Effects of Stimulation of Cervical Sympathetic Trunks with Various Frequencies on the Local Cortical Cerebral Blood Flow Measured by Laser Doppler Flowmetry in the Rat

Yuka SAEKI, Akio SATO,* Yuko SATO,
and Andrzej TRZEBSKI**

*Department of Physiology, Tokyo Metropolitan Institute of Gerontology,
Itabashi-ku, Tokyo, 173 Japan*

Abstract The effects of electrical stimulation of cervical sympathetic trunks for 1-min duration at supramaximal intensity with various stimulus frequencies on local cortical cerebral blood flow were investigated in urethane-anesthetized rats. Electrical stimulation with low frequency (1–2 Hz) produced a significant increase in local cortical cerebral blood flow during the stimulation. The local cortical CBF reached 111% of the resting value during the stimulation at 10 Hz, and slightly decreased for about 30 s after the end of stimulation. High-frequency stimulation (20–30 Hz) produced the short-term increase during the stimulation, which was followed by the dominant and long-lasting decrease, and the local cortical CBF reached 87% (at 30 Hz) of the resting value after the end of stimulation. The response of increase in flow was abolished by intravenous administration of β adrenergic blocking agent (propranolol, 1.3 mg/kg i.v.), while the response of decrease in flow was abolished by α adrenergic blocking agent (phenoxybenzamine, 0.5 mg/kg i.v.).

Key words: cervical sympathetic trunks, cortical cerebral blood flow, laser Doppler flowmetry, vasodilatation, vasoconstriction.

Sympathetic innervation of cerebral blood vessels including small vessels of cortical microcirculation is supplied mainly by the superior cervical ganglion (NIELSEN and OWMAN, 1967; IWAYAMA *et al.*, 1970; PEERLESS and YASARGIL, 1971; EDVINSSON *et al.*, 1972; PURDY and BEVAN, 1977). The superior cervical ganglion innervates predominantly the cerebral blood vessels branching from the internal

Received for publication November 30, 1989

* To whom all correspondence should be addressed.

** Present address: Department of Physiology, Warsaw Medical Academy, 26/28 Krakowskie Przedmiescie, 00927 Warsaw, Poland

carotid arteries and to a lesser extent, those branching from the vertebral arteries (EDVINSSON, 1975).

Despite the great deal of effort that has been devoted to elucidation of the sympathetic neural regulation of the cortical cerebral blood flow (CBF), the results of the cervical sympathetic trunk (CST) stimulation in physiological conditions have been controversial. Some authors observed decreases both in diameter of pial arteries (KOBAYASHI *et al.*, 1971; KUSCHINSKY and WAHL, 1975; WEI *et al.*, 1975; AUER *et al.*, 1981) and in CBF (LACOMBE *et al.*, 1977; SERCOMBE *et al.*, 1979; BUSIJA, 1985), while others could not produce any significant effects (HARPER *et al.*, 1972; HEISTAD and MARCUS, 1978; HEISTAD *et al.*, 1978). Doubt has been raised as to whether there is any functional role of sympathetic neural regulation of the cortical CBF under normal conditions (HEISTAD and MARCUS, 1978).

These conflicting results may be accounted for by several factors, e.g., the species of experimental animals used, sets of experimental conditions such as the metabolic state of the brain, different types of anesthesia, various vascular beds of the brain analyzed, and, most of all, different methods applied for measuring CBF.

The sympathetic stimulatory effects on the cerebral blood vessels are apparently rapid and transient. A proper approach to the study of rapid and transient changes of CBF has required a method suitable for recording continuously the time course of instantaneous changes in local CBF, and also for providing repeated measurements in the same experiments. Most of the methods used so far have mainly provided data on local CBF under static conditions, as microspheres or autoradiographic techniques including the most reliable iodoantipyrine autoradiography have contributed. Other techniques for repeatable measurements, such as radioactive inert gas clearance or hydrogen clearance, have provided averaged flow values over a longer time period, usually not less than 1 min, as relatively long sample acquisition time is needed for computing. Only the heat clearance technique (BETZ *et al.*, 1966) and pulse Doppler crystal technique (BUSIJA *et al.*, 1981) have enabled continuous recording of local CBF. The last method, however, could be applied only to relatively large pial arteries (diameters larger than 250 μm) and does not record nutritional capillary flow.

A new possibility for the continuous tissue blood flow measurement emerged with the laser Doppler velocimetry based on the principle of frequency broadening of the spectrum of scattered laser lights reflected from moving blood cells in microcirculation as proposed originally by RIVA *et al.* (1972) and by STERN (1975). At present, a new laser Doppler flowmeter employs gas He-Ne lasers and thin optical fibers both for emission of red visible rays and for recording of reflected scattered lights. Processing and analysis of the broadening Doppler spectrum multiplied by the fractions of light that is Doppler-shifted provide a reliable index (mV) of tissue flow (HABERL *et al.*, 1989). Although laser Doppler flowmetry (LDFM) estimates only the integrated motion of blood cells in small tissue volume, the values obtained in the rat cerebral cortex correlate well with the hydrogen clearance method as has recently been reported by SKARPHEDINSSON *et al.* (1988). A

great advantage of the new generation of the LDFM instrument is its high spatial resolution which allows recording of local microcirculation in small animals and in restricted brain areas.

Therefore, we applied the LDFM to examination of the effects of CST stimulation on the local cortical CBF in the rat. The present study focused on (1) the time course of cortical CBF responses during and after CST stimulation for 1-min duration, (2) the effect of frequency of CST stimulation on the cortical CBF responses, (3) the lateralization of the cortical CBF responses, and (4) the cortical topography of the effects. Finally, (5) a pharmacological analysis of the mechanisms involved was carried out by applying the α and β adrenergic receptor blockers (phenoxybenzamine and propranolol, respectively), as well as atropine as the cholinergic muscarinic receptor blocker and cimetidine as the histaminergic H_2 receptor blocker.

