Cerebral and Cerebellar Blood Flow Autoregulations in Acutely Induced Cerebral Ischemia in Spontaneously Hypertensive Rats — Transtentorial Remote Effect

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SUMMARY Autoregulation of cerebral (CBF) and cerebellar blood flow (CeBF) was studied before, during and after acutely induced cerebral ischemia in spontaneously hypertensive rats. Cerebral ischemia of the supratentorial portion was induced for one hour by bilateral carotid artery ligation (BCL). The animals were artificially ventilated and the blood flow was measured with a hydrogen clearance technique. To test the autoregulation, the blood pressure was stepwise lowered by bleeding and maintained at a new level, i.e. 15% or 30% lower than the baseline values before, during and after cerebral ischemia. At the pre-ischemic state, CBF and CeBF were 52.1 ± 6.2 and 58.9 ± 4.6 ml/100 g/min (mean ± SEM), of which autoregulations were normally preserved. Following BCL, CBF was markedly decreased to about 10% of control value while CeBF was minimally reduced to 46.9 ± 8.6 ml/100 g/min (80%). At the ischemic state, CBF became almost zero flow during hypotension. CeBF was also reduced to 74% and further to 58% of the resting value by 15% and 30% decrease in the blood pressure, respectively, indicating impaired CeBF autoregulation. At the 30 min post-ischemic state, CBF was recovered to 48.0 ± 4.9 and CeBF to 53.9 ± 5.4 ml/100 g/min. Autoregulation of CBF was still abolished, whereas CeBF was kept constant by 15% fall of blood pressure and slightly reduced to 84% by 30% hypotension, indicating almost recovery of CeBF autoregulation. The present results suggest that autoregulatory function of the cerebellum may be modulated to some degree by the supratentorial brain but a more likely explanation for the results in the present work is the loss of perfusion pressure in cerebellar vessels.

CEREBRAL VESSELS have a function of maintaining the blood flow relatively constant in response to a wide range of changes in blood pressure, i.e. autoregulation. A well known fact is that the autoregulatory capacity is impaired when associated with metabolic disorders of the ischemic brain. Recently, cerebral metabolism and circulation in the remote area apart from the ischemic lesion also have been found to be diminished, namely transhemispheric or transtentorial diaschisis, with the advent of the positron emission tomography. Another known fact is that during the acute stage of cerebral infarction, autoregulation is impaired, and that such dysautoregulation persists for a certain period of time, although its mechanism is still under dispute.

Bilateral carotid artery ligation (BLC) readily produces cerebral ischemia in spontaneously hypertensive rats (SHR) but hardly in normotensive rats. Induced ischemic lesion is located in the cerebral cortex of the supratentorium but not in the cerebellum during the first few hours following BCL. Therefore, by using this animal model, the autoregulation in the supratentorial portions was examined to clarify whether supratentorial ischemia influences autoregulatory capacity in the cerebellum.

Materials and Methods

Eight female SHRs, aged 5 to 7 months, were anesthetized with intraperitoneal amobarbital, 100 mg/kg body weight. Both femoral arteries were cannulated, one for continuous recording of the blood pressure and the other for anaerobic sampling of arterial blood and for exsanguination to reduce the blood pressure. Both common carotid arteries, separated carefully from the vagosympathetic trunks, were loosely encircled with sutures for later ligation. After tracheostomy, the rats were paralyzed with d-tubocurarine (0.45 mg/100 g body weight) and artificially ventilated with a Harvard respirator (HARVARD Co., MA, USA) in the room air. The hydrogen clearance technique was used to measure blood flow to the cerebral and cerebellar cortices. Details for this method have been described elsewhere. Briefly, the animal’s head was fixed in a head holder and two small burr holes were made on the right skull; one was 2 mm lateral to the bregma and the other 3 mm posterior and lateral to the confluens of the ipsilateral side. Teflon coated platinum electrodes, 200 μm in diameter, with platinum black on the tips were placed in the cerebral cortex (2 mm in depth from the surface of the brain) and in the cerebellum (2 mm in depth) by using a stereotaxic apparatus. The reference electrode was an Ag-AgCl inserted under the skin. Rectal temperature was kept close to 37°C by heat lamp. Arterial pH, pCO2 and pO2 were determined with an IL meter model 113 (Instrumentation Laboratory Inc., MA, USA).

