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Blood Flow Changes in the Hypothalamus during Pyrogen-induced Fever in Rabbits

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Abstract: It has been generally accepted that local brain temperature has three physical factors, i.e., metabolic heat production in local cerebral tissue, local blood flow, and the temperature of inflowing blood. From the thermoregulatory point of view, it is particularly important to determine how these three factors regulate the hypothalamic temperature. The present study was designed to evaluate changes in hypothalamic blood flow during pyrogen-induced fever in rabbits. A hydrogen clearance method was used to measure cerebral blood flow with conscious animals restrained stereotaxically. Blood flows were calculated from the initial slope of hydrogen clearance curves. After intravenous injection of LPS-pyrogen (from E. coli), the blood flow was increased or decreased with similar shifts of biphasic character in hypothalamic temperature. This suggests that blood flow changes in the hypothalamus may play an important role in fluctuations of hypothalamic temperature during pyrogen-induced fever.

INTRODUCTION

Bacterial pyrogens are known to induce fever in most experimental animals, including the rabbit, and have been used to investigate mechanisms of thermogenesis (for review see Cranston, 1976; Rosendorff, 1976; Hellon, 1978). We recently showed that during pyrogen-induced fever in rabbits, hypothalamic temperature was increased with a decrease in blood flow in the common carotid artery, and we suggested that this hypothalamic temperature change might be induced by an alteration of hypothalamic blood flow (Inomoto et al., 1979). From the thermoregulatory point of view, it is particularly important to determine the possible effect of hypothalamic blood flow changes on fluctuations in hypothalamic temperature.

Cranston and Rosendorff (1968b) and Rosendorff (1973) have shown an increase in hypothalamic blood flow during pyrogen-induced fever in rabbits using a \(^{133}\)Xe injection technique.

The present study was also designed to evaluate blood flow changes in the hypothalamus in response to intravenous injection of pyrogen in rabbits. However, we used a

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hydrogen clearance method to measure blood flow because repeated measurements can be readily performed. The hydrogen clearance method has been employed by many investigators since Fieschi et al. (1965) to measure local cerebral blood flow in a variety of species, including the rabbit (Halsey et al., 1977). The theoretical principle of the method was described by Aukland et al. (1964). In this study, hypothalamic temperature was also measured, and the possible roles for hypothalamic blood flow changes in fluctuations of hypothalamic temperature were discussed.

MATERIALS AND METHODS

Male albino rabbits weighing 2.7–3.4 kg were used in this study. The following surgical procedures and experiments were carried out with unanesthetized animals restrained stereotaxically under an ambient temperature of about 28°C.

Surgical procedures

After the animal's head was fixed in a prone position using a stereotaxic apparatus, two holes were drilled in the exposed skull bilaterally to the midline above the preoptic area of the hypothalamus according to the atlas of Monnier and Gangloff (1961). For the measurement of hypothalamic blood flow, a Pt/Pt black electrode (tip; 300 μ in diameter and 1 mm in length) was inserted stereotaxically into the hypothalamic region. The indifferent Ag/AgCl electrode was placed under the incised skin and sutured with the surrounding tissues. For the measurement of hypothalamic temperature, a copper-constantan thermocouple (1 mm in diameter) was inserted stereotaxically into the hypothalamus, through another craniotomy hole. All Pt/Pt black electrodes and thermocouples were anchored rigidly to the skull by a piece of gum.

Measurements of hypothalamic blood flow

A polarizing voltage of 10–50 mV 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 then 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 electrical circuit of the recording system is shown in Fig. 1. The first 40 seconds of the clearance curve recorded after inhalation of hydrogen had been stopped was discounted in order to correct for the arterial recirculation of hydrogen as discussed by Pasztor et al. (1973) and Halsey et al. (1977). The curves were replotted on semi-logarithmic paper, and blood flows were calculated from the two-minute initial slope of the curve (see "initial slope technique" of Olesen et al., 1971).
**Experimental design**

After the electrode system had been stabilized, individual basal flow values for 15 rabbits were determined over a 30 minute interval. The animals were then divided into a test and control group of 8 and 7 animals, respectively. Test animals were given 1–3µg/kg LPS–pyrogen (Lipopolysaccharide from *E. coli*, B-8, SIGMA), distilled in 2 ml physiological saline solution, through the retroauricular vein. Control animals received 2 ml of a physiological saline solution without pyrogen. Blood flows were measured repeatedly over four consecutive intervals of 30 minutes each, and differences in the flow changes from the basal value were compared between the two groups. In addition to hypothalamic temperature, temperatures of the rectum and ear skin, measured with copper-constantan thermocouples connected to an electric thermometer (ELLAB), and the respiratory rate were recorded in order to monitor effects of the drug administration.

