarture sensors but also as an efferent information to thermoregulatory effectors which are closely associated with the process of habituation. Brück and Wünnenberg (1967) reported that in cold-acclimated guinea-pigs the level of deep body temperature regulated was lowered. In a further investigation of this problem, Brück et al., (1970) found that there was also a shift in the level of core temperature at which the processes of both heat production and heat dissipation were achieved in the cold-acclimated guinea-pig. They found that the animal could simultaneously be acclimated to both heat and cold. The similar experimental results on heat- and cold-acclimated PO/AH impaired rabbits were reported by Ohwatari et al., (1983) and Kosaka et al., (1984). Although heat loss capability was slightly reduced, the time courses of the changes in peripheral vasodilation and in thermal panting induced by external heating in unanesthetized PO/AH impaired rabbits closely resembled those observed in intact rabbits. In a treatise on intact heat-acclimated rabbits, when the changing rate of Ta was set to be 0.5℃/min, pattern change in rectal temperature (Tre) during Ta displacement was 1.1±0.2℃ (Mean ± S.D.) in heat-acclimated rabbit, and 1.6±0.3℃ in control rabbit, respectively. Mean threshold temperature of Ta at the beginning of vasodilation of the ear

skin was 27.5 ± 1.2℃ in heat-acclimated rabbit, but in control rabbit, vasodilation already occurred under the thermoneutral condition at 25.0℃ of Ta. On the other hand, vasoconstriction of the ear skin in heat-acclimated rabbit occurred at 21.8 ± 3.3℃ of Ta, about 7.0℃ higher than that of control rabbit $(14.7\pm2.9$℃). It is supposed that during heat acclimation, shift of threshold temperature of Ta for inducing peripheral vaso-dilation and -constriction appeared, resulting in prevention of the change of core temprerature, that is habituation phenomenon (Fujiwara *et al.*, 1986). Furthermore, in the heat-acclimated PO/AH impaired rabbits, the level at which rectal temperature was regulated was higher than that in the cold-acclimated PO/AH impaired animals. These findings, that gains of heat dissipation to general heating increased in the heat-acclimated PO/AH impaired rabbits compared with those in the cold acclimated PO/AH impaired rabbits, were similar to those reported in thermally acclimated PO/AH intact rabbtis. These results suggest that the thermal acclimation and temperature regulation system must be composed of hierarchically organized control loops located in thermosensitive tissues of central nervous axis (Ohwatari *et al.*, 1983; Kosaka *et al.*, 1984).

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温度順化過程における視床下部血流変化

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中枢神経幹軸に分布する幾多の温度センサーからの求心性情報と同様に，視床下部血流量変化は視床下部レベルにおける体温調節機構に重要な役割を果たしている．温度順化は体温調節機構に変化をもたらすことも考えられるので，本研究では，温度順化が視床下部血流量に及ぼす影響を解明するため企画された．暑熱・寒冷順化および温度非順化ウサギの全身加温によって誘発される視束前野・前視床下部 (PO/AH) の血流量 (CBF) 変化を，水素クリアランス法（Initial slope 解析）で測定した．CBF は外気温およびその他の体温調節指標の変化とよく平行推移した．同度非順化ウサギでは加温および冷却により CBF が大きく変化したのに対し，暑熱順化ウサギでは，加温による CBF の増加が小さいのみならず，冷却による CBF の減少も少量であった。また，寒冷順化ウサギにおいても，同様の結果が得られた．つまり暑熱および寒冷順化ウサギの両者において，加温および寒冷刺激に対する体温調節反応量の減少が観察された．温度順化の過程における PO/AH の血流量変化の抑制は，いわゆる温度刺激によって誘発される体温調節反応の慣れの現象 (Habituation) の結果と考えられる．