Figure 1 shows the experimental protocol. Autoregulation of blood flow in the cerebrum and cerebellum was tested at three different stages; i.e. resting state, 30 min ischemia and 30 min recirculation following 1 hour ischemia. Stepwise reduction of the blood pressure, by 15 and 30% of the resting value, was induced by withdrawing blood and maintained at each level for
5 to 10 min during CBF measurement in each study. Immediately after testing, blood withdrawn was reinfused into the cannulated femoral artery.

The rat’s brain was macroscopically examined after termination of the experiment. When either an improper placement of the electrode or macroscopical tissue damage by inserting the electrode was found, data were excluded from the present results.

CBF and CeBF before hypotension at pre-ischemic state were compared by Student’s t-test. The statistical significance of the differences of the blood flow before and during stepwise blood pressure reduction was assessed by paired t-test. Acid-base balances and blood gases were analyzed by analysis of variance. Significance was determined by a $p$ value of less than 0.05.

**Results**

Arterial $pCO_2$ and $pO_2$ remained unchanged throughout the experiment while pH was slightly lowered during and after ischemia (table 1).

**Before Brain Ischemia**

Resting mean arterial pressure (MAP) was 187 ± 4 mm Hg (mean ± SEM). Baseline cerebral and cerebellar blood flows (CBF and CeBF) were 52.1 ± 6.2 and 58.9 ± 4.6 ml/100 g/min, respectively with its difference being not significant (table 1). When MAP was reduced to 85% (159 mm Hg) and 70% (131 mm Hg) of the resting MAP, CBF was actually unchanged, 52.4 ± 7.0 (101% of the resting flow) and 48.3 ± 5.8 ml/100 g/min (93%), respectively, indicating the preserved autoregulation. Similarly, CeBFs were also preserved well during stepwise hypotension, 58.9 ± 4.4 (100% of the resting flow) and 54.1 ± 4.2 ml/100 g/min (92%), respectively (figs. 2 and 5). The total amount of withdrawn blood was 3.6 ± 0.3 ml to reduce MAP by 30%.

**During Brain Ischemia**

At 30 min after BCL, MAP was elevated to 198 ± 7 mm Hg but CBF was reduced to 5.8 ± 2.0 ml/100 g/min (11% of the CBF before BCL, $p < 0.005$) and CeBF was also slightly reduced to 46.9 ± 8.6 ml/100 g/min. Under graded hypotension to 168 mm Hg (85% of the resting MAP) and 139 mm Hg (70%), blood flow to the cerebrum was markedly reduced to almost zero flow. CeBF was also significantly decreased to 34.5 ± 6.6 (74% of the resting CeBF, $p < 0.01$) at 168 mm Hg and 27.3 ± 5.4 ml/100 g/min (58%, $p < 0.01$) at 139 mm Hg (figs. 3 and 5), indicating that autoregulatory activity was impaired in the cerebellum during ischemia. Withdrawn blood during hypotension amounted to 1.9 ± 0.2 ml.

**After Recirculation**

At 1 hr after BCL, the occluded carotid arteries were reopened by releasing the sutures.

At 30 min following recirculation, the reduced CBF and CeBF were recovered to 48.0 ± 4.9 and 53.9 ± 5.4 ml/100 g/min, respectively. MAP was slightly lower (156 ± 6 mm Hg) than that prior to BCL (table 1). During graded hypotension to 133 and 109 mm Hg, blood flow to the cerebrum was reduced to 38.4 ± 4.7 (80% of the CBF before hypotension, $p < 0.05$) and 27.9 ± 3.7 ml/100 g/min (58%, $p < 0.01$), respectively. CeBF was kept constant (50.7 ± 6.3, 94% of the CeBF before hypotension) at 133 mm Hg, and was slightly reduced to 45.2 ± 7.0 (84%, $p < 0.05$) at 109 mm Hg (figs. 4 and 5). Total amount of withdrawn blood during hypotension was 2.3 ± 0.3 ml.

