A New Model of Bilateral Hemispheric Ischemia in the Unanesthetized Rat

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SUMMARY A new model of transient, bilateral hemispheric ischemia in the unanesthetized rat is described. During ether anesthesia the rat's vertebral arteries were electrocauterized through the alar foramina of the first cervical vertebra and reverses clasps placed loosely around the common carotid arteries. Twenty-four hr later, the awake rats were restrained and the carotid clasps tightened to produce 4-vessel occlusion. The carotid clasps were removed after 10, 20 or 30 min of 4-vessel occlusion and the animals killed by perfusion fixation 72 hr later. Rats which convulsed during the ischemic or post-ischemic period were excluded from further study. All rats subjected to 20 or 30 min of 4-vessel occlusion demonstrated ischemic neuronal damage. The H1 and paramedian hippocampus, striatum and layers 3, 5 and 6 of the posterior neocortex were the regions most frequently damaged. The advantages of this model are the ease of preparation of large numbers of animals, a high rate of predictable ischemic neuronal damage, a low incidence of seizures and the absence of anesthesia.

THE STUDY of the pathophysiology of cerebral ischemia is limited by the lack of a small animal model uncomplicated by anesthesia, systemic hypoxia, hypertension or generalized seizures. Cerebral hypoxia-ischemia in the rat represents one well-studied preparation where cerebral energy metabolism and histopathology are concerned. The rat is sufficiently large to permit easy monitoring of physiological variables (body temperature, electroencephalogram, arterial pressure and arterial gases) yet small enough to be used in the number requisite for statistical analysis without excessive costs. An efficient collateral circulation in the rat prevents either unilateral or bilateral carotid artery ligation from consistently altering cerebral metabolism or producing ischemic brain damage. Present methods to produce cerebral ischemia in this animal require systemic insults of hypoxia and/or hypotension anesthesia either at the initiation of ischemia or throughout the ischemic interval and, finally, the ischemia is frequently permanent rather than transient.

Occlusion of all 4 major arteries supplying the rat brain might decrease cerebral blood flow sufficiently to produce ischemic changes. We report here a method for producing reversible common carotid artery occlusion, which, combined with permanent interruption of the vertebral arteries in the awake, free-running rat, results in bilateral hemispheric ischemia with a high incidence of predictable brain damage and without the complications of previous models in small animals.

Methods

Male Wistar rats weighing between 250 and 300 gm were allowed free access to Purina Laboratory Chow and tap water in day-night regulated quarters at 24°C. They were anesthetized with ether and both common carotid arteries isolated via a ventral, midline incision. An atrumatic arterial clamp was loosely placed around each common carotid artery without interrupting carotid blood flow and the incision closed with a single suture. A second incision, 1 cm in length, was made behind the occipital bone directly overlying the first two cervical vertebrae. The paraspinal muscles were separated from the midline and with the use of an operating microscope, the right and left alar foramina of the first cervical vertebrae were exposed. As described by Green the rat's vertebral arteries travel within the vertebral canal and...
pass beneath the alar foramen before entering into the posterior fossa (fig. 1). A 0.5 mm diameter electrocautery needle (Bowie Monopolar Electrocautery, Cincinnati, Ohio) was inserted through each alar foramen and both vertebral arteries electrocauterized and permanently occluded. The electrocautery was grounded through the animal’s foreleg with operational settings that produced minimal, local muscle contraction.