MATERIALS AND METHODS

Thirty-five male Fischer rats weighing 290–500 g were anesthetized with urethane (initial dose 1.1 g/kg, i.p., supplemented later during experiment by further i.v. administration of 0.2–0.3 g/kg), paralyzed by gallamine triethiodide (initial dose 20 mg/kg i.v., supplemented during experiment), and artificially ventilated by respirator (Harvard pump 683, U.S.A.) through a tracheal cannula. All precautions were taken to maintain respiratory gases at a constant level, i.e., end-tidal CO_2 (F_{ETCO_2}) at about 4% and end-tidal O_2 (F_{ETO_2}) at about 16–17%. End-tidal gas concentrations were continuously monitored by a combined CO_2 - O_2 gas analyzer (NEC San-ei, Tokyo). Tidal volume was set at 0.9–1.2 ml/100 g b.w. Frequency of ventilation was adjusted so as to maintain F_{ETCO_2} at about 4%. In some experiments in which hypercapnia was imposed, CO_2 was injected into the inspiratory line of the respiration system under control of F_{ETCO_2} at a stable level. Body temperature was monitored in the rectum and automatically controlled at $37.0 \pm 0.5^\circ C$ with a DC heating pad and infrared lamp.

A polyethylene catheter was placed in the femoral artery for continuous recording of systemic arterial blood pressure by transducer and heart rate with a rate meter driven by the systolic peaks of pulse pressure. The rat's head was fixed in a head holder, and the animal was placed in a prone position. CSTs were exposed bilaterally by dorsal approach, separated from the vagi and aortic depressor nerves, and cut. Their peripheral ends were fixed on the bipolar platinum-iridium wire stimulating electrodes, and all areas including the nerves were covered by liquid paraffin. In experiments in which only one CST was stimulated, the left CST was prepared.

An approximately rectangular-shaped skull bone about 5.5–10 mm in length and 3–5 mm in width was drilled out on one side, strict precaution being taken to cool the drilled area by continuous application of cooled saline. The bone was removed, leaving dura mater intact. In some experiments, similar procedures were

used on bilateral sides to expose bilateral brain hemispheres. Dura remained intact throughout experiments, except for measurements of the correlation of hydrogen clearance values with laser Doppler recordings. In these experiments the cortical surface was covered by liquid paraffin. The exposed area provided the access to frontal, parietal, and occipital cortices. The laser Doppler flowmeter (ALF 2100, Advance Co., Ltd., Tokyo) used in this study had an optical output power of the He-Ne laser beam of 2 mW at the probe and a wavelength of 632.8 nm. Moving red blood cells backscattered the light and caused a frequency shift which was determined by photodetector (detection ranges of frequency shifted: from 30 Hz to 30 kHz). Electrical signal generated from photodetector contains the frequency and power information related to blood cell velocity and blood volume, respectively. The laser Doppler flowmeter processed all the voltage information (HABERL *et al.*, 1989). The flow signal was averaged with a 5 s time constant. The flowmeter with a probe diameter of 0.8 mm provided high spatial resolution for measurements of blood flow within a depth of about 1 mm just below the recording probe. The probe held in a balancer exclusive for the laser Doppler flowmeter (ALF-B, Advance Co., Ltd., Tokyo) was placed just above the dura mater surface. In some experiments in which the topography of cortical blood flow was analyzed, three probes were placed simultaneously over the frontal, parietal, and occipital lobes at the distance of about 4–5 mm. Great precautions were taken to avoid the placement of the probe in the proximity of large pial artery because, otherwise, the Doppler spectrum broadening from large-volume, high-velocity vessels would have dominated over the averaged microcirculatory signals, and abnormally high flow recordings would have been obtained. Electric signals in mV from the LDFM, arterial blood pressure, and heart rate all were simultaneously recorded on the polygraph (Nihon Kohden Ltd., Tokyo).

In 7 rats, hydrogen clearance was measured by an epoxyite-insulated platinum electrode with a tip diameter of 0.3 mm with exposed noninsulated end of 1.0 mm length (Unique Medical, Co., Ltd., Tokyo). After removal of the dura mater, the platinum electrode was inserted about 1 mm into the cortex at an angle of 45° in the closest proximity to the LDFM probe. The standard calomel reference electrode was placed at a hindlimb region. The current from the platinum electrode corrected by the calomel electrode potential was fed to the amplifier and recorded on the polygraph. Hydrogen gas of a concentration of 2–4% was introduced into the inspiratory line of the respiratory pump. Two minutes were allowed for stabilization of cerebral hydrogen. Hydrogen clearance curves were recorded for 1 min starting 3 min after the beginning of clearance slope as this section of curve provided the highest flow values. Only monoexponential curves were observed.

Electrical square pulse stimuli of 0.5 ms duration, 10 V intensity, and of varying frequency were applied on CST either unilaterally or bilaterally for 1 min. The 10 V stimulus was well above the suprathreshold level, as stimuli of 5 V usually produced the maximal responses.

The LDFM signals in mV were continuously recorded in the prestimulus control

conditions, as well as in conditions during and after subsequent sympathetic stimulation. The mean value of the LDFM signals for 30 s prior to the onset of stimulation was taken as the control value. Then, the sets of values during and after the CST stimulation, expressed as changes relative to the control values, were compared, and statistical significance of difference were checked by Student's or the paired *t*-test.

β adrenergic receptor blocker, propranolol (Inderal, Sumitomo Pharmaceutical Co., Ltd., Osaka), or α adrenergic blocker, phenoxybenzamine (Nacalai Tesque, Kyoto) was infused i.v. slowly within 1 min in the total dose of 0.25–2 and 0.5 mg/kg, respectively. For blockade of cholinergic muscarinic receptor, atropine (atropine sulfate, Tanabe Seiyaku Co., Ltd., Osaka) and for histaminergic H_2 receptor blockade, cimetidine (Tagamet, Fujisawa Pharmaceutical Co., Ltd., Osaka) were injected i.v. in the doses of 0.5 and 30 mg/kg, respectively.

