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The Measurement of Brainstem Blood Flow in the Experimental Recirculation Model of Brainstem Ischemia in the Rat

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Abstract This report describes a surgical approach and measurement of brainstem blood flow (BBF) used in experimental ischemic model of the brainstem. The vertebral artery of Wistar rat was exposed and prepared embolic thread was inserted into the vertebral artery. The lesions of the basilar artery occluded animals were restricted to the brainstem which, in the rat, is normally supplied by the vertebrobasilar artery. Using the hydrogen clearance techniques, blood flow of the brainstem was measured in 21 rats. The mean value of mean blood pressure (MBP) was 96.2 ± 6.2 mmHg before ischemia. It dropped to 62.0 ± 12.4 mmHg after transient hypertension. The preischemic BBF was 35.5 ± 2.4 ml/100g/min. It showed a significant decrease to 4.3 ± 2.2 ml/100g/min immediately after ischemia. This was followed by a gradual increase to hyperperfusion after recirculation. After 1 hour of recirculation, BBF rose to a level above 78.9 ± 5.9 ml/100g/min. These findings suggest that this model may be useful in the evaluation of pathophysiological changes in brainstem ischemia.

Key Words: Brainstem, Ischemia, Recirculation, Hyperperfusion Vasomotor center

Introduction

Recently, experimental permanent and transient ischemic model have been developed using various laboratory animals. Rats in particular have many advantages: a) They are relatively inexpensive when a large number of experiments is necessary. b) The employment of small brain ischemic models has become important as a result of the development of quantitative autoradiographic techniques for investigating functions of the cerebrovascular system. Though there have been many supratentrial ischemic models using a variety of techniques, there have been few concerning the posterior fossa. This report describes a surgical approach and measurement of brainstem blood flow used in the experimental recirculation model of the brainstem ischemia in the rat.

Materials and Method

1) First, a cast phenolic resin model of the vascular structure of the rat's brain was made. Using this model, the diameter and the length of the main artery were mesured (Fig.1). The diameter of the basilar artery was found to be approximately 0.3 mm long and the distance between the C2 portion and the basilar top was 18 mm.
2) A 0.3 mm diameter embolus was then made by coating a 6-0 monofilament nylon thread with silicon rubber latex (Fig.2).
3) Adult male Wistar rats (300-350g) were

anesthetized with pentobarbital sodium (40mg/kg, i.p.). Polyethylene catheters were then introduced into the femoral artery and vein to allow continuous monitoring of systemic blood pressure, infusion of the drug, and repeated sampling of arterial blood as appropriate. The animals were maintained in a normothermic state (approximately 36 ± 1.0 ℃) by means of a heat lamp system coupled to a rectal thermister probe. They were placed in the supine position and a cervical skin incision made along the midline. Tracheostomy was perfomed and subsequent steps were carried out using microsurgical techniques. The skin and cervical muscles were retracted and the left cervical vertebrae (C2 portion) exposed. The transverse process of C2 was carefully removed and the vertebral artery exposed for 3-4 mm. The distal portion of this artery was clipped with a Zen clip* and the proximal portion ligated and severed. Prepared embolic thread 18 mm long in length was inserted into the vertebral artery (Fig.3). Ventilation by a Harvard respirator (South Natick, Massachusetts) was then given using room air. Normocapnia was achieved by controlled respiration (PO_2: 75-120mmHg, PCO_2: 35-45mmHg, BE±5). Additionally, the rats were paralyzed with 0.45 mg/100g b.w.d-tubocurarine. The following experimental procedures were then performed: (a) In one group (10 rats), carbon black perfusion was carried out after 2 hours of ischemia.