**RESULTS**

**Basal hypothalamic blood flow**

After stabilization of the electrode system, basal blood flows were measured over a 30 minute interval for 15 animals. Either monoexponential or biexponential hydrogen clearance curves were obtained (Table 1). Ten out of 15 animals had only monoexponential clearances, while 5 showed only biexponential clearances. Monoexponential clearances gave a mean flow rate of 28.1 ml/100g per minute (SD±7.3). The flow rate of biexponential clearance calculated by the two compartmental analysis of Lassen *et al.* (1963) gave mean values of 168.7 ml/100g per minute (SD±29.0) and 39.0 ml/100g per minute (SD±5.0) for fast and slow components, respectively. We used the initial slope method to determine total blood flow within the hypothalamus. The validity of the method was discussed elsewhere (Symon *et al.*, 1974; Doyle *et al.*, 1975; Tamura *et al.*, 1978). Basal total blood flows estimated from the two-minute initial slope of the hydrogen clearance curves had a mean value of 33.3 ml/100g per minute (SD±10.1).

**Control experiment**

Repeated measurements of blood flow were carried out to determine stress-induced changes in blood flow and thermoregulatory parameters due to the physical restraint and

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<th>Clearance curves</th>
<th>Blood flows (ml/100g/min)</th>
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<tr>
<td>10</td>
<td>Monoexponential</td>
<td>28.1 ± 7.3</td>
</tr>
<tr>
<td>5</td>
<td>Biexponential</td>
<td>168.7 ± 29.0 (fast)</td>
</tr>
<tr>
<td></td>
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<td>39.0 ± 5.0 (slow)</td>
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* The difference between the slow component flow rate and the monoexponential clearance was not statistically significant (for details see text).
placement of the electrodes and thermocouples during a two hours experimental period.

Blood flow changes in the 7 control animals are summarized and shown in Fig. 2. The blood flows measured were grouped into sequential 30 minute intervals and expressed as a percentage of the basal values of each animal. Means and standard deviations for each interval are also presented. This figure illustrates a decrease in the blood flows compared with basal values at each time interval, but these changes were not statistically significant. Thermoregulatory parameters such as rectal, hypothalamic and ear skin temperatures as well as the respiratory rate were observed to be relatively constant over a period of two hours (Fig. 3).

Blood flow changes after pyrogen administration

Intravenous injection of LPS-pyrogen is known to cause a biphasic fever resulting from a decrease in heat loss mainly from the ear skin as well as respiratory passage, and from an increase in heat production due to shivering and non-shivering thermogenesis (NST) in the rabbit. In this experiment, 6 out of 8 pyrogen-injected animals showed biphasic fever. Monophasic fever was observed in the other two animals over a period of

Fig. 2. Change in hypothalamic blood flow over a period of two hours.

Fig. 3. Time course of a control experiment. HBF; hypothalamic blood flow, Thy; hypothalamic temperature, Tre; rectal temperature, Te; ear skin temperature, RR; respiratory rate.

Fig. 4. Effect of intravenous injection of LPS-pyrogen. Time course of a typical experiment. At the arrow, LPS-pyrogen (P.) was injected intravenously. For abbreviations see Fig. 3.
two hours. In 5 of the animals showing biphasic fever, and in 1 of the animals showing monophasic fever, similar shifts in hypothalamic blood flow were observed. However, the time course of the changes differed from animal to animal. Therefore, the blood flow changes after pyrogen administration could not be compared statistically with those in the control experiment at each time interval.

The thermoregulatory curves presented in Fig. 4 are representative of the data obtained in this experiment. Hypothalamic blood flow changes correlated more strongly with changes in hypothalamic temperature than with changes in rectal temperature.

**DISCUSSION**

It has been generally accepted that local brain temperature has three physical factors, i.e., metabolic heat production in local cerebral tissue, local blood flow, and the temperature of inflowing blood (Hayward and Baker, 1969). It is particularly important to determine how these factors regulate hypothalamic temperature during the febrile phase.

