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Measurement of Cerebral Cortical Blood Flow by Laser-Doppler Flowmetry in Rat Forebrain Ischemia

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Abstract Laser-Doppler flowmetry is a new method allowing convenient and continuous measurement of blood flow. The aim of our study was to evaluate the accuracy of Laser-Doppler flowmetry for measurement of blood flow in the central nervous system, compared with the established hydrogen clearance method, especially under ischemic conditions. Ten male Wistar rats were subjected to 60 min of forebrain ischemia, induced by occlusion of the bilateral common carotid arteries and unilateral vertebral artery with systemic hypotension. Under these conditions, the cerebral cortical blood flow was measured, by both methods simultaneously, three times in each animal. The cerebral blood flows measured by the hydrogen clearance method and Laser-Doppler flowmetry showed linear relationship ($r=0.89$, $p<0.01$). Laser-Doppler flowmetry facilitates accurate measurement of relative cerebral cortical blood flow under ischemic conditions.

Key Words : Laser-Doppler flowmetry, Cerebral blood flow, Forebrain ischemia, Rat

1. Introduction

Laser-Doppler flowmetry (LDF) allows instantaneous, continuous and easy measurement of microcirculatory blood flow in small tissue samples[1,2], and has been used extensively to monitor blood flow in various organs[3,4,5]. A growing number of laboratories are also adoping LDF to assess blood flow within the central nervous system[6,7,8,9], although only few studies have validated this method[10,11]. The aim of our study was to determine the accuracy of LDF, compared with the established hydrogen clearance technique[12], under experimental conditions.

2. Materials and Methods

Preparative surgery

Ten male Wistar rats, weighing 300-400g, were fasted overnight, but allowed free access to water. The animals were anesthetized initially with 4% halothane in oxygen, and were ventilated via an endotracheal tube with 1% halothane and 70% nitrous oxide in oxygen by a rodent respirator. The right femoral vein was cannulated for administration of pancronium bromide and sodium bicarbonate and for withdrawal of blood for induction of hypotension. The right femoral artery was cannulated for continuous monitoring of the mean arterial blood pressure (MABP) and serial measurements of the arterial oxygen and carbon dioxide tensions, pH and hematocrit. The rectal temperature was maintained at 37±1℃ by a heating lamp. The right vertebral artery was

electrocauterized in the lateral first and second cervical intervertebral space, by the anterior trans-cervical approach, and the bilateral common carotid arteries were separated and thread-rings were placed around them, ready for occlusion. Two cranial burr holes with diameter of 2 mm were made, 3 mm lateral and 2 mm rostral, and 2 mm caudal to the bregma on the right cranium, for a hydrogen clearance and a LDF probes, respectively. During drilling, care was taken to preserve a thin bone layer to avoid physical injury to the cerebral cortex. The thin bone layer was then carefully removed with micro-forceps without tearing the dura mater. Electrodes for electroencephalogram (EEG) monitoring then were inserted in the subcutaneous tissue behind the burr holes, the needle type hydrogen clearance probe was inserted via the rostral burr hole to a depth of 1 mm from the brain surface and the LDF probe (1 mm diamater, PF108d, Perimed, Sweden), which was attached to a micromanipulator, was advanced to touch the dura without indenting either the dura or cortex, visibly. Areas with large blood vessels were avoided. After stable readings of LDF were obtained, the probe was tightly fixed and the animals were immobilized with pancronium bromide (0.6-0.8 $\mu g/g$ body weight) to prevent the movement of the probe.

Induction of ischemia

Forebrain ischemia was induced by occluding the bilateral common carotid arteries with clips, the MABP was decreased simultaneously below 80 mm Hg and an isoelectronic EEG was obtained by withdrowing blood from the femoral vein. This is a modification of the previously established technique[13]. After 60 min of ischemia, the circulation to the brain was restored by removing the clips and reinfusing the withdrawn blood into the femoral vein. During ischemia, the MABP was allowed to change naturally and was not controlled.

Laser-Doppler flowmetry

We used a commercially available LDF monitor (PeriFlux PF3, Perimed, Sweden), which displays the flow values as DC signal outputs. This measurement does not represent the absolute cerebral blood flow. The principles of measuring the tissue surface blood flow by LDF have been described in detail elswhere[1,14]. In brief, light from a helium-neon laser is carried by an optical fiber to a probe, illuminates a 1-mm^3 volume of the tissue and is scattered by both stationary tissue and moving red blood cells. Scattering by moving red blood cells results in a Doppler frequency shift, whereas light scattered by stationary tissue remains unshifted. Analysis of the backscattered light yields the frequency of the Doppler shift, which is proportional to the red blood cell velocity. The fraction of the backscattered light which is Doppler shifted is proportional to the total volume of moving red blood cells.

