Different Susceptibilities to Cerebral Infarction in Spontaneously Hypertensive (SHR) and Normotensive Sprague-Dawley Rats

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

SUMMARY  Rapid occlusion of the middle cerebral artery (MCA) was undertaken in 5-6 week old rats to determine whether or not the young spontaneously hypertensive rat (SHR) or the normotensive Sprague-Dawley rat (SD) is protected against cerebral infarction by collateral circulation. Rats were killed 3 days after MCA occlusion and administration of Evans blue. As compared to SD, the SHR had elevated blood pressure prior to MCA occlusion, large cortical infarcts marked with Evans blue, and motor deficits contralateral to the occluded MCA. SHR did not develop an adequate collateral circulation, but SD were protected from infarction by it. Because the cerebral lesions were in young spontaneously hypertensive rats living prior to the established form of hypertension, the increased susceptibility to infarction was not secondary to it. Since normotensive rats usually do not infarct after sudden MCA occlusion, the infarction trait may be linked to the mechanism causing elevated blood pressure in spontaneously hypertensive rats.

RAPID OCCLUSION of the middle cerebral artery (MCA) near its origin at the circle of Willis almost always results in a large cerebral infarct in adult cat, and monkey. When the MCA distal to the striate branches is suddenly occluded in 5-6 week old normal Wistar (NW) or Wistar Kyoto (WKY) rats collateral circulation protects against the lesion. In contrast, young spontaneously hypertensive stroke-prone rats (SHRSP) invariably infarct after this occlusion.

One or more factors predisposing SHRSP to stroke may determine the occlusion test outcome. Because the spontaneously hypertensive rat (SHR) lacks the natural proneness to stroke, it may be protected by collateral circulation in the occlusion test. Alternatively, if SHR has an increased susceptibility to infarction after the occlusion, then this characteristic may be linked to the mechanism responsible for elevated blood pressure, a feature common to both rat strains.

This study differentiates outcomes of the occlusion test in young SHR with elevated blood pressure and normotensive Sprague-Dawley rats.

Methods

Source of Rats

Ten male spontaneously hypertensive rats (SHR) at 28 days of age were obtained from Charles River Breeding Laboratories, selected as a source outside of our breeding colony at The University of Michigan. The remaining f/53 generation SHR, 4 females and 2 males, were inbred in our colony of sibmated rats derived from NIH f/49 generation SHR. Ten female and 7 male Sprague-Dawley (SD) rats were outbred animals in our colony. Grand relatives of the SD were from Charles River Breeding Laboratories and less than 4 generations removed from those tested. Tail}

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systolic blood pressure readings were obtained between the hours of 8 AM and 3 PM from unanesthetized rats as previously described.

Surgical Exposure and Ligation of MCA

Rats were anesthetized with ketamine hydrochloride (132 mg/kg body weight, i.m.). The right MCA was exposed through a burr hole craniectomy (1-3 mm diameter, 0.8-7.1 mm² in area) made by a transtemporal route. The artery was dissected free of the meninges but it was not ligated in 4 sham operated rats. For 29 rats having rapid MCA ligation, the vessel was occluded with a 35 μm diameter monofilament nylon thread. The occlusion was distal to the striate branches of the MCA and 700-1000 μm dorsal to the rhinal fissure. Following wound closure with silk suture, 0.5–1.5 ml 2% Evans blue in physiologic saline was injected intraperitoneally in all rats except for 5 SHR that received the dye (0.007 ml/gram body wt) in the right femoral vein. This was done to mark injury to the Evans blue-albumin blood–brain barrier as occurs with severe ischemic injury, local surgical trauma in exposing the MCA or local surgical trauma in exposing the MCA.

Postsurgical Neurological Test

On the second postoperative day the rats were evaluated for motor deficits. An animal was placed on the 25 mm wide surface of a wooden meter stick suspended horizontally about 1 foot above a bench top. Observations were made on fore- and hind-paws, their locations and symmetries during station, gait, crawling or running on the meter stick.

Postmortem Determinations

Experiment termination was on the third postoperative day. Rats were deeply anesthetized with ether. Tissue fixation was initiated by injection of 50 ml 10% neutral buffered formalin into the ascending aorta. The brains were photographed several months after the rats were killed. Evans blue was no longer visible in the fixed brains, but the cortex where the dye marker had been was lighter in coloration than the surrounding
cortex (Figure 1a). Film and TV images were projected onto paper for tracing hemisphere and lesion boundaries. Coordinates (x, y) of points along the lesion and hemisphere boundaries were obtained as previously outlined and surface area of the lesion was computed after correction for brain curvature. 18, 19

**Tissue Histology.** Brains were placed in 25% sucrose-formalin several days before sectioning with a microtome. Frozen sections of the forebrain were cut at 25–50 μm in thickness and stored in fixative until mounted on glass slides and stained with hematoxylin and eosin or basic fuchsin.