Our observation that hypothalamic blood flow was increased during pyrogen–induced fever in the rabbit agrees with the previous reports of Cranston and Rosendorff (1968b) and Rosendorff (1973), suggesting a possible role for hypothalamic blood flow changes in fluctuations of hypothalamic temperature. On the other hand, it is true that a rise in arterial blood temperature results from a decrease of heat loss and an increase of heat production during pyrogen–induced fever. Therefore, changes in arterial blood temperature may also contribute to fluctuations of hypothalamic temperature, since the hypothalamus is considered to be directly affected by the temperature of arterial blood leaving the heart in "internal carotid" species such as the rabbit (Hayward and Baker, 1968 and 1969).

Furthermore, Rosendorff (1973) pointed out that blood flow alterations in the hypothalamus may be due to changes in body temperature, and are not pyrogen induced. Two interpretations for hypothalamic blood flow changes remain: blood flow changes may be attributed to neuronal activity, and/or they may be induced by neurogenic control of hypothalamic blood flow. Changes in the activity levels of thermo-sensitive neurons in the hypothalamus following pyrogen administration have been reported by many investigators since Wit and Wang (1968). The neurogenic control of hypothalamic blood flow was discussed previously (Inomoto et al., 1979).

It is of interest that recorded hydrogen clearance curves from the hypothalamus had both monoeXponential and biexponential characteristics. This suggests inhomogeneity in the blood flows within the hypothalamus, contrary to the findings of Cranston and Rosendorff (1968a) and Rosendorff (1972). In their experiments, $^{133}$Xe was injected into the hypothalamus to measure blood flow and $^{133}$Xe clearances were shown to be invariably monoeXponential. Differences in methodology may explain the discrepancy between these studies.
The clearance rate of the fast component was greater than the cortical grey matter or deep nuclear blood flows in other species (Pasztor et al., 1973; Choki et al., 1977). Using the hydrogen clearance method, however, Halsey et al. (1977) reported that when hydrogen was given by a brief inhalation of less than 5 minutes, the flow rate of the fast component from the rabbit cortex was 150 to 250 ml/100g per minute. Halsey et al. also suggested that the excess flow rate of the fast component may be attributed to diffusion of hydrogen gas from the fast flow compartment to the slow flow compartment in addition to direct delivery of the indicator by the blood.

In these experiments, we observed changes in hypothalamic blood flow after pyrogen administration. It is not known whether such changes were directly induced by pyrogen, or resulted from changes in body temperature. There is a growing body of evidence that thermo-sensitive neurons are widely distributed in the central nervous axis, and not restricted to the hypothalamus (for review see Kosaka, 1977). Nakayama and Hori (1973) reported that the activity of the thermo-sensitive neurons in the midbrain changes in response to pyrogen. Additional experiments are needed to determine whether or not similar blood flow changes occur during pyrogen-induced fever in the extrahypothalamic area containing thermo-sensitive neurons.

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発熱ウサギの視床下部温とその局所脳血流量
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体温調節の観点から、とくに発熱時の脳局部所血流量の変化について興味がもたれる。今回、水素ガス・クリアランス法によりウサギの視床下部（視床前野）血流量の測定を行い、pyrogen 発熱時の視床下部温に及ぼす脳局部所血流量の影響について検討した。室温を約28℃に保ち、無麻酔にて、ウサギの頭部を頭位固定装置で固定し、穿頭後視床下部の一側に視床下部温測定用熱電対を、対側に視床下部血流量測定のため白金一白金黒電極を挿入した。不関電極は頭部皮下に錆着した。血流量の算定は、水素ガス吸入と描記されたクリアランス曲線を片対数グラフにプロットし、吸入停止後の40秒間を除いた initial slope 法により行った。ウサギを2群に分け、コントロール群に生食水を、他群には LPS-pyrogen を生食水に溶解し、それぞれ耳介後静脈に投与した。視床下部血流量および温度測定に加え、直腸温・耳介皮膚温・呼吸数を測定し、それぞれの経時的変化を各群について比較した。pyrogen 投与後、視床下部血流量の変化はとくに視床下部温の2峰性の変化に同期し、また同様の増減傾向を示した。このことから、pyrogen 発熱時の視床下部温の変化には、局所血流量の増減も関与していると考えられる。視床下部血流量変化と同時記録した体温調節の諸反応指標から体温の神経性調節機序について二・三の検討が加えられた。

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