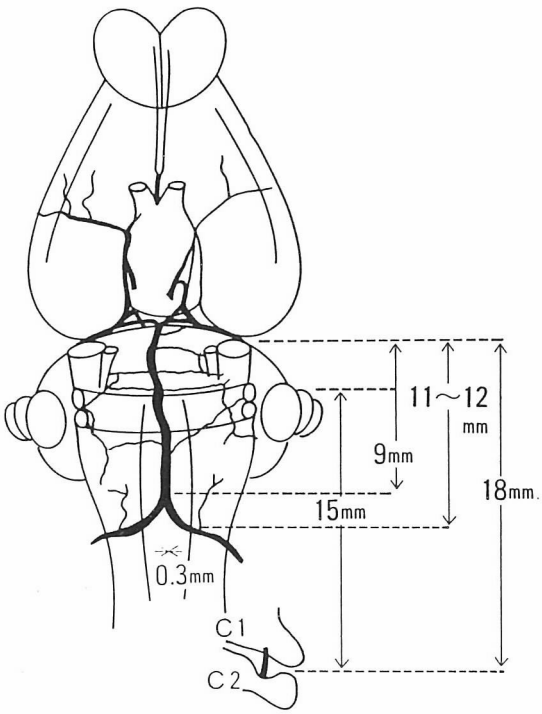

Fig.1 : Diagrammatic representation of vertebro-basilar system shows the diameter and the length of the main artery.

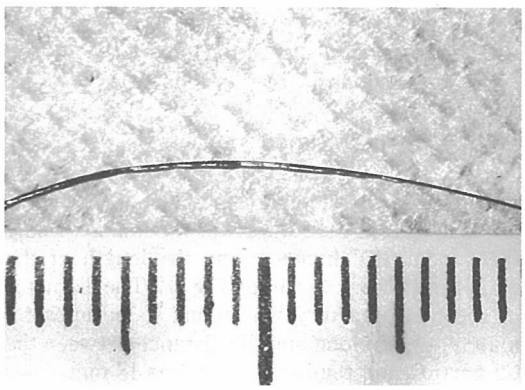

Fig.2 : Embolic nylon thread with silicon rubber latex.

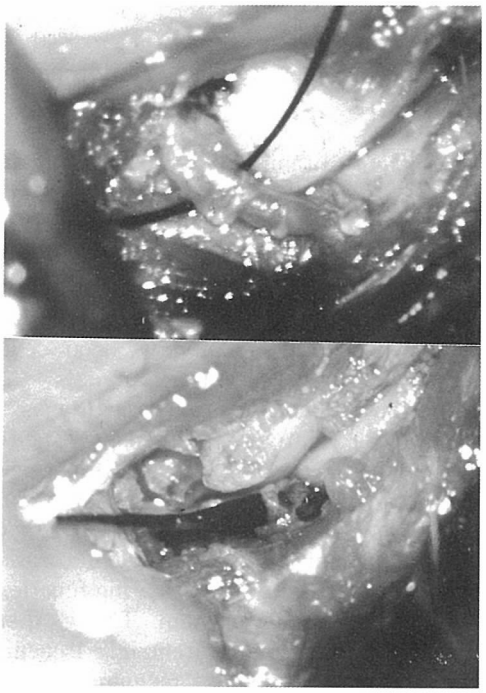

Fig.3 : upper : Exposed vertebral artery at the C1-C2 level.
lower : Inserted embolic thread into the vertebral artery at the same level.

(b) In another group (10 rats), circulation in the brain stem was restored by removing the embolic thread after 6 hours of ischemia, and Evans blue dye was then injected intravenously. Tissue fixation was after 2 hours of recirculation initiated by perfusion of formalin into the thoracic aorta. (c) In the sham-operated group (5 rats), left exposure of the vertebral artery were perfomed, and perfusion fixation was initiated 8 hours later.

4) Measurement of brainstem blood flow (BBF) by hydrogen clearance.

The hydrogen clearance technique was used to measure BBF in 21 rats. The rat's head was fixed in the supine position with a head holder and one burr hole was in the clivus. Teflon -coated platinum electrodes 200 μm in diameter were placed in the brain stem 2 mm superior and lateral to the basion, and 2 mm in depth from the surface, using a stereo-taxic apparatus (Fig.4). An Ag-AgCl referrence electrode was then inserted under the skin. Significance of the diffderences between blood flow before and after ischemia was assessed using the paired test. Gross examination of brain in all animals was carried out after tissue fixation. When either improper placement of the electrode or macroscopic damage of the tissue was found, the data were excluded.