**Premature Death**

Not all SHR or SD rats were sacrificed at termination time on the third postocclusion day. Three SHR with signs of morbidity were killed during the first postocclusion day and two died during the second day. Morbidity was characterized by non-movement and failure to maintain normal resting posture. Five Sprague-Dawley rats died during the first postoperative day. Two of the 5 SD were sham operated animals. All morbid rats and those dying prior to the scheduled termination of the experiment received Evans blue intraperitoneally.

**Statistical Procedures**

Tail systolic blood pressure and weight values before MCA occlusion were compared for rat strains with a two sample t-test. Before occlusion weight and blood pressure values were compared to values obtained after the occlusion with a paired t-test. Blood pressure and weight values were correlated with SHR infarct sizes. Correlation values were compared to 5 percent critical values differed significantly from zero. Mean values were expressed with the standard error of the mean (SEM) and p values less than 0.05 were considered to be significant. For measurement comparisons, data from the 23 rats surviving until experiment termination on the third postocclusion day were evaluated. This eliminated variable survival times confounding the measurements.

**Results**

**Blood Pressure**

Tail systolic blood pressure (BP) prior to MCA occlusion was significantly higher in SHR as compared to the Sprague-Dawley rats (table 1). There was no significant difference in preocclusion BP values between sexes for either SD or SHR. Blood pressure on the second postocclusion day was significantly lower than the value prior to surgery for SHR but not for SD (table 1). The value of -0.23 for blood pressure correlated with SHR infarct size was insignificant.

**Histologic Features of the Lesions**

In SHR, Evans blue marked the cortical lesion. Isolated focal patches of Evans blue were not grossly evident. Except near its border (fig. 1a), the lesion was continuous in extent. Tissue sections of the lesion were less intensely stained with hematoxylin and eosin than normal tissue. The lesion was characterized by the following features (fig. 1b). Pyknotic and fragmented nuclei of cells were present. Neutrophils were spongy in appearance and cell somas were not clearly outlined. Mitotic figures were evident and strands of endothelial cells were present. Polymorphonuclear leucocytes and phagocytes were common. This irreversible lesion usually included all cortical layers and was large; the infarct extended far distal to the occlusion site (fig. 1a) and was a consequence of the rapid MCA occlusion.

The lesion in Sprague-Dawley rats was characteristically small and localized to cortex adjacent to the burr hole craniectomy. The Evans blue marked lesion sometimes had a small amount of hemorrhage. Phagocytes containing vacuoles (possibly mineral oil or bone wax) were near the lesion surface. Pyknotic cells and mitotic figures were present. The lesion was limited to outer layers of the cerebral cortex. Deeper layers were spared from the lesion.

Sham operated rats undergoing the same surgery, but without ligation of the MCA, had small cortical lesions. Neither lesion histologic features nor sizes differentiated Sprague-Dawley rats with MCA occlusion from the sham operated rats. In SD, the small lesion was not a result of MCA occlusion, but rather, the injury was due to surgery required for the craniectomy, exposure, dissection of the MCA and placement of the ligature around the vessel. On this basis the small (<6mm²) lesions were classified as surgical lesions; larger ones were designated infarcts.

**Different Size Lesions in SHR and SD**

Mean size of the cortical infarct in SHR was 81.1 mm² (table 2). The infarct was about 40 times larger

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Rat Tail Systolic Blood Pressure and Weight Values Before and After MCA Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat strain</td>
<td>No. of rats (m,f)</td>
</tr>
<tr>
<td>SHR</td>
<td>11 (7,4)</td>
</tr>
<tr>
<td>SD</td>
<td>12 (5,7)</td>
</tr>
</tbody>
</table>

*p < 0.05 comparison of SD and SHR.†p < 0.05 comparison pre- and post-occlusion.‡p < 0.005 comparison of SD and SHR.§p > 0.05 comparison of pre- and post-occlusion. Data excludes rats not surviving 3 day post-occlusion period.
FIGURE 1. A. Infarct is large white region of the right cerebral hemisphere of a spontaneously hypertensive rat (SHR) 3 days after right middle cerebral artery (MCA) occlusion. B. Coronal cut section of an SHR rat brain 3 days after rapid MCA occlusion, basic fuchsin stain.

