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

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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 7: 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/g/min, 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/g/min, and no animal showed zero CBF. When animals were rendered hypotensive (MAP of 50 Torr) during vascular occlusion, mean CBF ranged between 0.03 and 0.10 ml/g/min 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 rat4,5 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, Somer-
ville, NJ 08876) was looped about it. Each ligature
was threaded through a 5-cm tube (PE-10, Clay Ad-
ams, Parsippany, NJ 07054) which had been tunnel-
led through the lateral neck to emerge caudal to the in-
ferolateral ear. The ends of the suture were tied to a short
piece of tubing such that blood flow through the carot-
id 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 hippo-
campus, and in the rostral and caudal cerebral neo-
cortex. The pin connectors (Anphenol #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 avail-
able 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/
mol, 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, Cam-
bridge, MA 02139). Arterial blood samples were col-
clected 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 re-
moved and frozen in isopentane chilled to — 60°C.

After warming to —18°C the brain was cut in 20-μm
sections that were applied to glass cover slips, dried at
60°C and mounted sequentially on cardboard with a
series of calibrated carbon-14 polymethylmethacrylate
standards (Amersham, Arlington Heights, IL 60005).
The sections were placed against a sheet of x-ray film
(Kodak SB-5) in a cassette for an exposure of 4 d
after development as recommended by the manufac-
turer. The arterial blood samples were pipetted in
50-µL aliquots into scintillation vials into which were
added hydrogen peroxide (30%, 250 µL), isopropanol
(250 µL), and a tissue solubilizer (500 µL, Scinti-
gest® 3, Fisher Scientific). After dissolution of the blood
by incubating at 60°C for 1 hr, 10 mL of scintillator
were introduced into each vial. After standing over-
night at 4°C, the vials were counted for 10 min in a
liquid scintillating counter (Packard TriCarb). The se-
quence of arterial blood counts was entered into a
computer employing a program from Dr. L. Soko-
loff, and the regional cerebral blood flows of various
gray and white matter structures were calculated on
line with optical density readings determined by a mi-
crodensitometer (model DR-2H, Gamma Scientific,
San Diego, CA 92123).

Monitoring and Occlusion Technique

After at least 48 hr of recovery from electrode im-
plantation, each rat was lightly anesthetized with hal-
othane for insertion of a tracheostomy tube and cath-
eters into the femoral artery and vein (PE-50, Clay Ad-
ams). The anesthetic was discontinued, and ani-
mals were paralyzed with D-tubocurarine (5U subcut)
and connected to rodent ventilator (model 680, Har-
vard Apparatus, Millis, MA 02054) set to maintain
satisfactory blood gases on 30% oxygen and 70% ni-
trogen. Body temperature was monitored with a rectal
probe and maintained at 37.5° ± 0.5°C with a heat
lamp regulated by a temperature controller (model 73
ATF, Yellow Springs Instruments, Yellow Springs,
OH 45387). Blood pressure was continuously mea-
sured with a pressure transducer (Statham model P-
37B, Gould Inc., Oxnard, CA 93030). In selected
animals blood pressure was lowered to 50 Torr by
permitting the heparinized (50 U IV) animal to bleed
into a saline-filled reservoir elevated to the desired
mean arterial blood pressure. After a series of control
of CBF, cerebral ischemia was induced by traction on
both carotid ligatures held fast by aneurysm clips.

Results

Control values for polarographically determined
CBF in four regions of the cerebrum are displayed in
table 1. These control data compare favorably with
those reported by other investigators. When normo-
tensive animals underwent carotid ligation, mean CBF
of the four monitored regions fell between 0.11 and
0.18 ml/g/min with the CBF of hippocampus and neo-
cortex in individual animals exceeding 0.20 and even
0.30 ml/g/min. When another group of nine rats was
rendered hypotensive (MABP = 50 Torr) before
ischemia, three of the four monitored regions mani-
fested some decline in local CBF though the values
were not significantly different from the control meas-
urements made during normal blood pressure (MABP =
122 ± 4 Torr). During ischemia, the hypotensive
rats exhibited a fall in CBF of the rostral thalamus that
differed highly significantly (p < 0.001) from the
value in ischemic normotensive rats. In the remaining
three regions CBF was 30–50% lower than that in the
ischemic normotensive animals, but statistical signifi-
cance was narrowly missed. By hydrogen-clearance
measurements, no normotensive animals attained zero
CBF, though total arrest of regional cerebral circula-
tion was observed in 4 of the 9 hypotensive animals.
The most abundant collateral flow in the cerebrum
after four-vessel occlusion was into the deep caudal
gray matter of the hippocampus followed in descend-
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 and in the hypotensive group also in ventral midline cerebral structures (data not included in table 2).

Discussion

The technique of Pulsinelli and Brierley produces widespread ischemia in the rat brain and represents an improvement over other methods such as the Levine preparation in regard to cardiorespiratory stability. The present study confirms that the brunt of the ischemia is borne by the cerebral hemispheres. Nevertheless, the severity of the ischemia is not uniformly distributed within the hemispheres, being more pronounced in the more rostral cerebral structures. Presumably, this pattern reflects the relative preservation of collateral flow chiefly from the spinal circulation via 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 that 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-
Middle Cerebral Artery Occlusion in the Young Rat

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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.

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. 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, distant 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 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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