Results

There were no areas of ischemia in the brain of any of the sham-operated animals. By contrast, in most of the basilar artery occluded animals, there was pallor of the brainstem indicated by the lack of carbon black perfusion (Fig.5). The recirculation group showed blue from the Evans blue staining (Fig.6). These lesions were similar in extent in both groups and were restricted. to the brainstem which, in the rat, is normally supplied by the vertebrobasilar artery. These change were detected in the brainstem of 18 out of 20 rats and were apparent in both sagittal and cornal sections (Fig.7). Both groups showed wedge shaped patterns in the pons and medulla, predominantly in the ipsilateral side. Using the hydrogen clearance techniques, BBF was measured in 21 rats. In 4 cases, however, the reduction of blood pressure and blood flow were too rapid and severe to allow measurement. Fig.8 summarizes the results in the other 17 cases, showing the mean value of mean blood pressure (MBP) and brainstem blood flow (BBF). The mean value of MBP was 96.2 $\pm$ 6.2 mmHg before ischemia. It dropped to 62.0 $\pm$ 12.4 mmHg after transient hypertension,

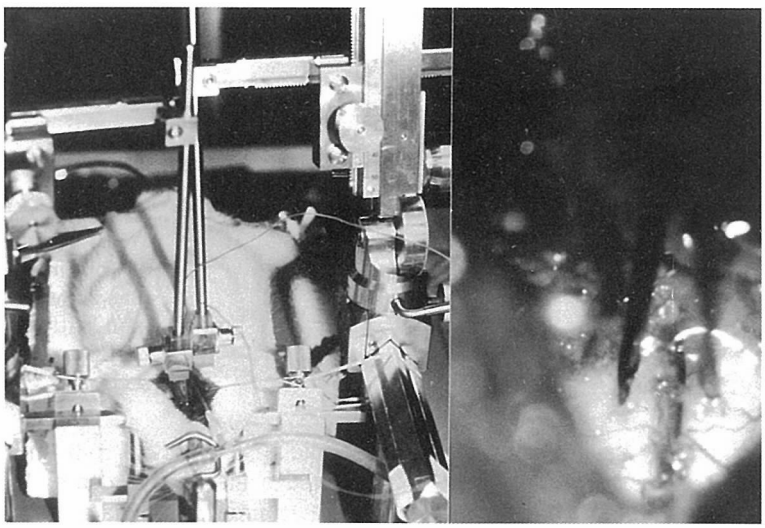

Fig.4 : left : A stereotaxic apparatus for measurement of CBF by hydrogen clearance technique.
right : Teflon-coated platinum electrodes placed in the brain stem.

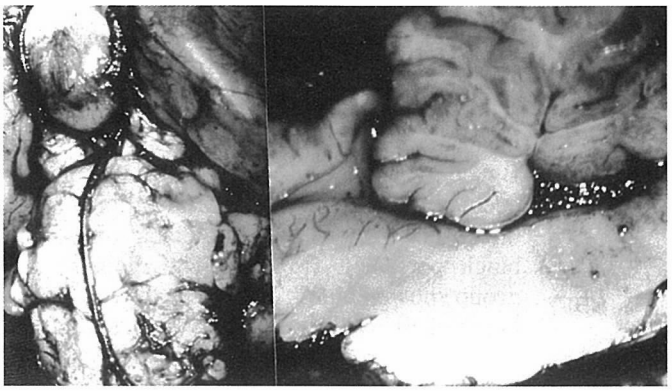

Fig.5 : The non-perfused area in the brain stem revealed by carbon black perfusion.
The embolic thread which occludes the basilar artery can be seen in the AP view.