The lesion size in SHR ranged from 51.4-113.4 mm², whereas in Sprague-Dawley rats the surgical lesion varied from 0.5-5.3 mm². One SHR with MCA occlusion had a 1.1 mm² surgical lesion and one SD developed a 19.0 mm² infarct. Thus, the size difference between the largest surgical lesion and the smallest infarct was more than 40 mm² except for one rat where the difference was about 14 mm², more than twice the size of the largest surgical lesion.

**Table 2**

<table>
<thead>
<tr>
<th>Strain and lesion type</th>
<th>No. of rats</th>
<th>Lesion size (mm²±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>infarct</td>
<td>9</td>
<td>81.1±6.1*</td>
</tr>
<tr>
<td>surgical</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>infarct</td>
<td>1</td>
<td>19.0</td>
</tr>
<tr>
<td>surgical</td>
<td>10</td>
<td>2.0±0.5</td>
</tr>
<tr>
<td>Sham (SHR,SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>infarct</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>surgical</td>
<td>2</td>
<td>1.8±0.1</td>
</tr>
</tbody>
</table>

*p < 0.0005 comparison of SHR infarct size with SD surgical lesion size.

**Table 3**

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Sex &amp; no. of rats</th>
<th>No. rats died (postop day)</th>
<th>No. rats morbid*</th>
<th>No. rats tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA occluded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>M 11</td>
<td>2 (2nd)</td>
<td>3</td>
<td>5/6</td>
</tr>
<tr>
<td></td>
<td>F 4</td>
<td>0</td>
<td>0</td>
<td>3/4</td>
</tr>
<tr>
<td>SD</td>
<td>M 7</td>
<td>2 (1st)</td>
<td>0</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>F 7</td>
<td>1 (1st)</td>
<td>0</td>
<td>0/6</td>
</tr>
<tr>
<td>Sham operated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>M 1</td>
<td>0</td>
<td>0</td>
<td>0/1</td>
</tr>
<tr>
<td>SD</td>
<td>F 3</td>
<td>2 (1st)</td>
<td>0</td>
<td>0/1</td>
</tr>
</tbody>
</table>

*Experiment was terminated if a rat became morbid.

**Motor Deficits**

Motor deficits were not obvious in SHR during movement on the table top. Most, but not all, SHR were observed to have motor deficits when tested on the horizontally suspended wooden meter stick during the second day of MCA occlusion (table 3). Deficits consisted of asymmetry of the right and left paws at station. Toes of the left hind foot did not grasp the stick because the foot was extended beyond its edges. Attempts by the rat to place the foot on the stick were often repeated in quick succession and unsuccessful in execution. The left hind limb was fully extended during periods between attempts to place it on the stick.

During gait, the left hind foot was not always placed on the meter stick. This caused the SHR to lose balance. Position was either regained or the rat fell to the table top. After some foot misplacements, the rat shifted its left rear quarter to the right side of the stick to provide more surface area for placement of the left hind foot. Rats with large (mean infarct size >85 ± 8.4 mm²) infarcts also had forelimb motor deficits. Unequivocal left forepaw placements that missed the stick occurred in 4 rats. Forebody shift towards the right side of the stick was observed during gait. None of the Sprague-Dawley rats nor the sham operated rats was observed to have a limb motor deficit.

**Rat Weights**

Five-six week old Sprague-Dawley rats of each sex weighed significantly more than SHR of the corresponding sex even though the SHR were slightly older (table 1). Male rat body weights were not significantly different from body weights of female rats of the same strain. Body weight was not significantly different on the third day of occlusion as compared to the value on the second day.
the occlusion day for either sex of either strain although the SHR tended to lose weight and SD gained weight. The correlation value of body weight to lesion size in SHR was 0.48 but this value was not significant.

Discussion

Differential Findings in Rat Strains

The most important differential finding was the presence of a large infarct in SHR but not in Sprague-Dawley rats. Irreversible histologic changes occurred and SHR had demonstrable motor deficits. These features were the result of sudden MCA occlusion in SHR. Similar histologic, neurologic and Evans blue findings were noted after sudden occlusion of the MCA in the stroke-prone SHR (SHRSP).

Cerebral infarction in SHR after rapid MCA occlusion indicates the young SHRSP is not a unique rat model.

