Cerebral Ischemia Produced By Four-Vessel Occlusion in the Rat: A Quantitative Evaluation of Cerebral Blood Flow

THOMAS W. FURLOW, JR., M.D.

SUMMARY Cerebral ischemia was produced in the rat by simultaneous occlusion of the vertebral and carotid arteries according to the method of Pulsinelli and Brierley (Stroke 10: 267, 1979). Local cerebral blood flow (CBF) was determined by polarographic and autoradiographic techniques. Hydrogen-clearance measurements showed that mean CBF fell in four monitored regions of the hemispheres to between 0.11 and 0.18 ml/min/g, being least in deep rostral gray, intermediate in superficial gray, and greatest in deep caudal gray. However, individual animals had local CBF in excess of 0.20 and even 0.30 ml/min/g, and no animal showed zero CBF. When animals were rendered hypotensive (MABP of 50 Torr) during vascular occlusion, mean CBF ranged between 0.03 and 0.10 ml/min/g in the same regional order. With hypotension, total arrest of flow occurred. Autoradiographic data confirmed the above findings and indicated adequate CBF to the lower brainstem. During vascular occlusion, sufficient CBF may be present to sustain cerebral tissue as in animals with a well-developed spinal circulation or an inadvertently patent vertebral artery.

THE MULTITUDE OF METHODS for producing cerebral ischemia in animals attests to the difficulty of imitating stroke in man.1,2 For instance, mere ligation of the carotids is ineffective in many animals3 including rats4,5 owing to an ample collateral blood supply. Thus, the model of cerebral ischemia in the rat introduced by Pulsinelli and Brierley in 1979 was welcome.6 These investigators first electrocoagulated the vertebral arteries in the atlas and then occluded the carotid arteries. The experimental approach results in global ischemia of the cerebral hemispheres with relative sparing of the brainstem as assessed by dye perfusion and by neuropathologic changes. Owing to the lack of quantitative hemodynamic data in the original report,6 the following study was undertaken to determine the distribution and magnitude of reduced cerebral blood flow (CBF) in the Pulsinelli-Brierley model.

Materials and Methods

The method of Pulsinelli and Brierley6 was employed for producing experimental cerebral ischemia in male Sprague-Dawley rats (Charles River Farms) weighing 200–400 g. Under halothane-oxygen anesthesia a midline incision was made in the dorsal neck, and the cervical muscles were divided down to the atlanto-occipital junction. The alar foramina of the atlas were identified, and a monopolar microelectrode was passed into each foramen in turn. A current sufficient to cause slight muscle contraction was applied in order to electrocoagulate the underlying vertebral artery. The foramina were packed with bone wax, and the muscles and fascia were closed in layers. Through a ventral midcervical incision each carotid artery was
isolated and a 9 × 0 nylon ligature (Ethicon, Sommerville, NJ 08876) was looped about it. Each ligature was threaded through a 5-cm tube (PE-10, Clay Adams, Parsippany, NJ 07054) which had been tunnelled through the lateral neck to emerge caudal to the ipsilateral ear. The ends of the suture were tied to a short piece of tubing such that blood flow through the carotid remained unimpeded.

**Measurement of CBF by Hydrogen Clearance**

Epoxy-coated (epoxy resin, A-M Systems, Inc., Everett, Washington 98204) platinum-10% iridium electrodes (5 mil diameter, Engelhard, Carteret, NJ 07008) with an exposed 1-mm tip were stereotactically implanted into the rostral thalamus, the caudal hippocampus, and in the rostral and caudal cerebral neocortex. The pin connectors (Amphenol #220-S03, Bunk er Ramo, Danbury, CT 06810) of the electrodes were embedded in methylmethacrylate cement to form a damage-resistant cap. Electrodes were polarized at +650 mV during experiments. After saturation of the brain to a breathing mixture containing 7% hydrogen, the hydrogen was turned off and the descent of the hydrogen-induced potential was recorded. Regional cerebral blood flow was calculated in ml/g/min as the quotient of 0.693 divided by the time in minutes for the clearance curve of the hydrogen to decline halfway from its maximum during tissue saturation to the zero potential indicating complete washout of the hydrogen indicator. Additional details of the method are available elsewhere.