Four percent Ficoll 70 (Pharmacia Fine Chemicals AB, Sweden) was intravenously infused in some animals with the purpose of maintaining systemic arterial blood pressure at the control level.

RESULTS

1. *Evaluation of laser Doppler signals for local cortical CBF by hydrogen clearance methods*

Figure 1 shows a regression line of the cortical CBF values of LDFM signals in mV plotted against the cortical CBF values calculated with hydrogen clearance technique ($n=7$). Low values of CBF were obtained by artificial bleeding with a syringe, or by occluding an ipsilateral common carotid artery, and high values of CBF were obtained either by hypercapnia with an increase in CO_2 in inspiratory respiration line, or by increasing systemic arterial blood pressure after occluding the descending aorta. The cortical CBF values calculated by hydrogen clearance method under the resting condition in 7 animals ranged between the 22–37 ml/(100 g·min). The correlation between the cortical CBF values measured by the laser Doppler method and hydrogen clearance method was significant ($p<0.01$).

2. *Dependence of the local cortical CBF response on the frequency of cervical sympathetic stimulation*

Systolic and diastolic arterial blood pressures under the resting condition during the experiments were about 90–160 and 50–130 mmHg, respectively, and they were within the ranges for cortical CBF autoregulation.

The local cortical CBF under the resting condition exhibited irregular slow fluctuations of varying amplitudes (Figs. 2–7). These fluctuations seemed to accord with the spontaneous vasoconstrictions that were related to low-frequency oscillations due to the cortical oxidative metabolism observed in both anesthetized (DORA and KOVACH, 1981; TOMITA *et al.*, 1982) and conscious animals (VERN *et*

Fig. 1. Correlation curve between the cortical CBF measured by hydrogen clearance method (abscissa) versus the cortical CBF measured by laser Doppler method (ordinate) obtained by using 7 rats. Closed circles show values under the resting conditions. Low values of CBF were obtained by bleeding or by occlusion of an ipsilateral common carotid artery and high values of CBF were obtained by hypercapnia or by increasing systemic arterial blood pressure after occlusion of the descending aorta (open circles). A linear regression analysis was significant ($Y=8.26X+25.92$, $r=0.867$, $p<0.01$).

al., 1988).

Figure 2 demonstrates three examples in which stimulation of CSTs for 1 min with a frequency of 5 Hz produced an immediate sharp increase in local cortical CBF. This response of the cortical CBF started within a few seconds after the onset of CSTs stimulation, and lasted over the total 1-min period of stimulation, usually subsiding slowly in the course of stimulation (Fig. 2A and B). After the cessation of CSTs stimulation, cortical CBF rapidly declined, and showed a slow and slight decrease, and then gradually returned to the basal control level within a few minutes. Systemic arterial blood pressure was slightly increased during the stimulation in some cases, as shown in Fig. 2A and B, evidently due to vasoconstriction in the extracranial vascular beds supplied by fibers in CST. Small and transient pressor effects could not necessarily account for the CST-induced increases in cortical CBF, because similar cortical CBF responses were also observed in those experiments in which arterial blood pressure did not change during CSTs stimulation, as shown in Fig. 2C.

The responses of the cortical CBF to CSTs stimulation of 1-min duration with various stimulus frequencies were systematically analyzed in 6 rats. Figure 3 demonstrates example responses to stimulations with different frequencies in one rat (A), and summarizes the results from 6 rats (B) presented in percentages. Our

Fig. 2. Three typical examples of CBF changes in three different rats induced by electrical stimulations of bilateral CSTs for 1-min (shown by horizontal bars) at a 10 V intensity with a 0.5 ms pulse duration at 5 Hz. A: CBF change accompanied with a marginal blood pressure increase. B: CBF change accompanied with a slight blood pressure change. C: CBF change without any change in blood pressure.

preliminary examination showed us that there was a close correlation ($n=6$, $r=0.985$, $p<0.01$) between the LDFM signals in the prestimulus control conditions and changes in the LDFM signals during and after the same stimulation of CST, despite different LDFM signals in the prestimulus conditions in different animals. Therefore, the present expression by percentages for the responses of CBF to CSTs stimulation is sufficient for describing the present data quantitatively. Electrical stimulation with low frequency (1–2 Hz) produced a significant increase in local cortical CBF during the stimulation. The local cortical CBF reached 111% of the resting value during the stimulation at 10 Hz, and slightly decreased for about 30 s after the end of stimulation. High-frequency stimulation (20–30 Hz) produced the short-term increase during the stimulation, which was followed by the dominant and long-lasting decrease, and the cortical CBF reached 87% (at 30 Hz) of the resting value after the end of stimulation. In some experiments a rapid rebound effect, i.e., a transient increase in cortical CBF, was observed shortly after the cessation of stimulation with high frequencies, especially when a decline of the response of increase in cortical CBF dominated during the stimulation (Fig. 5A₂, B₂, and C₂).

In general, the variability of the cortical CBF responses among different rats and even in the same rat, in the course of experiments lasting for several hours,

Fig. 3. CBF responses during electrical stimulations of bilateral CSTs with various frequencies (indicated at the left side of each trace) for 1-min (shown by horizontal bars) at a 10 V intensity with a 0.5 ms pulse duration. A: typical responses of CBF. B: summarized responses of CBF for 6 rats. The time course of the CBF response was calculated every 5 s and expressed as the percentage of the basal CBF for 30 s prior to the onset of stimulation. The dashed lines indicate the time at which the stimulus was presented. Each point and vertical bar shows the mean $\pm$ S.E. Each * and ** shows statistical significance between the basal CBF and the response following stimulation tested by the paired *t*-test ($p < 0.05$ and $p < 0.01$).

was great despite efforts to keep the experimental conditions (blood pressure, ventilation, end-tidal $F_{ET}O_2$, $F_{ET}CO_2$, etc.) as stable as possible.