**Discussion**

Many previous studies have reported that cerebral autoregulatory function in the ischemic regions be-
Cerebellar blood flow autoregulation became severely disturbed. The failure of Na-K pump of cell membrane, tissue acidosis and brain edema are found to induce vasoparalysis and thus, impair the autoregulation of cerebral arteries. Recently, Baron et al. found metabolic depression in the cerebellar hemisphere contralateral to the supratentorial ischemic lesion using the positron emission tomography. Although they explained the phenomenon as a crossed cerebellar diaschisis, this mechanism is still under discussion. Besides metabolic disorders, the autoregulatory function in the non-ischemic area apart from the ischemic lesion has only recently received attention.

The major findings in the present study were as follows: first, the autoregulation of cerebral cortical blood flow was severely impaired during BCL-induced cerebral ischemia and such dysautoregulation persisted for a while even after restoration of the blood flow to the ischemic lesion; and secondly, cerebellar autoregulatory function was also impaired but less markedly during cerebral ischemia, and its function was almost recovered after restored flow to the cerebrum. These results indicated that acute cerebral ischemia exerts undesirable effects on the blood flow regulation in the non-ischemic area apart from the ischemic lesion.

Although the mechanism as to why supratentorial ischemia leads to impaired infratentorial autoregulation was uncertain from this present study, we considered several possibilities to explain our findings. First, the increased intracranial pressure secondary to ischemic edema of the brain might have reduced the perfu-
sion pressure to infratentorial tissue resulting in cerebellar ischemia. In our study, this was not the case because blood flow to the cerebellar cortex was 46.9 ml/100 g/min which was sufficiently higher than critical level of blood flow with induction of ischemic damage. Our model found that one hour of ischemia was an inadequate length of time to develop brain edema. Also, our previous histological or biochemical studies in our model did not show any ischemic changes in the infratentorial tissue.

Thus, the present results could not be explained by ischemic metabolic disorder in the cerebellum. Secondly, exsanguination to lower MAP may have reduced the cardiac output and the perfusion to capillary or precapillary beds. A known fact is that CBF remains unchanged even when 20 ml/kg of blood is withdrawn or the cardiac output is decreased by about 25%. The amount of blood withdrawn to reduce the blood pressure by 30% during ischemic period in this study averaged 1.9 ml/rat or approximately 10 ml/kg of body weight. Thus, cerebral blood flow reduction due to blood loss seems unlikely. Thirdly, in recent years, cerebral circulation and metabolism have been considered to be modulated in part by a neurogenic mechanism, e.g. activation of sympathetic and parasympathetic nerves or other neurotransmitters. Cerebral arteries are evidenced to be innervated by rather dense sympathetic nerve fibers. Intensive investigations have focused on the effect of that innervation on the responsiveness of cerebral vessels to the changes in intraluminal pressure. Although α-receptors in cerebral vessels are less sensitive to their agonist than those in other vessels, cervical sympathetic stimulation has been found to constrict cerebral vessels to some extent. Evidence shows that enhanced nonspecific discharge of norepinephrine occurs in response to any kinds of "stress." Myers et al reported 1.54 times increase in plasma epinephrine in acute cerebral ischemia. Robinson et al and Kajihara et al also suggested that an excessive amount of catecholamines are released in non-ischemic as well as in ischemic brain in cerebrovascular accidents. If this is relevant to our model, the adrenergic nervous system in the cerebellum was activated by cerebral ischemia and thus, dilation of cerebellary vessels was inhibited in response to reduction of intraluminal pressure, leading to a decrease in CeBF during hemorrhagic hypotension. Fourthly, MAP during hypotension was maintained much higher than the lower limit of autoregulation in SHR (110–120 mm Hg). Therefore, it is more likely that perfusion pressure to the cerebellum was decreased during BCL, resulting in autoregulatory vasodilatation which was insufficient to totally compensate for the pressure fall. BCL reduces markedly the blood pressure in the circle of Willis and the blood flow in the vertebral and basilar artery will increase in an attempt to maintain a residual perfusion of the supratentorial tissue via the posterior cerebral and posterior communicating arteries. Thus, cerebellar perfusion pressure will be considerably lowered, and autoregulatory capacity may already be used up in response to carotid ligation, although the perfusion pressure in the cerebellum was not estimated in the present study. As in this study, a possible consideration is that when an additional fall in blood pressure is induced by bleeding, concomitant fall in CeBF can be demonstrated.