**Measurement of CBF by 14 C-Iodoantipyrine Autoradiography**

Over the final 60 sec of the experiment, selected rats received an intravenous infusion of 40 μCi of 4-(N-methyl-14C) iodoantipyrine (specific activity 54 μCi/mmol, New England Nuclear, Boston, MA 02118) in normal saline delivered at a rate of 17 μL/sec by a syringe pump (Sage model 355, Orion Research, Cambridge, MA 02139). Arterial blood samples were collected at time zero and then at precisely 5-sec intervals during isotope infusion. At 1 min the circulation was arrested by decapitation. The brain was quickly removed and frozen in isopentane chilled to −60°C. The sections were placed against a sheet of x-ray film (Kodak SB-5) in a cassette for an exposure of 4 d (Kodak SB-5, Rochester, New York 14650). The slides were processed and a tissue solubilizer (500 μL, Scinti-gestg<sub>8</sub>, Fisher Scientific). After dissolution of the blood by incubating at 60°C for 1 hr, 10 mL of scintillator was introduced into each vial. After standing overnight at 4°C, the vials were counted for 10 min in a liquid scintillating counter (Packard TriCarb). The sequence of arterial blood counts was entered into a computer employing a program from Dr. L. Sokoloff, and the regional cerebral blood flows of various gray and white matter structures were calculated on line with optical density readings determined by a microdensitometer (model DR-2H, Gamma Scientific, San Diego, CA 92123).
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ing order by the caudal neocortex, rostral neocortex, and deep rostral gray matter. Autoradiographic CBF studies were performed in small groups of normotensive and hypotensive rats undergoing ischemia (table 2). The three normotensive animals displayed unexpectedly high CBF, one having normal CBF, another low-normal CBF, and the third only mild ischemia. In contrast, the three hypotensive animals suffered uniformly severe ischemia rostral to the upper brainstem. In both groups local CBF was greater in the lower brainstem into the caudal regions of the brain. Besides the variation in CBF among regions of the brain, the magnitude of CBF within a given region also showed major variability. During normal blood pressure approximately one-third of animals exhibited a level of CBF that would be considered only borderline ischemic or low normal. As expected, this problem was especially evident in caudal parts of the brain closest to the remaining collateral channels but also in superficial structures such as neocortex. Unpredictable and patchy ischemic injury would, of course, be the consequence of such variability in local CBF. Combining four-vessel occlusion with arterial hypotension considerably enhanced the severity of the ischemia by nearly eliminating levels of CBF capable of sustaining tissue. Indeed, total circulatory arrest in the brain, i.e. zero CBF, was observed only when systemic blood pressure was normal.

In their initial report, Pulsinelli and Brierley noted a sizable minority of experimental animals failed to develop the behavioral syndrome of global cerebral ischemia. One explanation that they offered is the variability of collateral circulation to the brain among different strains of inbred rats. However, the Sprague-Dawley rats used in the present study responded similarly to the Wistar rats used by the original authors. Another explanation is to be found in the unintentional failure to occlude a vertebral artery. Because electrocoagulation is done blindly, vertebral occlusion cannot be verified till postmortem. In fact, dye infusion confirmed that one vertebral artery remained patent or recanalized in two animals examined in trials preliminary to this study. Both animals had evidenced low normal CBF in the hemispheres.

In conclusion, cerebral ischemia resulting from four-vessel occlusion in the rat shows variation in its severity among regions of the brain and within a given region. Fortunately, the blood supply to the cardiorespiratory centers in the medulla is relatively spread thereby maintaining the viability of the animal prep-

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**TABLE 1** Polarographically Measured CBF During Four-vessel Occlusion in Normotensive and Hypotensive Rats*