Comparisons of Spontaneously Hypertensive Rats

There were differences between SHR and SHRSP for the measured characteristics. Whereas SHRSP invariably develop an infarct, lesion frequency in SHR obtained from the commercial breeding source was 89 percent; genetic heterogeneity was not excluded as a factor accounting for a non-infarcting rat. A previous study gave evidence for a heterogeneous response to MCA occlusion in F1 progeny derived from mating a heterozygous normotensive animal with several female SHRSP. The SHRSP was characterized by its originators for its proneness to stroke, but this characteristic may not be the major factor predisposing rats to infarct after MCA occlusion, since SHR infarct but are not "stroke-prone." Lesion size in SHRSP was 63 ± 8 mm² but in SHR 81 ± 6 mm². Mean tail systolic blood pressure in 35-40 day old SHRSP was 140 mm Hg but for SHR 133 mm Hg. There was no significant linear correlation of lesion size with the preocclusion blood pressure value in SHR.

Relationship of Infarction Trait to Hypertension

The relationship of the trait of infarction after MCA occlusion to hypertension is not clear. The trait may relate to either the mechanism responsible for initiating hypertension or to the increase in wall stress resulting from elevated blood pressure. SHR and SHRSP are hypertensive as adults. Both rat types were tested before the established phase of hypertension which is accompanied by vascular structural change including hyaline degeneration, fibrinoid necrosis, and thrombotic occlusions that would most likely impede collateral circulation via the dorsal cerebral anastomoses. Such vascular structural defects could not be responsible for the large infarct since they do not exist in 5-6 week old rats.

Newborn SHR have higher blood pressure, larger hearts and there is evidence 14-15 day old SHR have thicker walled blood vessels, than normotensive animals. Tail systolic blood pressure in 5-6 week old SHR was elevated above that of SD in this study and similar BP differences were found for young SHR compared to normotensive WKY by others. Vascular differences resulting in increased resistance to collateral blood flow may make SHR and SHRSP, even at 5-6 weeks of age, susceptible to infarction after MCA occlusion. Clearly, this susceptibility in SHR and SHRSP was not dissociated in time from the initiation period for hypertension and its associated vascular differences.

Morbidity, Premature Death and the Infarct

All SHR and SD who became morbid or died received Evans blue by the intraperitoneal route. Premature death did not appear to be secondary to the process of infarction because sham operated rats and rats without infarcts died, yet not one of 5 SHR having an infarct died, even though each received Evans blue intravenously. High doses of Evans blue administered intraperitoneally may be linked to the morbidity and deaths although the mode of action is an enigma. Evans blue does not initiate cerebral lesion development because hypertensive rats never receiving the dye infarct after MCA occlusion and survival rate for months is high (unpublished observation).

Rat Weights

Sprague-Dawley rats were significantly heavier than the slightly older SHR animals. This was not unexpected because inbred rats are usually lighter in weight than outbred animals. Whereas the correlation value between body weight and lesion size in SHR was positive, the value was insignificant. There is no evidence that body weight per se is a meaningful predictor of lesion size in SHR.

Outcome to MCA Occlusion in Sprague-Dawley Rats

Young Sprague-Dawley rats were protected from large infarcts by collateral circulation. This finding is compared with data obtained by others. Tamura and coworkers occluded the MCA proximal to striate branches in adult Sprague-Dawley rats. Blood flow to cortex distal to the occluded vessel was reduced to 13 percent of control levels. Their data were for collateral blood flows estimated 30 minutes after MCA occlusion involving older rats and a larger collateral field that included not only the cortex but the caudate nucleus supplied by striate branches. Histologic evidence for an infarct was not presented. Robinson and coinvestigators occluded the MCA in adult SD at approximately the same location as done here. Cortical lesion diameters were estimated 5, 20, or 40 days after the occlusion. Lesion size varied from 0.8 to 19.6 mm² in adult Sprague-Dawley rats.

Lesion size range for young SD of this study was not unlike that of adult SD studied by the Robinson group. A precise comparison of lesion size can not be made because neither the mean lesion size nor the lesion size for individual rats was reported. Nor was cortical injury due to surgery of exposing the MCA differentiated from that resulting from the MCA occlusion.
In the current study with one SD having a 19.0 mm² lesion, the infarct was most likely the result of MCA occlusion. In the remaining 10 SD, all with lesions less than 6.0 mm², there was injury due to surgery necessary to expose and ligate the MCA, but no infarct occurred as a result of the MCA occlusion. As reported by others for human, MCA occlusion does not necessarily carry a poor prognosis.