3. Lateralization of the local cortical CBF response to CST stimulation

In this series of experiments, either the left, the right, or both CSTs were simultaneously stimulated. The LDFM probe was always placed over the left parietal lobe. Figure 4 demonstrates an example of the biphasic response of the left cortical CBF, an initial increase followed by a later decrease, following repetitive stimulation with high frequencies at 20 Hz delivered at ipsilateral (A), contralateral (B), and bilateral (C) CSTs. Although responses varied from rat to rat, the responses to ipsilateral and bilateral stimulations had an insignificant tendency to be slightly larger than that to contralateral stimulation.

Fig. 4. A–C represent sample recordings demonstrating CBF response to ipsilateral (A), contralateral (B), and bilateral (C) CSTs stimulation at 20 Hz (10 V, 0.5 ms) for 1-min (shown by horizontal bar).

Fig. 5. A typical example of the CBF response recording at three different cortical regions (A_1 and A_2 : frontal; B_1 and B_2 : parietal; C_1 and C_2 : occipital) simultaneously to the low frequency 5 Hz (A_1 , B_1 , C_1) and high frequency 20 Hz (A_2 , B_2 , C_2) stimulations of bilateral CSTs (10 V, 0.5 ms, 1 min; shown by horizontal bars).

4. Cortical topography of the local cortical CBF responses produced by CSTs stimulation

In this series of experiments, three probes of three laser Doppler flowmeters were placed simultaneously over the frontal, parietal, and occipital lobes about 4–5 mm apart from each other in order to avoid interference of recording from 3 different sites. Stimulations of bilateral CSTs were applied to produce as marked CBF responses as possible. In all 7 experiments, the basic patterns of cortical CBF responses in these 3 lobes were similar. Cortical CBF increased during the stimulation with a low frequency of 5 Hz, and there was a biphasic, increase-decrease cortical CBF response during the stimulation with high frequencies of 20–30 Hz. Figure 5 shows examples of responses in the 3 lobes for 2 different stimulations with a low

Fig. 6. The effects of β adrenergic blocking agents (propranolol) on CBF response to the CSTs stimulation (10 V, 0.5 ms, 5 Hz) for 1-min (shown by horizontal bars). A and B: sample recordings of control response (A) and response after administration of propranolol (1.3 mg/kg i.v.) (B). C: summarized responses of CBF for mean of 5 rats. The time course of the CBF responses was calculated every 5 s and expressed as the percentage of the basal CBF for 30 s prior to the onset of stimulation. The dashed lines indicate the time at which the stimulus was presented. Each point and vertical bar shows the mean $\pm$ S.E. Each * and ** shows statistical significance between the basal CBF and the response following stimulation tested by Student's *t*-test ($p < 0.05$ and $p < 0.01$).

frequency of 5 Hz (A_1 , B_1 , and C_1) and with a high frequency of 20 Hz (A_2 , B_2 , and C_2) in the same animal. The magnitudes of cortical CBF responses seemed to be slightly more pronounced in the frontal and parietal lobes than in the occipital lobe. Yet the paucity of data did not allow an analysis of statistical significance of these local differences.

5. Effects of β and α adrenergic receptor blockers on the cortical CBF responses to CSTs stimulation

The responses of increase in parietal cortical CBF during the CSTs stimulation of 1-min duration were significantly reduced by an intravenous injection of the β receptor blocker, propranolol (0.25–2 mg/kg), within 5–10 min, but the responses of decrease in parietal cortical CBF appearing after the end of CSTs stimulation were not influenced, as shown in Fig. 6A and B (sample recording), and in Fig. 6C (summarized results).

Following an intravenous injection of the α receptor blocker, phenoxybenzamine (0.5 mg/kg), the response of decrease in parietal cortical CBF observed in the later period of the CSTs stimulation of 1-min duration and also observed for a period more than 1 min after the end of CSTs stimulation with high frequency at 30 Hz reversed to the response of increase in CBF, as shown in Fig. 7A and B (sample recording) and in Fig. 7C (summarized results). These effects of phenoxybenzamine were highly significant (Fig. 7C), and were tested using only

Fig. 7. The effects of α adrenergic blocking agents (phenoxybenzamine) on CBF response to the CSTs stimulation (10 V, 0.5 ms, 30 Hz) for 1-min (shown by horizontal bars). A and B: sample recordings of control response (A) and response after administration of phenoxybenzamine (0.5 mg/kg, i.v.) (B). C: summarized responses of CBF for 7 trials of 6 rats. See Fig. 6 for other details.

high frequency at 30 Hz for CSTs stimulation in order to obtain larger control responses of decrease in the cortical CBF before administration of phenoxybenzamine. The slight responses of increase in CBF observed in an early period for about 10 s of CSTs stimulation did not change following administration of phenoxybenzamine. The present administration of phenoxybenzamine always produced a decrease in systemic arterial blood pressure, and the depressor responses could not fully be recovered even after intravenous infusions of Ficoll solution. No single systemic pressor responses were observed during CSTs stimulation after the administration of phenoxybenzamine, and this result was a confirmation of the effectiveness of phenoxybenzamine as a pharmacological blocker of the α adrenergic receptors.

6. Cortical CBF responses to CSTs stimulation after blocking the cholinergic muscarinic receptors and the histaminergic H_2 receptors

Neither cholinergic muscarinic blocker, atropine (0.5 mg/kg i.v.), nor histaminergic H_2 blocker, cimetidine (30 mg/kg, i.v.), influenced the cortical CBF responses to CSTs stimulation.

7. Cortical CBF responses to CSTs stimulation under hypercapnic state

During systemic hypercapnia, with the end-tidal CO_2 elevated up to 9–10%, the cortical CBF responses to CSTs stimulation were still clearly observed despite the tremendous background increase in cortical CBF reaching almost 200% of the control values under the normocapnia.