While the rats were still anesthetized, stainless steel screws were mounted on the calvarium to record the electroencephalogram (EEG) and a tail artery cannula inserted to record the blood pressure, $Po_2$, $PCO_2$ and pH. The cannula was secured under the animal’s skin and kept patent overnight by injecting a dilute solution of heparin (2 units/ml) into the cannula tip which was then sealed. The rats were allowed to recover from anesthesia for 24 hours at which time they were indistinguishable from normal animals by clinical and EEG criteria. The awake rats were then hand-held in a simple restraint, the ventral neck suture removed and both carotid clasps were tightened to produce 4-vessel occlusion. Body temperature was monitored and maintained at 37°C with a rectal thermistor (Yellow Springs Inst., Yellow Springs, Ohio) coupled to a heating lamp. Carotid clasps were removed following 10, 20 or 30 min of 4-vessel occlusion and restoration of carotid blood flow was verified by direct observation. The animals were observed, clinically, for their level of consciousness, the presence or absence of a corneal reflex, their ability to walk and to climb.

At 72 hr of postischemic survival the rats were anesthetized and their brains perfusion-fixed with FAM (40% formaldehyde: glacial acetic acid: methanol, 1:1:8) via the ascending aorta after briefly (30 sec) washing out the cephalic circulation (abdominal aorta clamped) with heparinized, physiological saline. The brains were left in situ for 1-4 hours at 4°C before removal and then stored in FAM. Paraffin sections (7 and 12 μm) were stained with cresyl violet, Luxol fast blue and cresyl violet, phosphotungstic acid and hematoxylin, and with hematoxylin and eosin. The sections were examined with the light microscope and ischemic neuronal damage graded on a scale of 0 = normal, 1 = a few neurons damaged, 2 = many neurons damaged, 3 = majority of neurons damaged.

**Results**

With this method of 4-vessel occlusion, 98 of 127 rats (77%) became unresponsive and lost their righting reflex within 15-30 sec after bilateral carotid artery ligation; 19 (15%) of the rats became only lethargic and the remaining 10 (8%) died within 2-3 min from respiratory failure. In contrast, bilateral carotid artery ligation in 10 rats with intact vertebral arteries resulted in no change in their level of consciousness. The 4-vessel occluded rats that became only lethargic were excluded from further studies. Following completion of this study the incidence of hemispheric ischemia in this model, i.e. the rapid loss of consciousness and the inability to right upon carotid artery ligation, decreased below the 77% level quoted above. Although all rats were purchased from the same supplier, inbreeding may have improved the collateral supply to the brain in the latter animals. Wistar rats from several suppliers were tested before finding a strain which resulted in a similar 70-80% success rate observed earlier. Payan et al. reported similar variability in the incidence of brain damage between several strains of rats subjected to permanent bilateral carotid artery ligation. Differences in collateral blood supply to the brain were most likely responsible for this latter observation. These collateral vessels probably originate from cervical branches of the subclavian arteries.

Four-vessel occlusion resulted in hyperventilation with a mild elevation of arterial $Po_2$, a moderate hypocapnia and respiratory alkalosis (fig. 2). The mean arterial blood pressure rose 20-30 mm Hg immediately after bilateral carotid artery ligation, presumably as a consequence of reduced carotid baroreceptor discharge. Arterial blood gas values, hydrogen ion concentration, and blood pressure returned to control values within 15 min of carotid clasp removal. Mean rectal temperature decreased 1°C during 4-vessel occlusion and returned to control values after 4 hours. The EEG became isoelectric within 2-3 min of 4-vessel occlusion (fig. 3) in those animals that became unresponsive and lost their righting reflex. The EEG remained isoelectric throughout the ischemic period. Intermittent slow waves appeared after 15 min of reperfusion but the EEG remained abnormally slow for at least 4 to 6 hr.
Clinically, the degree of neurological deficit was proportional to the duration of ischemia. The animals continued to breathe spontaneously and never lost their corneal reflex during the ischemic or postischemic period suggesting that the brainstem was unaffected. Animals subjected to 10 min and 20 min of 4-vessel occlusion developed no permanent neurologic deficit other than bilateral ptosis which resulted from carotid artery manipulation. Animals subjected to 30 min of 4-vessel occlusion remained unresponsive for up to 2 hr after release of the carotid clasp; spontaneous movements were negligible for up to 6 hr and, although they could walk by 12 hr, the majority never regained normal motor activity. The percentage of animals that convulsed during a 72 hr survival period following 10 min or 20 min of 4-vessel occlusion was 0% and 8% respectively. Following 30 min of ischemia 20% of the rats convulsed by 24 hr and 40% by 72 hr of survival. All rats which convulsed during the postischemic period were excluded from further study.

