A new model of transient hindbrain ischemia in gerbils

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A model of transient hindbrain ischemia in Mongolian gerbils is described. The vertebrobasilar junction of gerbils was exposed by a transcervical approach through the space between the atlas and occipital bone. The origin of the basilar artery was occluded by a clip, and the local cerebral blood flow (CBF) was measured with carbon-14-iodoantipyrine autoradiography. This gerbil model produces ischemia in the thalamus, midbrain, pons, medulla, and cerebellum, where blood flow is supplied from the vertebrobasilar system. Recirculation of blood flow was easily accomplished by removing the clip. Local CBF returned to normal levels immediately after recirculation, then decreased at 30 minutes after recirculation (postischemic hypoperfusion). Almost no effects of local CBF in the forebrain structures were noted during and after hindbrain ischemia. The model may be useful to study the pathophysiological, metabolic, and histopathological effects of ischemia in the vertebrobasilar system.

KEY WORDS • vertebrobasilar artery • hindbrain ischemia • cerebral blood flow • 14C-iodoantipyrine autoradiography • gerbil model • experimental ischemia

In the past few years, several experimental models of cerebral ischemia in small animals have been established for morphological, biochemical, and physiological studies. However, most of these models are of forebrain ischemia, and no purely hindbrain ischemia model has yet been developed.

In the present report, we describe a model of transient hindbrain ischemia in Mongolian gerbils. The model produces ischemia in the thalamus, midbrain, pons, and cerebellum exclusively. The distribution and degree of ischemia developed in this model is presented.

Materials and Methods

Operative Procedure

Twenty-one Mongolian gerbils, each weighing 70 to 80 gm, were subjected to experiment. They were starved for 3 to 4 hours before the procedure was begun. Animals were lightly anesthetized with ketamine hydrochloride (30 mg/kg), and placed in the supine position with their extremities restrained by rubber bands. The right femoral artery and right jugular vein were catheterized with Silastic tubes for blood pressure monitoring, blood sampling, and administration of radioactive tracers.

Under the operating microscope, a small midline incision was placed in the neck. The trachea was exposed and incised for tracheostomy. The tracheal edge was attached to the lower corner of the skin incision with a 4-0 nylon suture. The esophagus was then cauterized and cut. Both sides of the incision were retracted and fixed to the skin with stay sutures. The longus colli muscles were dissected, and the C-1 vertebral body and the lower edge of the occipital bone were exposed.

The operating microscope was then adjusted for high-power magnification, and the space between the occipital bone and atlas was dissected with fine microsurgical forceps and bipolar cautery. The dura was opened, and the junction of the vertebral and basilar arteries was exposed (Fig. 1 left). The arachnoid around the vertebrobasilar junction was dissected and a Zen clip* was applied to the vertebrobasilar junction (Fig. 1 right). Oxycel was applied around the clip to close the dural opening and to prevent leakage of cerebrospinal fluid.

In the group of experimental animals with blood flow restoration, the clip was carefully removed under the operating microscope at high-power magnification, and the dural opening was closed with Oxycel. In the control group, with sham operation, the arachnoid was dissected and a clip was placed on each side of the basilar artery. However, the basilar artery was not occluded and the clip was removed. Oxycel was applied to the dural opening.

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

### TABLE 1
**Physiological data in experimental group**

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Gerbils</th>
<th>pH</th>
<th>PaO2 (mm Hg)</th>
<th>PaCO2 (mm Hg)</th>
<th>MABP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>preocclusion</td>
<td>9</td>
<td>7.402 ± 0.040</td>
<td>112.9 ± 8.5</td>
<td>38.9 ± 2.3</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>during occlusion</td>
<td>9</td>
<td>7.395 ± 0.037</td>
<td>106.0 ± 3.8</td>
<td>40.1 ± 1.7</td>
<td>89 ± 5</td>
</tr>
<tr>
<td>after recirculation</td>
<td>6</td>
<td>7.395 ± 0.043</td>
<td>115 ± 11.3</td>
<td>39.5 ± 2.4</td>
<td>91 ± 6</td>
</tr>
</tbody>
</table>

* Each value represents mean ± standard error of the mean. No statistically significant changes (t-test) were detected in the physiological parameters in the preocclusion, intraocclusion, and postrecirculation periods.