DISCUSSION

LDFM has been used for continuous recordings of local CBF both in experimental animals such as rats (CHEN *et al.*, 1986) and monkeys (WILLIAMS *et al.*, 1980) and in human patients during neurosurgery (ROSENBLUM *et al.*, 1987). LDFM has a great advantage in the analysis of instantaneous responses of local CBF to various stimulations. However, only recently has the method been quantitatively compared with a more direct and well-established technique of hydrogen clearance by SKARPHEDINSSON *et al.* (1988). They obtained a significant correlation between the two values of local CBF measured by LDFM and by hydrogen clearance. However, their experimental conditions differed from those of the present study, as they used hypertensive rats (SHRs) under chloralose anesthesia, and their animals were breathing spontaneously. In addition, as the diameter of their LDFM probe was three times greater than the present one (2.5 mm versus 0.8 mm), spatial resolution in both the horizontal and vertical dimensions could apparently be more diffuse than in our experiments and flow of larger pial arteries could influence their recordings. Therefore, first of all, we had to compare the present LDFM signals for the local cortical CBF values with the values obtained by the well-established hydrogen clearance method in our experimental conditions. Our results also showed a significant correlation between the two values. The slope of the regression line in the present study was flatter than that reported by SKARPHEDINSSON *et al.* (1988), because the present CBF values calculated by hydrogen clearance were significantly lower than values obtained by SKARPHEDINSSON *et al.* (1988). The difference may result from the urethane anesthesia used in our experiments. Rats anesthetized with urethane seem to provide lower CBF (SCREMIN *et al.*, 1973) as compared to conscious rats or those anesthetized with other anesthetic agents (for a review see SKARPHEDINSSON *et al.*, 1988). Different strains of rats, different experimental conditions except for F_{ETCO_2} , and different LDFM probes may also explain this discrepancy in calculated values. Such a discrepancy necessitates great caution in interpreting experimental data in terms of absolute values of blood flow. The main significance of LDFM consists in providing an instantaneous response and continuous recording of relative changes in microcirculatory blood flow in very restricted brain areas rather than absolute values of microflow.

The present values of cortical CBF are slightly lower than those measured previously (SCREMIN *et al.*, 1973), and the present low value seems to depend on slight hypocapnia with the end-tidal CO_2 at around 4% in the present experiment. The present hypocapnia was employed, because this respiration gave more reproducible responses of the cortical CBF to CSTs stimulation than those under the normocapnic at 5% of the end-tidal CO_2 .

A major finding of the study is that instantaneous local cortical CBF responses to CST stimulation for 1-min duration were composed of both (1) the increase in cortical CBF during the CST stimulation and (2) the decrease in cortical CBF after

the end of stimulation. As these two components were elicited whether or not systemic arterial blood pressure changed, the increase and decrease in cortical CBF can be suggested to correspond to the local vasodilatation and vasoconstriction of small blood vessels in the cortex, respectively.

A further important finding of this study is that the cortical CBF response to CST stimulation was stimulus frequency dependent. CST stimulation with low frequencies (1–10 Hz), in the ranges of resting discharge frequencies observed in the rat cervical preganglionic fibers (GILBEY *et al.*, 1982) produced a significant increase in local cortical CBF during the stimulation, and its value maximally reached 111% of the resting value. Stimulation with high frequencies (20 and 30 Hz) produced the initial increase followed by late decline or recovery during the stimulation. After the cessation of stimulation, there were frequency-dependent decreases in cortical CBF with frequencies of about 5 Hz, reaching down to 87% at 30 Hz. The local cortical CBF computed with the LDFM technique was from averaged velocities of red cells at different directions in the local cortical microcirculation. BAUMBACH and HEISTAD (1983), using low-frequency sympathetic stimulation (6–8 Hz), computed small pial vessel resistance applying the microspheres technique for blood flow measurements. Their calculation indicated a decrease in vascular resistance of cortical microcirculation in cats and rabbits. BUSIJA *et al.* (1982) observed in cats that velocity of CBF in relatively large pial arteries more than 250 μm in diameter was increased during CST stimulation with high frequency (20 Hz). However, a parallel decrease in arterial diameter indicated that calculated cortical CBF remained unchanged. Our findings of the cortical CBF response during CST stimulation using LDFM were substantially consistent with those results.

It has been proposed that increased velocities of blood flow in pial arteries are due to autoregulatory dilatations of small downstream arteries following constrictions of large arteries (BUSIJA *et al.*, 1982). However, the following findings of the present study strongly suggest that CST stimulation with low frequency produces, rather, an active neurogenic vasodilatation in cortical microcirculation. First, blocking of the β adrenergic receptors by propranolol reduced significantly or abolished the response of increase in cortical CBF during CST stimulation, leaving the response of decrease in cortical CBF after the cessation of stimulation unchanged. Secondly, the α adrenergic receptor blocker, phenoxybenzamine, augmented and extended the increase in cortical CBF, and reversed a decrease in cortical CBF to the increase occurring at high-frequency CST stimulation. Thirdly, hypercapnia, which dilates small pial arteries and prevents their compensatory autoregulatory response (HARPER *et al.*, 1972), did not abolish the increase in cortical CBF induced by CST stimulation with low frequency.