An estimate of cerebral perfusion was made by injecting a blue dye into the ascending aorta at 120-130 mm Hg. This was done in control rats, in rats with either the carotid or vertebral arteries occluded and in rats with 4-vessel occlusion. Well-perfused areas of the brain stained dark blue while ischemic areas appeared pale. When only the carotid or vertebral arteries were occluded, perfusion of all brain structures was well maintained (fig. 4A). Four-vessel occlusion resulted in a marked decrease in perfusion of the cerebral hemispheres but did not produce complete ischemia, i.e. a colorless brain. Perfusion of the brain stem in 4-vessel occluded rats was preserved but was less than in controls (fig. 4B). Perfusion of the cerebellum and olfactory lobes was variable but usually preserved even when the cerebral hemispheres were ischemic (fig. 5). Blood flow to the brainstem and cerebellum in 4-vessel occluded rats was probably due to filling via the anterior spinal artery. The source of blood flow to the olfactory lobes in this model remains unknown.

Histological examination of the brains from 8 control (vertebral arteries electrocauterized and clamps placed loosely around both carotids) rats showed no pathological alterations. Neuropathological damage in brains from ischemic rats was characterized by ischemic cell change with and without incrustations and homogenizing changes in neurons as described previously by Brown and Brierley\(^5\) in other ischemic models. All animals exposed to 20 or 30 min of 4-vessel occlusion demonstrated ischemic neuronal cell damage.

**Figure 2.** Physiological variables in the rat prior to, during (shaded) and following 4-vessel occlusion. Vertical bars represent mean ±1 SE of arterial P\(_{O_2}\), P\(_{CO_2}\), pH and BP.

**Figure 3.** Electroencephalograms representative of rats which became unresponsive and lost their righting reflex within 15–30 seconds of 4-vessel occlusion. Post-ischemia = after carotid clasp removal.
damage; the grade of damage varied among regions and was proportional to the duration of ischemia. The table demonstrates the distribution and incidence of grade 2–3 damage in rats subjected to 10, 20 and 30 min of 4-vessel occlusion. The HI and paramedian (PM) hippocampus were the most vulnerable structures. Ten minutes of 4-vessel occlusion resulted in grade 2–3 damage to 40% of these structures while the remaining regions were less frequently damaged. Following 20 min of ischemia 85%–90% of the HI and PM areas of the hippocampus demonstrated grade 2–3 damage. When 4-vessel occlusion was increased to 30 min there was an increase in the incidence of grade 2–3 damage in the striatum (93%) and thalamus (61%), the latter being focal in nature. Neocortical damage was limited to layers 3, and/or 5 and 6, the posterior cortex being more frequently involved than the anterior cortex. The cerebellum was infrequently damaged while the brainstem appeared histologically normal in all animals except one which had grade 3 damage of the right substantia nigra. The integrity of the cerebellum and brainstem was attributed to perfusion via the anterior spinal artery.

### Table 1. Percentage of Hemispheres with Grade 2–3 Ischemic Damage

<table>
<thead>
<tr>
<th>Region</th>
<th>Operated controls*</th>
<th>10 Min ischemia</th>
<th>20 Min ischemia</th>
<th>30 Min ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant. Cortex (Layers 3, 5, 6)</td>
<td>0</td>
<td>10</td>
<td>37</td>
<td>57</td>
</tr>
<tr>
<td>Post. Cortex (Layers 3, 5, 6)</td>
<td>0</td>
<td>15</td>
<td>47</td>
<td>64</td>
</tr>
<tr>
<td>Striatum</td>
<td>0</td>
<td>0</td>
<td>35</td>
<td>93</td>
</tr>
<tr>
<td>H1 Hippocampus</td>
<td>0</td>
<td>40</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>H3-5 Hippocampus</td>
<td>0</td>
<td>25</td>
<td>55</td>
<td>79</td>
</tr>
<tr>
<td>PM Hippocampus</td>
<td>0</td>
<td>40</td>
<td>90</td>
<td>93</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