Cerebral blood flow (CBF) analysis

Before ischemia was induced, the baseline cerebral blood flow by the hydrogen clearance method (baseline CBF_{H2}) and the baseline LDF value (baseline LDF) were assessed. A mean LDF value over the 5-min period which corresponded to the hydrogen clearance time measured was chosen as the LDF value. Cerebral blood flow measurement by the hydrogen clearance method is an established technique and the procedure involved has been described in detail elswhere[12]. CBF was measured three times, i. e. immediately, 20 min and 40 min after induction of ischemia, by the hydrogen clearance method (CBF_{H2}) and LDF (CBF_{LDF}), simultaneously. As LDF show the flow value as a DC signal, the CBF obtained by LDF was calculated as follows;

CBF_{LDF} = baseline $CBF_{H2} \times LDF_X$/ baseline LDF

x: time in minute after induction of ischemia, that is immediately, 20 min and 40 min.

CBF_{LDF} was compared with CBF_{H2} in 30 measurements (3 measurements per animal), and the relationship between these values was evaluated by linear regression analysis and calculation of Pearson's correlation coefficient, which was examined by Fisher's test. Correlations at p values of less than 0.05 were considered to be significant.

3. Results

Physiological variables including the hematocrit were measured just prior to induction of ischemia and at the termination of ischemia. The mean decrease of hematocrit was 3.0 % (range 0-10 %). Arterial pO_2, pCO_2 and pH were maintained within the normal range (pO_2 100-200 mm Hg, pCO_2 35-45 mm Hg, pH 7.35-7.45).

Recording LDF from the cerebral cortex

The baseline LDF values were highly sensitive to minor changes in the positioning of the probe and showed wide variations, which

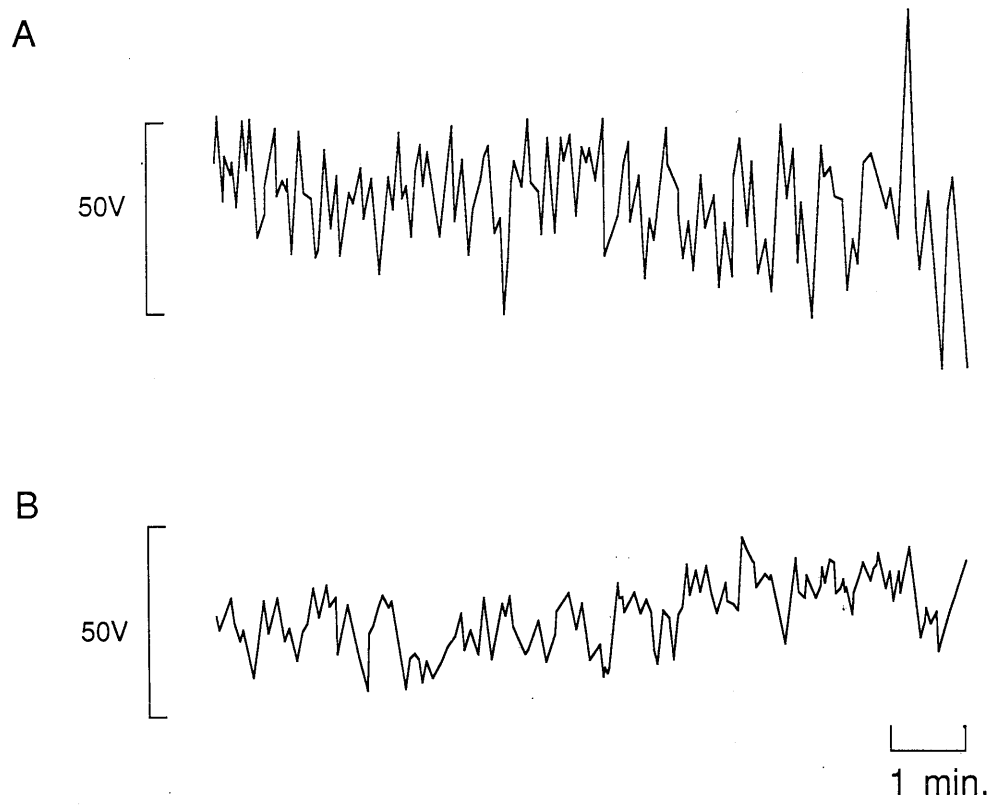

Fig. 1 Cyclic variations of LDF readings. The oscillatory frequency was between 0.5 and 8 cycle/min; the amplitude was variable and was smaller under ischemic (B) than non-ischemic (A) conditions.
V: volt