Both the α and β adrenergic receptors have been reported to exist in the cortical pial vessels (EDVINSSON, 1975; WAHL and KUSCHINSKY, 1977; EDVINSSON *et al.*, 1979). HARDER *et al.* (1981) observed in their *in vitro* experiments a relaxation of the pial artery by applying a small dose of norepinephrine in a concentration of

less than $1\text{ }\mu\text{M}$, and a contraction of the same artery with a larger dose of norepinephrine, and the relaxation and contraction were abolished by propranolol (β adrenergic blocker), and phentolamine (α adrenergic blocker), respectively. Apparently, the amount of release of norepinephrine from the postganglionic sympathetic nerve terminals in the pial arteries during CST stimulation seems to depend on stimulus frequency, i.e., a small amount of release with low frequency and a large amount of release with high frequency. The vasoconstriction by activating the α adrenergic receptors and the vasodilatation by activating the β adrenergic receptors may compete, although there is a more predominant vasodilatory response than the vasoconstrictive one at the initial stage of CST stimulation. Therefore, we hypothesize that (1) a small amount of norepinephrine released during CST stimulation with low frequencies activates predominantly the β adrenergic receptors and dilates small pial arteries in the cerebral cortex, because the present response in increase in cortical CBF was abolished by intravenous administration of propranolol, and also that (2) a large amount of norepinephrine released in the cortex during CST stimulation with high frequencies activates additionally the α adrenergic receptors and tends to constrict small arteries in the cortex, because the present response in decrease in cortical CBF was abolished by intravenous administration of phenoxybenzamine. We do not exclude the possibility that a small, propranolol-resistant increase in cortical CBF observed at the onset of CST stimulation is due to an autoregulatory mechanism secondary to constriction of larger basilar arteries (HARPER *et al.*, 1972; BUSHA *et al.*, 1982).

The present late vasoconstrictive response of cortical CBF (i.e., decrease in cortical CBF) observed after the end of CST stimulation seems to be produced by activation of the α adrenergic receptors of small arteries in the cortex. As phenoxybenzamine reversed this late vasoconstrictive response to the vasodilative response, the β adrenergic receptors of small arteries in the cortex seem to be activating potentially or latently also after the end of CST stimulation. Especially for a certain period after the end of CST stimulation, the α adrenergic receptor-induced vasoconstrictive response of small arteries in the cortex is apparently more dominant than the β adrenergic receptor-induced vasodilative response. It is unknown at this moment why only the vasoconstrictive response is produced after the end of CST stimulation under the control condition without administration of the α adrenergic receptor blocker.

As atropine did not influence the cortical CBF response to CST stimulation, muscarinic receptors do not seem to play any role in the neurogenic vasodilatory response. Also histamine, which dilates pial vessels via H_2 receptors (WAHL and KUSCHINSKY, 1979), does not seem to be involved in the mechanism of the vasodilatory effect of CST stimulation because the cortical CBF effect remained unchanged after H_2 receptor blocker, cimetidine, was applied. Both atropine and cimetidine are known to penetrate the blood-brain barrier. Our present results do not indicate whether neuropeptide Y (NPY) in postganglionic sympathetic nerve terminals (a powerful vasoconstrictor and peptidergic co-transmitter) (EDVINSSON

et al., 1984, 1987) might contribute to the reduction of cortical CBF.

The present result that both the vasodilatory and vasoconstrictive responses in cortical CBF to CST stimulation were slightly more pronounced during ipsilateral or bilateral stimulation than during contralateral stimulation, is in agreement with anatomical data on predominant ipsilateral innervation of the cerebral blood vessels (EDVINSSON, 1975), and on some overlaps of ipsilateral and contralateral sympathetic innervation (NIELSEN and OWMAN, 1967; PEERLESS and YASARGIL, 1971). It has been reported that bilateral sympathetic stimulation induced more pronounced and sustained vasoconstrictor effects (BUSIJA and HEISTAD, 1984; BUSIJA, 1986; SADOSHIMA *et al.*, 1987).

Typical patterns of responses of the vasodilatation and vasoconstriction in cortical CBF following the CST stimulation were similar in all frontal, parietal, and occipital lobes examined. A tendency of slightly pronounced responses in frontal and parietal lobes requires more data and further research.

The significance of sympathetic vasoconstrictors in hypertension (SADOSHIMA and HEISTAD, 1983; SADOSHIMA *et al.*, 1986; TAMAKI and HEISTAD, 1986) for shifting the low level of CBF autoregulation upwards to protect the brain against excessive CBF, high cerebral vascular pressure, and edema (HEISTAD and MARCUS, 1979; BUSIJA and HEISTAD, 1984) has been emphasized. The cerebral microvasculatures contribute to about a half of the total brain vascular resistance in the rat (HARPER *et al.*, 1972) and cat (KONTOS *et al.*, 1978) within the autoregulatory ranges of systemic arterial blood pressure. If the thresholds for the vasoconstriction of the large arteries and of the small arterioles are different, a substantial and sustained increase in the local CBF can be achieved in microcirculation with no obvious effects on the total CBF. In physiological ranges of sympathetic nerve activations on such occasions as mental excitation, emotional response, or alerting reaction, an active increase in cortical CBF due to sympathetic excitation could be of significance for providing a fast and immediate oxygen supply to the brain tissue in addition to supplementing metabolic vasodilatation. In contrast, the vasoconstriction elicited by sympathetic excitation with a higher frequency especially for a certain period after the end of sympathetic excitation could play a protective role in hypertension against an increase in small arterial pressure, brain overperfusion, and edema (HEISTAD and MARCUS, 1979; SADOSHIMA *et al.*, 1983, 1986, 1987; BUSIJA and HEISTAD, 1984).

Quantitative contribution of these two opposite sympathetic effects, vasodilatation and vasoconstriction, to the local cortical CBF may be variable in different experimental conditions. Also, in our experiments, the dispersion of quantitative data was remarkable. In some experiments a vasodilatory effect dominated and could be observed even by CST stimulation with high frequencies. The two opposite vasodilatory and vasoconstrictory sympathetic effects could possibly explain the great discrepancies among results reported by various authors using different techniques with no strict control of (1) the stimulus frequency dependence of the cortical CBF responses, and (2) the different cortical CBF

responses during and after CST stimulation.

In conclusion, we suggest that sympathetic innervation of the cortical cerebral microvasculatures may be of functional importance for the control of nutritive microcirculation in physiological ranges of arterial blood pressure. Sympathetic control may rapidly adjust local cortical blood supply in behavioral situations anticipating more delayed changes in cortical CBF that are secondarily regulated by the local metabolism.

The authors are grateful to Miss Harumi Hotta for her excellent technical assistance.