* 8 rats X (left and right) = 16 hemispheres
10 rats X (left and right) = 20 hemispheres
14 rats X (left and right) = 28 hemispheres

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Figure 4. Dorsal (A) and ventral (B) view of brains from 4 rats perfused with a blue dye via the ascending aorta. a. control rat, b. bilateral carotid artery ligation, c. bilateral vertebral artery occlusion, d. bilateral carotid and vertebral artery occlusion. Note that the cerebellum (Ad) is partially perfused and that there is dye in the basilar artery and circle of Willis of this brain (Bd).

Figure 5. Dorsal view of brains of 3 rats perfused with a blue dye during 4-vessel occlusion. Note that the ischemia is not complete. The cerebellum and olfactory lobes are variably perfused while the cerebral hemispheres appear pale.
Comment

The study of pathophysiological mechanisms of ischemic brain damage in small animals has been complicated by factors such as anesthetics, systemic hypoxia, hypotension and generalized seizures. The advantage of the Mongolian gerbil is that ischemic brain damage can be produced by carotid occlusion per se as a consequence of a functionally incomplete circle of Willis.\textsuperscript{18, 19} Its disadvantage is that only 30–40\% of gerbils treated this way will develop clinical signs of hemorrhagic ischemia.\textsuperscript{14, 18} Moreover, the gerbil is genetically predisposed to convulsions induced by mild stimuli\textsuperscript{20, 21} and following unilateral carotid artery ligation 75\% of clinically affected animals developed generalized seizures.\textsuperscript{19–22} Since seizures increase cerebral energy metabolism\textsuperscript{23} they may jeopardize neurons in the ischemic and postischemic periods.

In man, seizures are a rare complication (<10\%) of ischemic stroke\textsuperscript{24, 25} and therefore exclusion of convulsing animals in pathophysiological studies of ischemic brain damage is justified.

Various methods are available for producing cerebral hypoxia-ischemia in small animals. However, most animals have a well-developed circle of Willis and, therefore, unilateral or bilateral carotid artery ligation must be combined with hypoxia and/or hypotension to consistently derange cerebral metabolism and produce ischemic damage. Systemic hypoxia and hypotension may damage organs (heart, kidney) other than brain and thereby alter physiological mechanisms of postischemic survival. Moreover, the above methods, as well as cerebral embolization\textsuperscript{26} and middle cerebral artery occlusion,\textsuperscript{15} employ anesthetics which may depress cerebral energy metabolism,\textsuperscript{26–27} modify ischemic brain damage\textsuperscript{28} and hinder attempts to ameliorate post-ischemic encephalopathy. The method described herein of 4-vessel occlusion in the rat is devoid of systemic hypoxia, hypotension or anesthetic drugs; there is a sufficiently low incidence of seizures to permit exclusion of convulsing animals; the procedure is surgically simple so that large numbers of animals can be assessed statistically; there is a high incidence of predictable ischemic neuronal damage in certain regions of the brain. Regions most vulnerable to ischemic damage in this model were the H1, H3–5, and paramedian zones of the hippocampus, layers 3, 5 and 6 of the posterior neocortex, and the striatum. This pattern of selective vulnerability is similar to that found in other animal models of hypoxia-ischemia.\textsuperscript{29}