meant that the absolute LDF reading did not reflect the CBF. Therefore, care was taken to avoid small displacements of the probes or animals that could artifactually change the LDF reading. Cyclic fluctuations of the LDF reading with frequencies between 0.5 and 8/min, were detected under both non-ischemic and ischemic conditions. The amplitude was smaller under ischemic condition than that under non-ischemic condition (Fig. 1). The occurence of these oscillations were not related to oscillations in blood pressure, and the onset and disappearance of oscillatory activity was independent to the changes in any physiological parameters measured. When cyclic fluctuations of the LDF reading were present during data sampling, mean values were extrapolated from the curve. The LDF reading has dropped immediately after induction of ischemia, and changed variably thereafter according to the changes in MABP; it ranged from 20% to 80% of the baseline value (Fig. 2). Changes in the LDF readings during ischemia varied and were subjected to inter-individual variations.

Correlation of CBF_{LDF} and CBF_{H2}

Figure 3 shows the regression line, calculated from 30 paired measurements of CBF_{LDF} and CBF_{H2}. The regression line and correlation coefficient were $CBF_{LDF} = -0.32 + 1.07\ CBF_{H2}$, $r = 0.89$ and $r^2 = 0.79$, respectively. The correlation was statistically significant ($p < 0.01$).

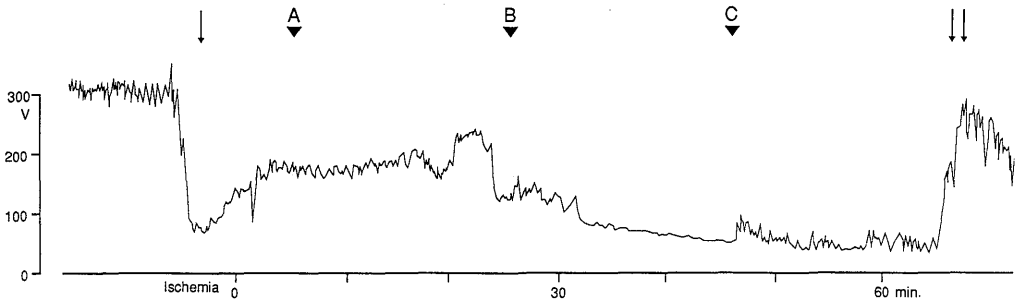

Fig. 2 Changes in absolute LDF readings displayed by the chart recorder. The LDF reading fell immediately after induction of ischemia (arrow) and changed variably thereafter, under ischemic conditions. The CBF was measured simultaneously by the two methods three times during ischemia (arrowheads A, B, C); the double arrow shows recirculation.
V: volt