#### Measurement of Local CBF

In 12 animals, local cerebral blood flow (CBF) was measured by the carbon-14 (14C)-iodoantipyrine autoradiographic technique. Details of the method are described elsewhere. Briefly, 100 μCi/kg of 14C-iodoantipyrine+ dissolved in 0.5 ml of normal saline was injected at a constant rate for 30 seconds through the catheter in the jugular vein. Arterial sampling was done every 5 seconds, and animals were sacrificed 30 seconds after the injection was begun. Brains were removed and frozen with Freon gas. Arterial samples of 15 μl were placed into counting vials and suspended in 5 ml of scintillation phosphor. They were radioassayed with a liquid scintillation counter. Serial brain sections 60 μm thick were cut on a cryostat. Sections were dried on a hot plate and placed on thick paper. They were attached to an SB-5 Kodak x-ray film, with the brain sections facing the emulsion, and placed in the darkroom. The 14C-methyl methacrylate standard plates, which were precalibrated for their autoradiographic equivalence to the brain sections, were also attached to the x-ray film. They were exposed for 1 week, and the x-ray film was developed. Densitometric measurement was made with a densitometer.

Calculation of local CBF was made according to the equation developed by Kety. The partition coefficient of 14C-iodoantipyrine was estimated to be 0.8 as described by Sakurada, et al. A desk-top computer was used for calculation.

#### Microcarbon Perfusion

Six gerbils were perfused with microcarbon 2 hours after occlusion of the vertebrobasilar junction to delineate the area of ischemia. Three sham-operated animals also underwent perfusion as a control. In those animals, a Silastic catheter was placed in the ascending aorta through the left ventricle, and the right atrium was opened for drainage of blood. Normal saline, 30 ml, was infused, followed by 20 ml of India ink.

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† Carbon-14-iodoantipyrine supplied by New England Nuclear Corp., 549 Albany Street, Boston, Massachusetts.
‡ Liquid scintillation counter, Mark III, manufactured by Tracor Analytic, Inc., 1842 Brummel Drive, Elk Grove Village, Illinois.
§ Sakura PDA-15 densitometer manufactured by Konishiroku, Tokyo, Japan.

#### Results

**Clinical Observations**

The majority of the animals (83%) that underwent occlusion of the basilar artery showed irregular respiration immediately after occlusion, but regained normal breathing within 1 minute. Neither mean arterial blood pressure nor arterial blood gas values changed significantly during ischemia or the postischemic recirculation period (Table 1). The animals were hypoactive and quadriplegic during ischemia despite light anesthesia. Convulsions were observed in the limbs in 15% of the animals.

**Local CBF Measurements**

Local CBF values of 15 anatomically discrete regions in ischemic animals and sham-controls are listed in Table 2. Occlusion of the proximal basilar artery produced considerable reduction of local CBF in the hindbrain structures. Local CBF values of the hindbrain structures at 30 minutes after occlusion were significantly lower than in sham-operated controls. Maximum reduction of local CBF was noted in the vestibular nucleus, where mean local CBF at 30 minutes after occlusion was 39% of values in the sham-operated controls. In the forebrain structures, significant local CBF changes were noted only in the sensorimotor cortex and thalamus. Local CBF after 30 minutes of ischemia and 5 minutes of recirculation returned to control values. However, postischemic hyperemia was not found in any part of the brain structures. Local
CBF decreased again at 30 minutes after recirculation. No significant changes in local CBF were noted in the forebrain structures during the recirculation period. Representative autoradiographs are shown in Fig. 2.

**Microcarbon Perfusion**

In the sham-operated animals, microcarbon perfusion was adequate throughout all brain structures. On the other hand, animals with occlusion of the vertebrobasilar junction showed ineffective perfusion in the tegmentum of the midbrain, upper part of the pons, and cerebellum (Fig. 3). Incomplete perfusion was also noted in part of the thalamus and tectum (inferior colliculus) of the midbrain.