Closing Statements

After rapid MCA occlusion, 5–6 week old Sprague-Dawley rats (SD) had a small lesion marked by Evans blue in cortex adjacent to the craniectomy. The SD had no detectable neurological deficit. An adequate collateral circulation protected SD for there was no large infarct in the MCA collateral field distal to the occlusion. The small lesion at the craniectomy site was the result of surgery necessary to expose and ligate the MCA. In contrast, spontaneously hypertensive rats (SHR) and the spontaneously hypertensive stroke-prone rat (SHRSP), each with systemic blood pressure significantly greater than in normotensive controls, develop a large cortical infarct marked by Evans blue. Motor deficits contralateral to the occluded vessel accompanied the lesion. The infarct appears to be the consequence of inadequate collateral circulation. That the MCA occlusion test outcome was similar in two lines of spontaneously hypertensive rats rules out the SHRSP being a unique model and suggests the susceptibility to infarction may be linked to the mechanism responsible for elevated blood pressure in young spontaneously hypertensive rats.

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References

CEREBRAL INFARCTION IN RATS/Coyle

The Effect of Dichloroacetate on Brain Lactate Levels Following Incomplete Ischemia in the Hyperglycemic Rat

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SUMMARY Dichloroacetate (DCA) is known to prevent the phosphorylation of the pyruvate dehydrogenase complex (PDHC) by blocking the action of PDH kinase. This action allows the active PDHC to exert its effect on the metabolism of glucose, lactate and alanine to acetyl CoA. DCA has been shown to reduce serum lactate levels in humans and animals in such conditions as diabetes, phenformin-induced hepatic failure, exercise, and endotoxin-induced shock. Lactic acidosis in the brain has often been postulated as a cause of neuronal damage following ischemia and hypoxia. Therefore, we examined the effect of intravenously administered DCA (100 mg/kg) in rats that were rendered hyperglycemic by intravenous glucose (2 g/kg), and then made to undergo 15 minutes of incomplete cerebral ischemia by bilateral carotid ligation and systemic hypotension (mean arterial pressure of 50 mm Hg). DCA significantly reduced serum lactate levels pre-ischemia, but had no effect on serum lactate levels after ischemia induction. Brain levels of lactate, ATP and PCr after 15 minutes of incomplete ischemia were unaffected by DCA. We conclude that in this in-vivo model the control of PDHC activity in the brain may be different than that in the periphery, and that DCA was not effective in reducing brain tissue lactate levels.

The Effect of Dichloroacetate (DCA) has been shown to reduce serum lactate levels in pathological conditions such as diabetes, phenformin-induced hepatic failure and endotoxin induced shock. Its mechanism of action appears to be as an inhibitor of the PDHC kinase which phosphorylates PDHC into an inactive form. PDHC is a rate-limiting enzyme in the conversion of glucose, lactate and alanine to acetyl CoA. DCA has been shown to reduce tissue lactate levels in heart, diaphragm, muscle and liver. Little work has been done on its effect on brain metabolism.

Rehncrona et al have postulated that brain damage following ischemia may be due in part to excess tissue lactate build-up and intracellular changes in pH. Hyperglycemia in the setting of cerebral ischemia in the rat, cat and monkey has been shown to be deleterious and brain tissue lactate levels have been higher. The present study was designed to see if DCA administered prior to the induction of cerebral ischemia could reduce the brain tissue lactate elevations found by previous workers. In order to maximize the brain lactate levels so as to increase any possible effect that DCA might have, rats were rendered hyperglycemic prior to ischemia induction. Since Eklof has shown inhomogeneity in cerebral blood flow reduction in incomplete ischemia in the rat under normotensive conditions, rats were rendered hypotensive (mean arterial blood pressure of 50 mm Hg) by withdrawal of blood at the induction of incomplete ischemia which was accomplished by bilateral carotid ligation; a model in which severe reduction of cerebral blood flow in forebrain cortical structures has been shown.

Materials and Methods

Wistar rats of either sex weighing 350—450 gms were fed ad libitum until the time of surgery. Anesthesia was induced and maintained with 0.6% halothane and 30% O2. Mechanical ventilation was maintained after intubation and administration of pancuronium (2 mg/kg IV), supplemented hourly (1 mg/kg). Catheters were placed in both femoral arteries and one femoral vein. Arterial pressure was monitored continuously (P23 ID Statham) and displayed on a strip chart recorder (Gould Brush 2400). Serial arterial blood gases were performed (Radiometer Mark II) and ventilator adjustments made to maintain PO2, PCO2 and pH in a physiological range. Temperature was measured via rectal probe and maintained at 37° by heat lamp. Both carotid arteries were dissected in the neck for later ligation. An incision was made in the scalp to facilitate subsequent freezing of the brain.

Two groups of rats were then studied. In the first group (n = 7) rats received 100 mg/kg DCA as an intravenous bolus at time 0 and at 75 minutes an intra-
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