This work was entrusted by the Science and Technology Agency, using the Special Coordination Funds for Promoting Science and Technology, and also supported by a research grant from the Ministry of Health and Welfare, Japan.

REFERENCES

- AUER, L. M., JOHANSSON, B. B., and LUND, S. (1981) Reaction of pial arteries and veins to sympathetic stimulation in the cat. *Stroke*, **12**: 528-531.
- BAUMBACH, G. L. and HEISTAD, D. D. (1983) Effects of sympathetic stimulation and changes in arterial pressure on segmental resistance of cerebral vessels in rabbits and cats. *Circ. Res.*, **52**: 527-533.
- BETZ, E., INGVAR, D. H., LASSEN, N. A., and SCHMAHL, F. W. (1966) Regional blood flow in the cerebral cortex, measured simultaneously by heat and inert gas clearance. *Acta Physiol. Scand.*, **67**: 1-9.
- BUSIJA, D. W. (1985) Sustained cerebral vasoconstriction during bilateral sympathetic stimulation in anesthetized rabbits. *Brain Res.*, **345**: 341-344.
- BUSIJA, D. W. (1986) Unilateral and bilateral sympathetic effects on cerebral blood flow during normocapnia. *Am. J. Physiol.*, **250**: H498-H502.
- BUSIJA, D. W. and HEISTAD, D. D. (1984) Factors involved in the physiological regulation of the cerebral circulation. *Rev. Physiol. Biochem. Pharmacol.*, **101**: 161-211.
- BUSIJA, D. W., HEISTAD, D. D., and MARCUS, M. L. (1981) Continuous measurement of cerebral blood flow in anesthetized cats and dogs. *Am. J. Physiol.*, **241**: H228-H234.
- BUSIJA, D. W., MARCUS, M. L., and HEISTAD, D. D. (1982) Pial artery diameter and blood flow velocity during sympathetic stimulation in cats. *J. Cereb. Blood Flow Metab.*, **2**: 363-367.
- CHEN, S. T., HSU, C. Y., HOGAN, E. L., MARICQ, H., and BALENTINE, J. D. (1986) A model of focal ischemic stroke in the rat: Reproducible extensive cortical infarction. *Stroke*, **17**: 738-743.
- DORA, E. and KOVACH, A. G. B. (1981) Metabolic and vascular oscillations in the cat brain cortex. *Acta Physiol. Acad. Sci. Hung.*, **57**: 261-275.
- EDVINSSON, L. (1975) Neurogenic mechanisms in the cerebrovascular bed. Autonomic nerves, amine receptors and their effects on cerebral blood flow. *Acta Physiol. Scand.* (Suppl.), **427**: 1-35.
- EDVINSSON, L., COPELAND, J. R., EMSON, P. C., MCCULLOCH, J., and UDDMAN, R. (1987) Nerve fibers containing neuropeptide Y in the cerebrovascular bed: Immunocytochemistry, radioimmunoassay, and vasomotor effects. *J. Cereb. Blood Flow Metab.*, **7**: 45-57.
- EDVINSSON, L., EMSON, P., MCCULLOCH, J., TATEMOTO, K., and UDDMAN, R.

- (1984) Neuropeptide Y: Immunocytochemical localization to and effect upon feline pial arteries and veins *in vitro* and *in situ*. *Acta Physiol. Scand.*, **122**: 155–163.
- EDVINSSON, L., LACOMBE, P., OWMAN, Ch., REYNIER-REBUFFEL, A.-M., and SEYLAZ, J. (1979) Quantitative changes in regional cerebral blood flow of rats induced by alpha- and beta-adrenergic stimulants. *Acta Physiol. Scand.*, **107**: 289–296.
- EDVINSSON, L., OWMAN, Ch., ROSENGREN, E., and WEST, K. A. (1972) Concentration of noradrenaline in pial vessels, choroid plexus, and iris during two weeks after sympathetic ganglionectomy or decentralization. *Acta Physiol. Scand.*, **85**: 201–206.
- GILBEY, M. P., PETERSON, D. F., and COOTE, J. H. (1982) Some characteristics of sympathetic preganglionic neurones in the rat. *Brain Res.*, **241**: 43–48.
- HABERL, R. L., HEIZER, M. L., MARMAROU, A., and ELLIS, E. F. (1989) Laser-Doppler assessment of brain microcirculation: Effect of systemic alterations. *Am. J. Physiol.*, **256**: H1247–H1254.
- HARDER, D. R., ABEL, P. W., and HERMSMEYER, K. (1981) Membrane electrical mechanism of basilar artery constriction and pial artery dilation by norepinephrine. *Circ. Res.*, **49**: 1237–1242.
- HARPER, A. M., DESHMUKH, V. D., ROWAN, J. O., and JENNETT, W. B. (1972) The influence of sympathetic nervous activity on cerebral blood flow. *Arch. Neurol.*, **27**: 1–6.
- HEISTAD, D. D. and MARCUS, M. L. (1978) Evidence that neural mechanisms do not have important effects on cerebral blood flow. *Circ. Res.*, **42**: 295–302.
- HEISTAD, D. D. and MARCUS, M. L. (1979) Effect of sympathetic stimulation on permeability of the blood-brain barrier to albumin during acute hypertension in cats. *Circ. Res.*, **45**: 331–338.
- HEISTAD, D. D., MARCUS, M. L., and GROSS, P. M. (1978) Effects of sympathetic nerves on cerebral vessels in dog, cat and monkey. *Am. J. Physiol.*, **235**: H544–H552.
- IWAYAMA, T., FURNESS, J. B., and BURNSTOCK, G. (1970) Dual adrenergic and cholinergic innervation of the cerebral arteries of the rat. *Circ. Res.*, **26**: 635–646.
- KOBAYASHI, S., WALTZ, A. G., and RHOTON, A. L., Jr. (1971) Effects of stimulation of cervical sympathetic nerves on cortical blood flow and vascular reactivity. *Neurology*, **21**: 297–302.
- KONTOS, H. A., WEI, E. P., NAVARI, R. M., LEVASSEUR, J. E., ROSENBLUM, W. I., and PATTERSON, J. L., Jr. (1978) Responses of cerebral arteries and arterioles to acute hypotension and hypertension. *Am. J. Physiol.*, **234**: H371–H383.
- KUSCHINSKY, W. and WAHL, M. (1975) Alpha-receptor stimulation by endogenous and exogenous norepinephrine and blockade by phentolamine in pial arteries of cats. *Circ. Res.*, **37**: 168–174.
- LACOMBE, P., REYNIER-REBUFFEL, A.-M., MAMO, H., and SEYLAZ, J. (1977) Quantitative multiregional blood flow measurements during cervical sympathetic stimulation. *Brain Res.*, **129**: 129–140.
- NIELSEN, K. C. and OWMAN, Ch. (1967) Adrenergic innervation of pial arteries related to the circle of Willis in the cat. *Brain Res.*, **6**: 773–776.
- PEERLESS, S. J. and YASARGIL, M. G. (1971) Adrenergic innervation of the cerebral blood vessels in the rabbit. *J. Neurosurg.*, **35**: 148–154.
- PURDY, R. E. and BEVAN, J. A. (1977) Adrenergic innervation of large cerebral blood vessels of the rabbit studied by fluorescence microscopy: Absence of features that might contribute to non-uniform change in cerebral blood flow. *Stroke*, **8**: 82–87.
- RIVA, C., ROSS, B., and BENEDEK, G. B. (1972) Laser Doppler measurements of blood flow in capillary tubes and retinal arteries. *Invest. Ophthalmol.*, **2**: 936–944.