When MAP was reduced from 156 mm Hg to 133 mm Hg after recirculation, reduction of CeBF was insignificant (6% of the resting value) in contrast to 20% reduction in cerebrum (p < 0.05 vs. cerebellum). This finding suggests that autoregulatory activity in the cerebellum was quickly recovered after the recirculation of the blood flow to ischemic brain. Also, this supports our hypothesis that the impaired responsiveness of blood vessels in cerebellum is transient and reversible, and is probably induced by a functional mechanism as previously mentioned.

In conclusion, autoregulation of the cerebellum may be modulated to some degree by supratentorial brain but a more likely explanation for the results in the present work is simply the loss of perfusion pressure in cerebellary vessels.
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Behavioral Performance of Rats Following Neonatal Hypoxia-Ischemia

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SUMMARY The behavioral performance of rats subjected in the neonatal period to hypoxia-ischemia at either 37°C or 21°C was compared to that of sham-ligated animals. Performance on complex motor tests was significantly delayed only in the hypoxic-ischemic 37°C rats. However, cognitive testing disclosed significant delay of spatial learning in animals subjected to hypoxia-ischemia at 21°C and those with gross infarction at 37°C. There was enhanced avoidance learning in the animals with gross infarction in the hypoxia-ischemia 37°C group. Hypoxic-ischemic damage in the neonatal rat at 37°C results in transient delay of complex motor skills, but longer lasting cognitive changes. Hypoxia-ischemia during hypothermia produces no motor deficits, although there may be similar alterations in learning.

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THE LEVINE METHOD (unilateral carotid artery ligation and hypoxia) has been utilized by a number of investigators to study the histologic, metabolic, and neurotransmitter alterations following hypoxic-ischemic injury in the neonatal rat. However, the behavioral consequences of neonatal hypoxia-ischemia induced by the Levine method have not been described.

The behavioral consequences of perinatal hypoxic-ischemic injury are of great interest since there is no consensus regarding the degree of functional recovery following unihemispheric brain injury in the neonatal period in either the human or experimental animal. In addition, there is considerable debate regarding cognitive abilities following hypoxia-ischemia during conditions of hypothermia. Our purpose was to study the motor development and cognitive performance of rats subjected to the Levine procedure as neonates during conditions of normothermia and hypothermia.

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Methods

Seven day old Sprague-Dawley rats of both sexes (Charles River Laboratories) were anesthetized with halothane (1.5–3.5%) in oxygen by mask inhalation as previously described. The right common carotid artery was exposed and ligated. The animals were returned to their dams for a 3 hour recovery period. Following the recovery period, the animals were placed in air-tight 500 ml jars with continuous flow of humidified gas (8% O2, 92% N2) for 4 hours. During the hypoxic exposure, hypothermia was induced (or normothermia maintained) by immersing the jars in a water bath thermostatically regulated to maintain a temperature of 37°C or 21°C. Following the hypoxic exposure, animals were returned to their dams.

Control animals were similarly anesthetized; their carotid arteries were visualized, but not ligated. The control animals were then subjected to the same experimental protocol as the experimental animals, except that they were placed in jars containing room air, rather than the hypoxic gas mixture.

Behavioral Testing

Animals were examined daily for appearance of six motor milestones according to a standardized neurologic developmental battery for rats. The specific skills of head lifting, walking, righting, cliff avoid-
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