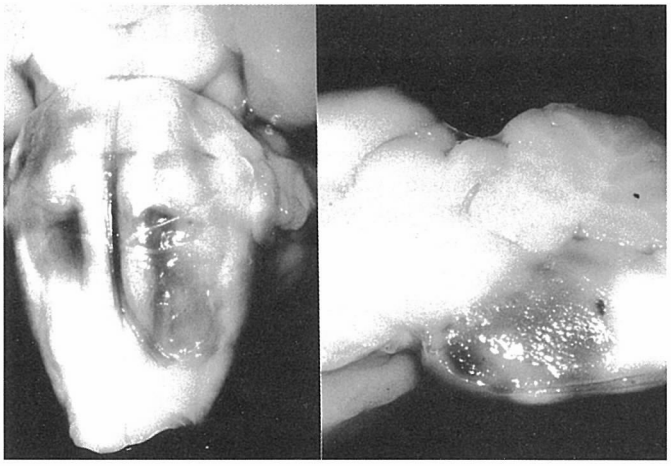

Fig.6 : Ischemic lesion in the brainstem stained by Evans blue.

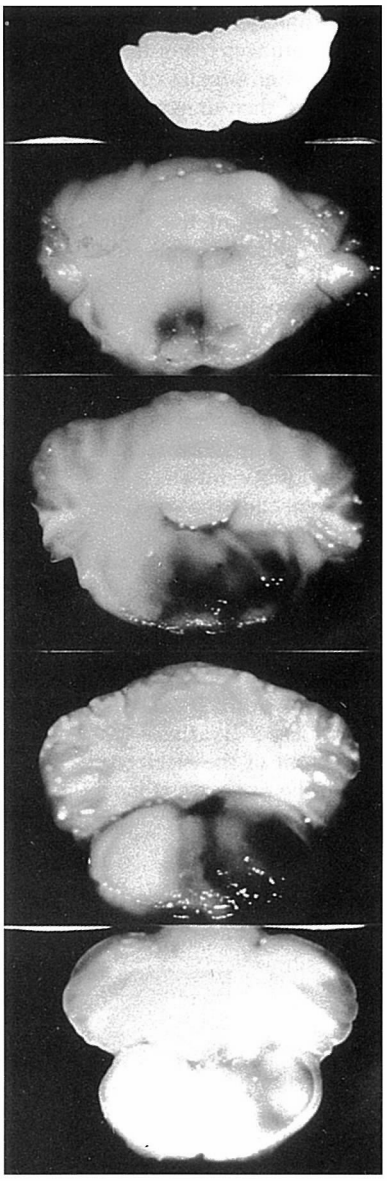

Fig.7 : Wedge shaped lesion in the brainstem stained by Evans blue showing the most common pattern in the basilar occlusion model (coronal section).

which was signigicantly lower than the basal value ($p<0.001$). After recirculation, MBP increased gradually to almost the control level in one hour (Fig.8). The change in BBF was, in general, similar to the pattern of MBP except for hyperperfusion. The preischemic BBF was 35.5 ± 2.4 ml/100g/min. It showed a significant decrease to 4.3 ± 2.2 ml/100g/min immediately after ischemia ($p<0.001$). This was followed by a gradual increase to hyperperfusion after recirculation. After 1 hour of recirculation, BBF rose

Brainstem blood flow and Mean blood pressure before and after brainstem ischemia

	control	10 min after ischemia	30 min	60 min	120 min	10 min after recirculation	60 min	120 min
Brainstem blood flow (ml/100g/min±S.E.)	35.5±2.4		4.3±2.2*	7.6± 2.5	7.5± 2.1	42.1± 6.9	78.9±5.9*	60.8±4.3
Mean blood pressure (mmllg ±S.E.)	96.2±6.2	155.0±13.2	62.0±12.4*	67.0±10.7	68.2±12.8	74.0±11.6	98.5±9.3	97.2±8.3