**Discussion**

Although many experimental models have been used for studying cerebral ischemia in the carotid system, only a few models for vertebrobasilar ischemia have been reported. In the mongrel dog, embolization of the basilar artery with a specially prepared silicone cylinder produced ischemia in the midbrain and pons. However, the cerebellum was spared because of abundant collateral channels. In rodents, no models of vertebrobasilar ischemia have yet been developed. Since in the Mongolian gerbil the circle of Willis is functionally incomplete, unilateral carotid ligation may produce symptomatic cerebral ischemia in 30% to 40% of animals. However, the cerebellum was spared because of abundant collateral channels. In rodents, no models of vertebrobasilar ischemia have yet been developed. Since in the Mongolian gerbil the circle of Willis is functionally incomplete, unilateral carotid ligation may produce symptomatic cerebral ischemia in 30% to 40% of animals. This is also useful in producing ischemia in the region of the posterior communicating artery. In the present study, we took advantage of this anatomical phenomenon to produce ischemia in the vertebrobasilar system. Since the basilar artery of Mongolian gerbils terminates as two superior cerebellar arteries, and no effective anastomosis between carotid and vertebrobasilar systems is present, occlusion of the basilar artery produces ischemia exclusively in the midbrain, pons, cerebellum, and a part of the thalamus and medulla oblongata. In unpublished studies, we found that similar occlusion produced no ischemia in Wistar rats, indicating that this approach is only useful in Mongolian gerbils. Although tracheostomy and general anesthesia are needed in this model and long-term follow-up studies may not be possible, the model might be useful in the investigation of the pathophysiology of ischemia in the posterior fossa.

We used 14C-iodoantipyrine autoradiography for CBF measurement because of the ability of this technique to identify regional changes in blood flow. The local CBF values in Mongolian gerbils measured by the present method compare favorably with values measured by the indicator-fractionation technique or the microsphere method. Local CBF values of the brain stem measured by the 14C-butanol indicator-fractionation method and selenium-85 microsphere method were 114 ml/100 gm/min and 113 ml/100 gm/min, respectively. The data obtained by those methods have considerable similarity to our present findings (Table 2). However, local heterogeneity in blood flow values was present in the hindbrain structures (Table 2), and this could be better observed by the autoradiographic method (Fig. 2).

In animals with proximal basilar artery occlusion, local CBF decreased significantly throughout the hindbrain structures. Reduction of blood flow was especially evident in the inferior colliculus, pontine gray matter, vestibular nucleus, and cerebellar cortex, where blood flow at 30 minutes after occlusion was 50% or less of the sham-operated control values. These flows were low enough to produce ischemic cerebral damage in Mongolian gerbils, since the critical level of CBF required to damage the gerbil brain is reported to be 50% of

