Hypertension and Acute Focal Cerebral Ischemia

Infarction and Edema after Occlusion of a Middle Cerebral Artery in Cats

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SUMMARY Hypertension was produced in 8 cats by nephrectomy and wrapping the opposite kidney. Subsequent occlusion of one middle cerebral artery caused ischemic infarcts that were larger than those of normotensive cats. The larger infarcts may have been caused by increases of ischemic cerebral edema resulting from changes in the cerebral endothelial barrier induced by hypertension. In addition to increasing the likelihood of strokes, hypertension in humans may predispose toward larger cerebral infarcts.

HYPERTENSION is recognized as the most important risk factor for the development of ischemic cerebral infarction in humans. Moreover, there is some evidence that in humans hypertension predisposes to cerebral infarcts that are larger and more severe than those occurring in normotensive patients. The present study is the first in which the influences of pre-existing hypertension on infarction and edema have been investigated in an experimental model of acute focal cerebral ischemia in animals.

Materials and Methods

Production of Experimental Hypertension

Eighteen unselected adult cats were used. Nine of the cats were anesthetized with sodium pentobarbital, 20 mg/kg injected intraperitoneally, and the right kidney was exposed retroperitoneally and removed. The left kidney also was exposed, and wrapped with a plastic film (Saran). After recovery from anesthesia, the cats were provided with their usual food and water. No salt or adrenal corticosteroids were added.

Production of Acute Focal Cerebral Ischemia

Three to six months after nephrectomy each cat, and each of 9 additional cats used as controls, was sedated with phencyclidine hydrochloride, 1 mg/kg injected intramuscularly, and the skin over a femoral artery and vein was anesthetized with procaine hydrochloride injected in a 1% solution. Polyethylene catheters were placed through the femoral artery into the abdominal aorta and through the femoral vein into the inferior vena cava. Mean aortic blood pressure (MAP) was recorded with a strain gauge and polygraph. The cats then were anesthetized with sodium pentobarbital, 20 mg/kg injected intraperitoneally.

The left middle cerebral artery (MCA) was exposed transorbitally with the aid of an operation microscope and occluded by bipolar coagulation, except for one cat in each group in which the MCA was gently grasped with the forceps (sham operation).

Measurement of Neurologic Deficits, Infarction, and Edema

Twenty-four and forty-eight hours after MCA occlusion each cat that survived was anesthetized with sodium pentobarbital, 20 mg/kg injected intraperitoneally. Sodium pertechnetate containing 100 microcuries of technetium-99m was injected intravenously. Fifty-nine minutes later a sample of arterial blood was removed from the catheter in the abdominal aorta, and one minute after that the cat was killed by rapid exsanguination through the same catheter. The brain was removed quickly (within 7 minutes) and a coronal sections approximately 3 mm thick were made at the levels of the tips of the temporal lobes and the medulla. The hemispheric section was cut into 38 samples weighing 10 to 50 mg (fig. 1) and the medullary section into 2 samples. Each sample was placed in a separate tared container and weighed immediately for determination of wet mass. The count rate for technetium-99m in each sample of brain and in the blood were measured in a well counter with appropriate corrections for background activity and natural decay. The samples then were heated in an oven at 60°C for 48 hours, exposed to a vacuum reaching 0.1 micron of mercury for at least 4 hours, and weighed again for determinations of dry mass. The difference between the wet weight and the dry weight of each sample was considered to be the water content, expressed as percent of wet weight. The relative concentration of pertechnetate in each sample was expressed as the brain:blood ratio, or the ratio of the count rate for technetium-99m per unit net weight of brain to the count rate per unit weight of blood. The remaining brain tissue was fixed in a 10 percent solution of formaldehyde and histologic sections were prepared from the surfaces adjacent to the sections.
HYPERTENSIVE