The method of initial, permanent occlusion of the vertebral arteries permits control of cerebral circulation by the occlusion of the common carotid arteries alone or with the addition of cervical branches of the subclavian arteries. The surgical approach and electrocauterization of the vertebral arteries through the alar foramen or groove of the first cervical vertebrae is simple,atraumatic, and may be accomplished before carotid artery occlusion. This 2-stage procedure should be applicable to other animals having a complete circle of Willis providing collateral circulation is adequate to maintain vital brain stem centers. Occlusion of the vertebral arteries does not compromise cardiorespiratory centers in the dog\textsuperscript{30,31} or monkey.\textsuperscript{32} It remains to be determined whether the brain stem of additional animals will tolerate permanent vertebral artery occlusion. If this were so, permanent occlusion of the vertebral arteries under anesthesia could be followed by carotid artery occlusion using techniques (pneumatic cervical cuff, arterial snare) that do not require anesthesia.

References


Survival of Rabbits After Prolonged Cerebral Ischemia

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SUMMARY—Cerebral ischemia was produced by a combination of vascular occlusion and mild systemic hypotension in 2 groups of rabbits. Arterial blood pressure, arterial pH, arterial blood gases, blood glucose and PCV were monitored and recorded before, during and for 3 hours after reperfusion. Return of EEG activity, vasomotor control, spontaneous ventilation and corneal reflex were also recorded. At 4, 8, 12, 24 and 48 hours after reperfusion, the rabbits' neurologic status was assessed according to an arbitrary scale based on motor function. The 2 groups differed in return of reflexes and motor function. Eighty percent of the rabbits ischemic for 20 minutes and 75% of the rabbits ischemic for 30 minutes survived. The graduated response of motor function to cerebral ischemia is attributed to the ventilatory and circulatory support given the rabbits for the first 3 hours after reperfusion. The graduated response of motor function to ischemia supports the suggestion that motor function can be used as an index of neurologic damage.

A VARIETY OF METHODS to control the cerebral circulation of rabbits has been used to study the effects of cerebral ischemia (CI) in these animals. The methods vary in their complexity. Simple clamping of the aorta just distal to the coronary sinus can cause CI. If a left ventricular outflow reservoir is also used, fatal cardiac dilatation is prevented and reversible cerebral ischemia is achieved. Most other methods of achieving CI involve arterial ligation and one or more other maneuvers. One method involves permanent basilar artery ligation, temporary bilateral carotid occlusion, and inflation of a cerebral pressure cuff to cause CI. A simplified method employs bilateral temporary carotid artery occlusion and drug-induced profound systemic hypotension. A similar and more simplified method uses a cerebral pressure cuff inflated to 1.5 atm pressure to cause CI. A method which does not employ arterial occlusion but achieves CI by using drug-induced profound hypotension, tilting the rabbit head up and ventilating it with a mixture of 96% N2 and 4% O2 has been described. These methods have been classified and commented on. Some have to be criticized because they require extensive or traumatic surgery to gain control of the cerebral circulation. Some of them, also, cause hypoxemia and ischemia of organs in addition to the brain, which could result in artifactual responses during recovery.

The method described herein overcomes many of these criticisms as it requires minor surgical intervention, allows venous return from the brain, maintains adequate blood oxygenation, and preserves adequate systemic blood pressure.

Methods

Surgical Preparation

Each rabbit is given 0.2 mg of atropine kg/body weight. Anesthesia is induced with a gas mixture of halothane and oxygen. After intubation, anesthesia is maintained with the same mixture. The rabbit is prepared and draped for aseptic surgery. A 3 cm incision is made in the lateral surface of the neck beginning at the caudal edge of the transverse process of the 1st cervical vertebra. The subcutaneous tissues are incised, and the splenius muscle is identified and reflected dorsally to expose the intertransversarius cervicus dorsalis muscle. The intertransversarius cervicus dorsalis and the intertransversarius intermedius muscles are separated to expose the vertebral artery as it passes out of the transverse foramen of the second cervical vertebra. The artery is identified and ligated.

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