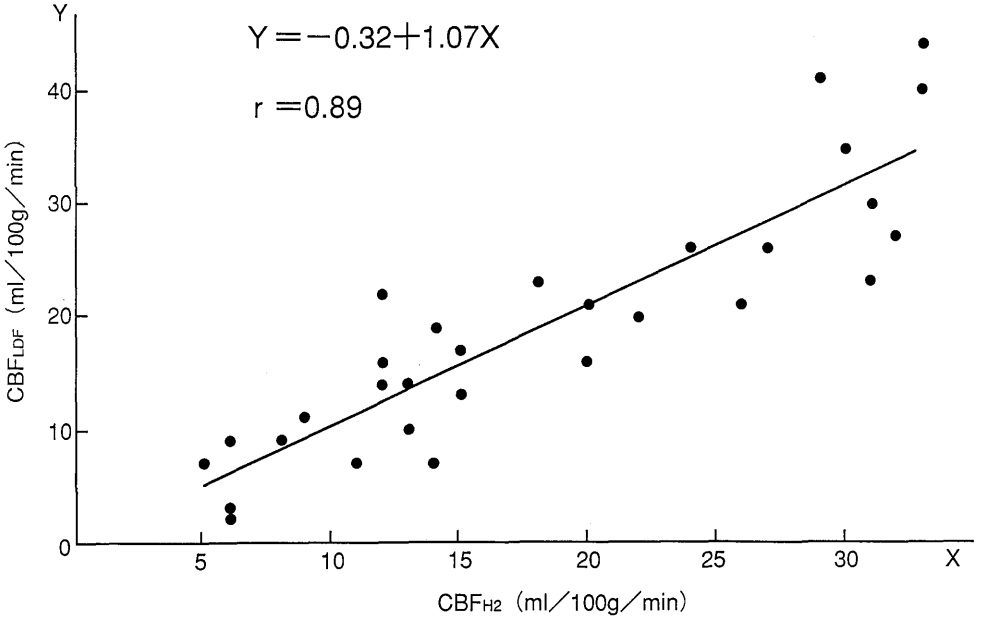

Fig. 3 Correlation of CBF_{LDF} and CBF_{H2}.

4. Discussion

Laser-Doppler flowmetry is a new method, which has been used extensively to measure blood flow in various organs[3,4,5]. It enables to measure blood flow easily, non-invasively and continuously. However, how accurate is this method for measurement of blood flow in the central nervous system? The absolute flow readings obtained are meaningless, as they are highly dependent on probe placement and position, i. e., minor changes in probe position sometimes result in major changes of the absolute flow reading. Johnson et al. also found that skin resting blood flow values

varied at various forearm sites by LDF, although skin blood flow determined by plethysmography did not differ between these sites[15]. This problem may be explained by several factors. In the rat, on most occations, the LDF probe has to be placed on pial arteries or veins with high flow velocities and volume components due to the dense pial microvasculature of this species, which may affect the true microvascular flow signal. Moreover, the absolute flow reading obtained by LDF could be influenced by several physiological factors and show major difference in two adjacent tissue areas, even though the regional CBF may be the same in both tissue areas due to compensatory phenomena. Therefore, mechanical stability of the probe attachment is of great importance in order to obtain correct results over longer observation period. We found the relative CBF value obtained by LDF, that means the percent change of the absolute value, to be very reliable in this study, when it was compared with CBF measured by the hydrogen clearance method. Other studies, in which the LDF flow value was compared with CBF measured by autoradiography, also have confirmed the reliability of the relative LDF value[11]. In our experiment, ischemia was induced by arterial occlusion and simultaneous hypotension, which was obtained by withdrawal of blood from the cannulated femoral vein, which induced a moderate decrease in the hematocrit. The CBF measured by LDF was correlated well with that measured by the hydrogen clearance method and, thus, it appears that a moderate decrease in the hematocrit dose not affect LDF, possibly because the decrease in blood volume is compensated by an increase in blood velocity.

Problems with LDF in the central nervous system

1. Laser-Doppler flowmetry recording through the dura mater or exposed cortex

In this study, the dura was left intact to protect the brain surface from physical injury. However, this had no adverse influence on LDF measurements, because the dura of the rat is a translucent, 2-5μm thick membrane with very few blood vessels and lymphatics[16], so damping of the laser signal is minimal. In clinical use, however, the LDF probe has to be applied to the cortical surface, because the human dura mater is a thick membrane with many blood vessels and is not translucent.

2. Sample volume of LDF

The LDF sample volume in various tissues appears to be approximately 1 mm^3, with a laser penetrating depth of 1 mm, so CBF in deep brain regions cannot be investigated. The results from some studies, however, have indicated that the sample volume may be larger, and penetration may be as deep as 2 or 3 mm[4,14,17]. No confirmatory data about this problem, particularly with respect to the central nervous system, are available at present.

3. Cyclic fluctuations

Laser-Doppler flowmetry showed cyclic fluctuations, with a range of 0.5-8 cycles/min, during pre-ischemic and ischemic conditions. This phenomenon is believed to be due to spontaneous rhythmic vasomotion in cerebral microcirculation, which has been observed in various species[18,19,20]. Its physiological meaning is unknowm. These cyclic fluctuations pose methodological limitation for measuring representative CBF values, particularly when they have high amplitudes, as LDF yields different flow values depending on whether the study was performed at peak, trough or intervenig levels of blood flow, while LDF is very sensitive to be able to detect such spontaneous vasomotion in microcirculation. Therefore, it is recommended that LDF values should be continuously recorded on the chart and the arithmetic mean values over a few minutes observation be chosen as a representative blood flow value, as performed in our study.

In summary, LDF has been shown to enable relative cerebral blood flow to be measured accurately in a rat model of forebrain ischemia. It is a good method, which provides continuous CBF measurements, although it dose have some disadvantages.

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