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**TABLE 2**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Sham Operation</th>
<th>30-Min Occlusion</th>
<th>30-Min Occlusion &amp; 5-Min Reflow</th>
<th>30-Min Occlusion &amp; 30-Min Reflow</th>
</tr>
</thead>
<tbody>
<tr>
<td>hindbrain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>peraqueductal gray matter</td>
<td>111 ± 12</td>
<td>66 ± 13†</td>
<td>99 ± 6†</td>
<td>70 ± 7†</td>
</tr>
<tr>
<td>superior colliculus</td>
<td>84 ± 7</td>
<td>51 ± 8†</td>
<td>76 ± 7†</td>
<td>57 ± 12†</td>
</tr>
<tr>
<td>inferior colliculus</td>
<td>160 ± 14</td>
<td>73 ± 18†</td>
<td>118 ± 15†</td>
<td>97 ± 10†</td>
</tr>
<tr>
<td>pontine gray matter</td>
<td>103 ± 15</td>
<td>55 ± 8†</td>
<td>87 ± 6†</td>
<td>67 ± 14†</td>
</tr>
<tr>
<td>vestibular nucleus</td>
<td>143 ± 13</td>
<td>56 ± 13†</td>
<td>105 ± 15†</td>
<td>84 ± 12†</td>
</tr>
<tr>
<td>medullary gray matter</td>
<td>91 ± 16</td>
<td>70 ± 13†</td>
<td>85 ± 10</td>
<td>71 ± 16‡</td>
</tr>
<tr>
<td>cerebellar cortex</td>
<td>88 ± 15</td>
<td>46 ± 11†</td>
<td>78 ± 14</td>
<td>55 ± 12†</td>
</tr>
<tr>
<td>cerebellar white matter</td>
<td>42 ± 6</td>
<td>27 ± 8†</td>
<td>49 ± 7†</td>
<td>26 ± 6†</td>
</tr>
<tr>
<td>forebrain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>frontal cortex</td>
<td>74 ± 8</td>
<td>71 ± 11</td>
<td>82 ± 8</td>
<td>68 ± 5</td>
</tr>
<tr>
<td>sensorimotor cortex</td>
<td>101 ± 9</td>
<td>90 ± 10†</td>
<td>93 ± 9</td>
<td>103 ± 7</td>
</tr>
<tr>
<td>parietal cortex</td>
<td>91 ± 7</td>
<td>94 ± 7</td>
<td>82 ± 10</td>
<td>96 ± 8</td>
</tr>
<tr>
<td>auditory cortex</td>
<td>70 ± 4</td>
<td>70 ± 10</td>
<td>73 ± 4</td>
<td>76 ± 10</td>
</tr>
<tr>
<td>visual cortex</td>
<td>80 ± 9</td>
<td>80 ± 8</td>
<td>69 ± 12</td>
<td>83 ± 11</td>
</tr>
<tr>
<td>caudate-putamen</td>
<td>74 ± 4</td>
<td>76 ± 9</td>
<td>78 ± 5</td>
<td>68 ± 8</td>
</tr>
<tr>
<td>thalamus</td>
<td>105 ± 8</td>
<td>80 ± 8</td>
<td>100 ± 7</td>
<td>78 ± 13†</td>
</tr>
</tbody>
</table>

* Local cerebral blood flow (CBF) in ml/100 gm/min (mean ± standard deviation of six observations in three animals for each group). Statistical significance by t-test; different from sham operation at †P < 0.01, ‡P < 0.05.
Experimental hindbrain ischemia

normal control values. On the other hand, local CBF changes in the forebrain structures were minimal (Table 2). Since the thalamus has a direct blood supply from the basilar artery, it is reasonable to expect a decrease in blood flow during occlusion. No forebrain structures other than the sensorimotor cortex showed a statistically significant difference in blood flow during hindbrain ischemia. This means that there may be no remote effect on blood flow during hindbrain ischemia.

Recirculation of blood was easily accomplished by removing the occluding clip. In the present model, no area of postischemic hyperemia was evident in the hindbrain structures. Instead, delayed postischemic hypoperfusion was evident in the hindbrain structures and thalamus at 30 minutes after recirculation. Levy, et al., described similar postischemic hypoperfusion following unilateral carotid occlusion in Mongolian gerbils, and discussed several possible mechanisms. No matter what the cause may be, hypoperfusion after recirculation is a good indication that hindbrain structures have indeed

FIG. 2. 14C-iodoantipyrine autoradiographs at two levels (upper and lower) in gerbils subjected to occlusion of the vertebrobasilar junction. A: Sections from a sham-operated control animal. B: Sections at 30 minutes after occlusion, demonstrating moderate reduction of blood flow in the midbrain, pons, and cerebellum. C: Sections after 30 minutes of occlusion and 5 minutes of recirculation. D: Sections after 30 minutes of occlusion and 30 minutes of recirculation, showing postischemic hypoperfusion in the hindbrain structures.

FIG. 3. Sections of gerbil brain after transcardiac microcarbon perfusion at 2 hours after occlusion of the vertebrobasilar junction. There was no perfusion of the microcarbon in the tegmentum of the midbrain, upper pons, or cerebellum. Incomplete perfusion was noted in part of the thalamus and tectum (inferior colliculus) of the midbrain.
been exposed to ischemia and that tissue damage during ischemia has occurred.

In summary, the model of transient hindbrain ischemia in Mongolian gerbils presented here may be useful in the study of the pathophysiological, metabolic, and histopathological effects of ischemia in the vertebrobasilar system.

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