82.2% 165** D 80.5 170 D

80.1 160 D 78.5 210

69.7 160 68.7 180

59.7 200 54.6 210 D

NORMOTENSIVE

78.5 115 D 67.8 135 D

64.9 150 62.9 145

45.7 120 28.1 150

4.9 125 4.2 135

* Size of infarct, per cent
** MABP before occlusion, mm Hg
D = Died before 48 hours

Figure 1. Pre-occlusion MABP and sizes of infarcts in the hypertensive and normotensive cats. Dotted lines indicate locations of individual samples of brain.

sampled for water and pertechnetate content. Histologic sections also were prepared from the brains of the cats that did not survive 48 hours after MCA occlusion. The sections were stained with hematoxylin and eosin and examined microscopically. Schematic drawings were made of the coronal sections and the infarcted regions of one hemisphere (fig. 1). The drawings of the coronal sections and of the infarcted regions were cut out and weighed on an analytic balance. The size of each infarct was expressed as percent of the corresponding coronal section, by weight.

Analysis of Data

Differences between the hypertensive and the nonhypertensive groups of cats, excluding the cats with sham operations, were analyzed. The Student t-test was used for analysis of pre-occlusion MABP, but the t-test based on the Behrens-Fisher distribution was used for analysis of sizes of infarcts because of the apparent unequal variances.7 To analyze differences in water and pertechnetate content, the values for each of the 20 samples from one hemisphere and ipsilateral medulla were compared with the values for the analogous samples from the opposite side. In addition, mean values for each of the 40 locations in the 2 groups of cats were compared.

Results

The values for MABP recorded before MCA occlusion are given in figure 1. The mean MABP was 182 mm Hg ± 22 (standard deviation) for the hypertensive group and 134 ± 13 for the normotensive group; this difference was significant at P < 0.001.

The 8 hypertensive cats all had large infarcts. Smaller infarcts were found in 4 of the normotensive cats (fig. 1); the other 4 had large infarcts. The mean infarct size was 71.8 ± 10.4 percent in the hypertensive group and 44.6 ± 29.1 percent in the normotensive group; this difference was significant at P < 0.05 using the Welch approximation.7

Within the 2 groups there were no clear or consistent relationships between the sizes of the infarcts and preocclusion MABP. No infarcts were hemorrhagic. Neurologic deficits were slightly more severe in the hypertensive cats, and generally in keeping with the sizes of the infarcts (table). Four hypertensive cats and 2 normotensive cats died within 48 hours of MCA occlusion.

The water content and pertechnetate content of samples from the hemispheres on the side of the occluded artery were consistently greater in the 4 hypertensive cats that survived for measurements than in the 6 normotensive cats (fig. 2). The pertechnetate content of samples from the hemispheres opposite occluded MCAs likewise was consistently greater in the hypertensive group of cats, but there were no differences in water content, or in medullary pertechnetate or water (fig. 2). Larger numbers of samples of brain were infarcted or ischemic in the hypertensive cats, accounting for the major differences that were observed. When only ischemic or only infarcted samples were compared, differences in water content and pertechnetate content did not reach statistical significance (P ≤ 0.05), but the values still were consistently greater in the hypertensive group.

No relationships between pre-occlusion MABP and water or pertechnetate content were identified in either group. The distribution of increased water and pertechnetate content in ischemic hemispheres were

Table 1 Neurologic Deficits, Graded 0 (none) to 4 (maximum)

<table>
<thead>
<tr>
<th>Size of infarct</th>
<th>24 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>W</td>
</tr>
<tr>
<td>Hypertensive group</td>
<td>82.2%</td>
<td>Dead</td>
</tr>
<tr>
<td>80.5</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>80.1</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>78.5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>69.7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>68.7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>50.7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>54.6</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>Normotensive group</td>
<td>78.5%</td>
<td>Dead</td>
</tr>
<tr>
<td>67.8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>64.9</td>
<td>3</td>
<td>3</td>
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<tr>
<td>62.9</td>
<td>4</td>
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<td>45.7</td>
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<td>3</td>
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<tr>
<td>28.1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4.9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4.2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

C = Disturbance of Consciousness
W = Focal Weakness
F = Forced Motor Activity
related to the sizes of the infarcts (fig. 3) but not to pre-occlusion MABP.