- ROSENBLUM, B. R., BONNER, R. F., and OLDFIELD, E. H. (1987) Intraoperative measurement of cortical blood flow adjacent to cerebral AVM using laser Doppler velocimetry. *J. Neurosurg.*, **66**: 396–399.
- SADOSHIMA, S., FUJII, K., KUSUDA, K., SHIOKAWA, O., YAO, H., IBAYASHI, S., and FUJISHIMA, M. (1987) Importance of bilateral sympathetic innervation on cerebral blood flow autoregulation in the thalamus. *Brain Res.*, **413**: 297–301.
- SADOSHIMA, S., FUJII, K., YAO, H., KUSUDA, K., IBAYASHI, S., and FUJISHIMA, M., (1986) Regional cerebral blood flow autoregulation in normotensive and spontaneously hypertensive rats—Effects of sympathetic denervation. *Stroke*, **17**: 981–984.
- SADOSHIMA, S. and HEISTAD, D. D. (1983) Regional cerebral blood flow during hypotension in normotensive and stroke-prone spontaneously hypertensive rats: Effect of sympathetic denervation. *Stroke*, **14**: 575–579.
- SCREMIN, O. U., ROVERE, A. A., RAYNALD, A. C., and GIARDINI, A. (1973) Cholinergic control of blood flow in the cerebral cortex of the rat. *Stroke*, **4**: 232–239.
- SERCOMBE, R., LACOMBE, P., AUBINEAU, P., MAMO, H., PINARD, E., REYNIER-REBUFFEL, A.-M., and SEYLAZ, J. (1979) Is there an active mechanism limiting the influence of the sympathetic system on the cerebral vascular bed? Evidence for vasomotor escape from sympathetic stimulation in the rabbit. *Brain Res.*, **164**: 81–102.
- SKARPHEDINSSON, J. O., HÄRDING, H., and THOREN, P. (1988) Repeated measurements of cerebral blood flow in rats. Comparisons between the hydrogen clearance method and laser Doppler flowmetry. *Acta Physiol. Scand.*, **134**: 133–142.
- STERN, M. D. (1975) *In vivo* evaluation of microcirculation by coherent light scattering. *Nature (Lond.)*, **254**: 56–58.
- TAMAKI, K. and HEISTAD, D. D. (1986) Response of cerebral arteries to sympathetic stimulation during acute hypertension. *Hypertension*, **8**: 911–917.
- TOMITA, M., GOTOH, F., TANAHASHI, N., TANAKA, K., and KOBARI, M., (1982) Ipsilateral increase in CBV by unilateral severing of the cervical sympathetic preganglionic fiber in cats. In: *Cerebral Blood Flow: Effects of nerves and neurotransmitters*, ed. by HEISTAD, D. D. and MARCUS, M. L., Elsevier North-Holland Inc., New York, Amsterdam, Oxford, pp. 403–408.
- VERN, B. A., SCHUETTE, W. H., LEHETA, B., JUEL, V. C., and RADULOVACKI, M. (1988) Low-frequency oscillations of cortical oxidative metabolism in waking and sleep. *J. Cereb. Blood Flow Metab.*, **8**: 215–226.
- WAHL, M. and KUSCHINSKY, W. (1977) Autonomic receptors as studied in pial vessels—Microapplication *in situ*. In: *Neurogenic Control of the Brain Circulation*, ed. by OWMAN, Ch. and EDVINSSON, L., Pergamon Press, Oxford, pp. 185–195.
- WAHL, M. and KUSCHINSKY, W. (1979) The dilating effect of histamine on pial arteries of cats and its mediation by H_2 receptors. *Circ. Res.*, **44**: 161–165.
- WEI, E. P., RAPER, A. J., KONTOS, H. A., and PATTERSON, J. L., Jr. (1975) Determinants of response of pial arteries to norepinephrine and sympathetic nerve stimulation. *Stroke*, **6**: 654–658.
- WILLIAMS, P. C., STERN, M. D., BOWEN, P. D., BROOKS, R. A., HAMMOCK, M. K., BOWMAN, R. L., and DiCHIRO, G. (1980) Mapping of cerebral cortical strokes in rhesus monkeys by laser Doppler spectroscopy. *Med. Res. Eng.*, **13**: 3–5.