* Denotes significant difference from control with $p<0.001$

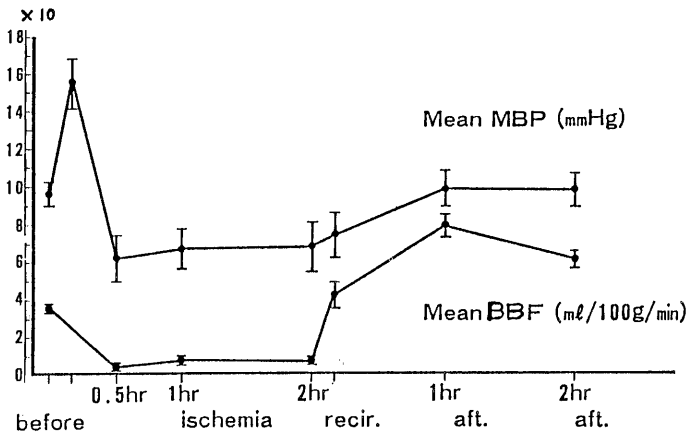

Fig.8 : Comparison of the change of brain stem blood flow and blood pressure.

to a level above 78.9 ± 5.9ml/100g/min; significantly higher than the basal value ($p<0.001$).

Discussion

There are a considerable reports of literature concerning experimental ischemic stroke[1,2] covering a variety of animal models. The Mongolian gerbil has been widely used in studies or regional cerebral ischemia because it has a functionally incomplete Willis circle. However, an ischemic model in the posterior fossa is rare. Yamada[3] reported a new model of transient hindbrain ischemia in gerbils. In rats, too, there have been various regional ischemic models reported by several authors. Tamura[4] and Colye[5] described a surgical approach to middle cerebral artery occlusion. Fujishima[6] has developed a basilar artery permanent occlusion model in the rat.

The objectives for this study were:

1) Introduction of a feasible surgical approach for occlusion and recirculation of the basilar artery as a standardized method; 2) Evaluation of size and location of lesions in the basilar artery occlusion model; and 3) Measurement of BBF changes in the recirculation model of brainstem ischemia. In most of the models, results showed reproducible wedge-shaped ischmic lesions based on the ventral surface of the the brainstem. This suggests that the main damage was caused by the occlusion of the median and paramedian perforating arteries[7] in the ver-

tebrobasilar artery. The data on systemic circulatory changes suggests that rapid decreases of blood pressure after transient hypertension may be caused by ischemic damage of the vasomotor center in the medulla oblongata. Montgomery[8] described in a clinical report that localized interference with arterial blood flow in the medulla may cause a hypertensive response. Experiments by Foster[9] and Cushing[10] emphasize the activity of the medullary vasomotor centers and indicate the participation of ischemia in the production of neurogenic hypertension. Recent experimental models[3,8,11] of brainstem ischemia generally show no remarkable blood pressure changes. However, our data indicated that severe ischemic damage in the lower brainstem can induce blood pressure reduction. In addition, Katuki[12] demonstrated that sectioning of the medulla caused an immediate drop in blood pressure. The hydrogen clearance method of flow assessment has the advantage of recording flow repeatedly at short intervals and is effective for observation of alterations in blood flow. Hyperperfusion describes the state when local perfusion is raised. In this model, it was generally observed within 1 hour after recirculation and lasted for approximately 1 hour. In the MCA occlusion model, it is likely that such hyperperfusion is a common reaction to ischemic recirculation[13]. During hyperperfusion, BBF increased by 78.9 ± 5.9 ml/100g/min and was significantly above the basal values ($p<0.001$). That hyperperfusion was not observed in all cases might be due to the degree of the damage in the medulla oblongata. Hypoperfusion or no reflow phenomena were also followed by severe ischemia with remarkable hypotension (4 cases). It is fairly clear from our results that the pattern of postischemic hyperperfusion is responsible for decreased oxgen availability in the brainstem and dysfunction of the autoregulation. These findings suggest that this model may be useful in the evaluation of pathophysiological changes in brainstem ischmia.

*Zen clip manufactured by Ohwa Tsusho Co., Ltd., Tokyo, Japan.

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