There were no infarcts and no side-to-side differences in water content in the sham-operated cats. Pertechnetate content was slightly greater in the left hemisphere of the sham-operated cat with the nephrectomy.

**Discussion**

In this experimental model of acute focal cerebral ischemia in cats, pre-existing hypertension produced by unilateral nephrectomy and wrapping the remaining kidney with plastic film was associated with larger cerebral infarcts and somewhat more severe neurologic deficits. Moreover, edema and a breakdown of the blood-brain barrier to pertechnetate (perhaps bound to serum albumin) were greater in the hypertensive cats than in a normotensive group without nephrectomy or wrapping. The increased edema and disruption of the cerebral endothelial barrier was not due solely to the larger infarcts in the hypertensive cats; in nonischemic, ischemic, and infarcted samples of brain, water and pertechnetate content were consistently greater in the hypertensive group although the differences did not reach statistical significance perhaps because of the small number of animals.

These results indicate that hypertension modifies the cerebral endothelial barrier even in normal, nonischemic brain, and that because of this modification endothelial structures are less able to withstand the deleterious effects of ischemia to prevent the transendothelial distribution of water and larger molecules. Increased edema with swelling, caused by increased "leakiness" of the endothelial structures in the hypertensive cats, perhaps was responsible in part for the larger infarcts, because of increases of local tissue pressure or general intracranial pressure producing increases of vascular resistance and secondary decreases of perfusion. Greater decreases of regional cerebral blood flow (CBF), caused by differences in pressure-flow relationships, also may have occurred in the hypertensive as opposed to the normotensive cats after MCA occlusion. The sizes of the infarcts in the hypertensive cats were near the maximum expected from occlusion of one MCA.

In squirrel monkeys, nephrectomy alone without
hypertension may influence the cerebral endothelial barrier.\textsuperscript{18} However, bilateral carotid occlusion causes larger infarcts and greater changes of cerebral metabolism in spontaneously hypertensive rats, without nephrectomy, than in normotensive rats.\textsuperscript{14, 17} It is likely that hypertension, rather than nephrectomy, was responsible for the greater edema and larger infarcts in the present study.

The morphologic and pathophysiologic abnormalities underlying an increased susceptibility of the cerebral endothelium to ischemia with hypertension are unknown. With hypertension the regulatory arterial mechanisms that maintain CBF are reset to higher levels of perfusion pressure.\textsuperscript{16, 18} Perhaps vascular constriction, caused by high intraluminal pressures, is responsible for a decrease in the availability of collateral circulation through pre-existing anastomotic channels. This could account for greater decreases of regional CBF\textsuperscript{12} but not for an increase of the transendothelial distribution of water and larger molecules during ischemia. Associated morphologic changes, such as fibrinoid necrosis or the deposition of collagen in arterial vessels,\textsuperscript{29} may play a role.

In the present study, pre-existing hypertension did not predispose to hemorrhagic infarction. In earlier studies, hemorrhagic infarction has been found in normotensive cats after MCA occlusion,\textsuperscript{19} and in studies by others hypertension induced after the onset of cerebral ischemia has caused hemorrhagic infarction.\textsuperscript{29} Perhaps hemorrhagic infarction is related more to early reperfusion of an ischemic zone than to pre-existing hypertension.

Extension of the results of studies of experimental models of cerebral ischemia to the clinical situations of strokes in humans may not be fully justified or advisable. However, the results of the present study suggest that hypertension may not only increase the likelihood of an ischemic cerebral infarct in humans, but it may also predispose toward an increased extent and severity of infarction.

Acknowledgment

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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 reversible clamps placed loosely around the common carotid arteries. Twenty-four hr later, the awake rats were restrained and the carotid clamps tightened to produce 4-vessel occlusion. The carotid clamps 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 HI 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, hypotension 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 and/or 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 cervical incision. An atrumatic arterial clasp 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...
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