<table>
<thead>
<tr>
<th>Neuroanatomical Structure</th>
<th>MABP</th>
<th>RT</th>
<th>CH</th>
<th>RN</th>
<th>CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal Cortex</td>
<td>1.39 ± 0.17</td>
<td>0.84 ± 0.30</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Parietal Cortex</td>
<td>1.70 ± 0.20</td>
<td>0.77 ± 0.13†</td>
<td>0.04 ± 0.01†</td>
<td>0.04 ± 0.01†</td>
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</tr>
<tr>
<td>Occipital Cortex</td>
<td>1.12 ± 0.08</td>
<td>0.78 ± 0.31</td>
<td>0.07 ± 0.02</td>
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<td>Caudate-Putamen</td>
<td>1.22 ± 0.24</td>
<td>0.65 ± 0.21‡</td>
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<tr>
<td>Hippocampus</td>
<td>1.24 ± 0.14</td>
<td>0.89 ± 0.34</td>
<td>0.04 ± 0.01</td>
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<td>Amygdala</td>
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<tr>
<td>Thalamus</td>
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<td>0.75 ± 0.06§</td>
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<tr>
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<td>1.80 ± 0.11</td>
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<td>Medullary Gray</td>
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*Local CBF in ml/g/min (mean ± SEM); n = 6 for control group and n = 3 for each ischemic group.

†p < 0.01; ‡p < 0.05; §p < 0.001, statistical comparisons between two ischemic groups.

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**TABLE 2** Autoradiographically Measured CBF During Four-vessel Occlusion in Normo- and Hypotensive Rats*

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<th>Neuroanatomical Structure</th>
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*Local CBF in ml/g/min (mean ± SEM); RT = rostral thalamus, CH = caudal hippocampus, RN = rostral neocortex, CN = caudal neocortex.
uation. Arterial hypotension during four-vessel occlusion considerably enhanced the degree of ischemia and permits complete circulatory arrest to be achieved locally in the cerebrum.

Acknowledgments
The author thanks Lynn Harrison and Patti Rotenberry for technical assistance and Carol Smitherman for typing the manuscript.

References

Middle Cerebral Artery Occlusion in the Young Rat

PETER COYLE, M.S., PH.D.

SUMMARY This investigation describes a surgical approach for ligation of the middle cerebral artery (MCA) in the young rat and evaluates consequences of the occlusion with a neurologic exam for motor deficits, Evans blue test for blood-brain barrier leaks, and light microscopy for histologic changes after 3 days. Evans blue extravasation and the lesion were limited to cortex at the burr hole site in occluded and sham operated rats. MCA occlusion beyond the point of origin of the striate branches in the young rat results in neither neurological deficits, dye markings, nor histologic changes in the distal vascular field to indicate an infarct. Apparently, the young rodent collateral supply maintains the tissue in a viable state.

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RAPID OCCLUSION of the middle cerebral artery (MCA) almost always results in a cerebral infarct.1-5 The lesion may be precipitated by an insufficient collateral supply due to too few collaterals,6 inappropriate regulation of existing ones, altered hemodynamics or failure in metabolism.7,8 A literature search disclosed neither a method for rapid occlusion of the MCA nor a description of its consequences for the young rat. Arterial collaterals are known to exist in this animal where numerous anastomotic junctions join distal branches of the three major cerebral vessels.3 Even so, the collateral supply may not protect against infarction. If an infarct should occur, the responsible mechanisms and possible preventive interventions could be studied. Conversely, the young rat may develop a cerebral collateral circulation without complications imposed by an infarct. In this case interventions that promote infarction could be investigated.

Objectives for this study were 1) to introduce a feasible surgical approach for rapid occlusion of the MCA at a standardized location, 2) to compare sizes of lesions marked intravitaly in MCA occluded and sham operated animals, 3) to identify histologic changes in the cortex at the craniotomy site and 4) to characterize the infarct, if any, distal to the ligation after 3 days.

Methods

Surgical Exposure of MCA

Eleven 36 day old normal Wistar rats of either sex were anesthetized with ketamine hydrochloride (136-150 mg/kg body weight, i.m.) Five animals were sham operated. The MCA was occluded on the right side in 6. Skin of the temporal-parietal region was shaved and an incision was made over the right eye (fig. 1A). The incision above the zygoma was deep, cutting through the temporalis muscle to the squamosal bone. Deep surgery was performed with aid of a Bausch and Lomb StereoZoom 7 Microscope and Nikon MK II

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