Dorsal Cerebral Collaterals of Stroke-Prone Spontaneously Hypertensive Rats (SHRSP) and Wistar Kyoto Rats (WKY)

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ABSTRACT Earlier studies established that stroke-prone spontaneously hypertensive rats (SHRSP) invariably infarct after middle cerebral artery (MCA) occlusion. Normotensive rats are usually protected from infarction after the occlusion. Objectives of this study were to characterize the anastomosing collaterals that may determine the different outcomes to MCA occlusion in SHRSP and Wistar Kyoto rats (WKY). Young (5–10 week) and old (40–69 week) rats of each sex were anesthetized, then administered papaverine to produce maximal vasodilatation of the cerebrovascular bed. Under control conditions latex was injected into the arterial tree to measure the internal diameter of branches of the anterior cerebral artery (ACA), the MCA, and the ACA-MCA anastomosing collaterals. Large diameter ACA and MCA rami in old, but not young, SHRSP were significantly smaller in diameter than the respective ACA and MCA branches in old WKY. The number of ACA-MCA anastomoses was the same for SHRSP and WKY. Mean internal diameter of the ACA-MCA anastomoses was significantly ($p < 0.0001$) smaller in SHRSP than WKY in both age groups. There were significant negative correlations between age and 1) the internal diameter of the ACA-MCA anastomoses in WKY but not SHRSP, and 2) the largest diameter ACA and MCA rami in SHRSP but not WKY. The findings suggest that vascular resistance of fully relaxed collaterals is greater in SHRSP than WKY, thereby compromising the dorsal collateral circulation before large diameter vessel changes occur that accompany the established form of hypertension.

Sudden occlusion of the middle cerebral artery (MCA) just above the rhinal fissure reduces blood flow to the territory of the occluded MCA and invariably results in infarction in the stroke-prone spontaneously hypertensive rat (SHRSP) Coyle and Heistad, 1986; Coyle and Jokelainen, 1983). Normotensive rats usually do not have infarction after this occlusion (Bederson et al., 1986; Chen et al., 1986; Coyle, 1986; Coyle and Jokelainen, 1983; Coyle, 1982). Following MCA occlusion in normotensive rats, the anastomoses that provide protective collateral supply enlarge with time, and blood flow to the territory of the occluded MCA returns to virtually normal levels (Coyle, 1984; Coyle, 1985; Coyle and Heistad, 1987). Objectives of this study were to characterize the anastomosing collaterals that may account, at least in part, for infarction in SHRSP and protection against it in Wistar Kyoto rats (WKY) after middle cerebral artery occlusion.

METHODS

Animal Preparations

Six SHRSP and 5 WKY, including members of each sex, with ages ranging from 5–10 weeks (young), 23 weeks, and 40–69 weeks (old) were anesthetized with ketamine hydrochloride (136 mg/kg body weight, i.m.). Skin and periosteum over the skull were reflected. Bone overlying the occipital cortex 0.5–3 mm from the midsagittal suture and at similar distance from the lambdoidal suture was thinned with a dental burr to permit microscopic observation of the collaterals through a closed cranial window. The observation permitted assessment of vessel filling during latex injection. Details of the procedure are given elsewhere (Coyle and Jokelainen, 1982).

Visualization of Vessels

Papaverine hydrochloride (40–50 mg/kg body weight in water) was injected intravenously to standardize all vessels at maximal vasodilatation and for animal sacrifice. Undiluted Vultex, a white latex-based compound (Chicago Latex Products No. 563) was warmed to 38–42°C and injected through an 18-gauge cannula in the ascending aorta in order to visualize the lumenal size and course of cerebral arterioles. The right atrium was not opened because venous back pressure facilitates vessel filling. Thirty to 45 minutes after latex injection, brains were removed from the skull and fixed in 10% neutral buffered formalin. All brains were photographed in the standard dorsal orientation (see Fig. 1). Prints were made at 35–40 X for measurements.

Measurements and Computations

Dorsal collaterals join distal branches of the major cerebral arteries in the rat (Coyle and Jokelainen, 1982).

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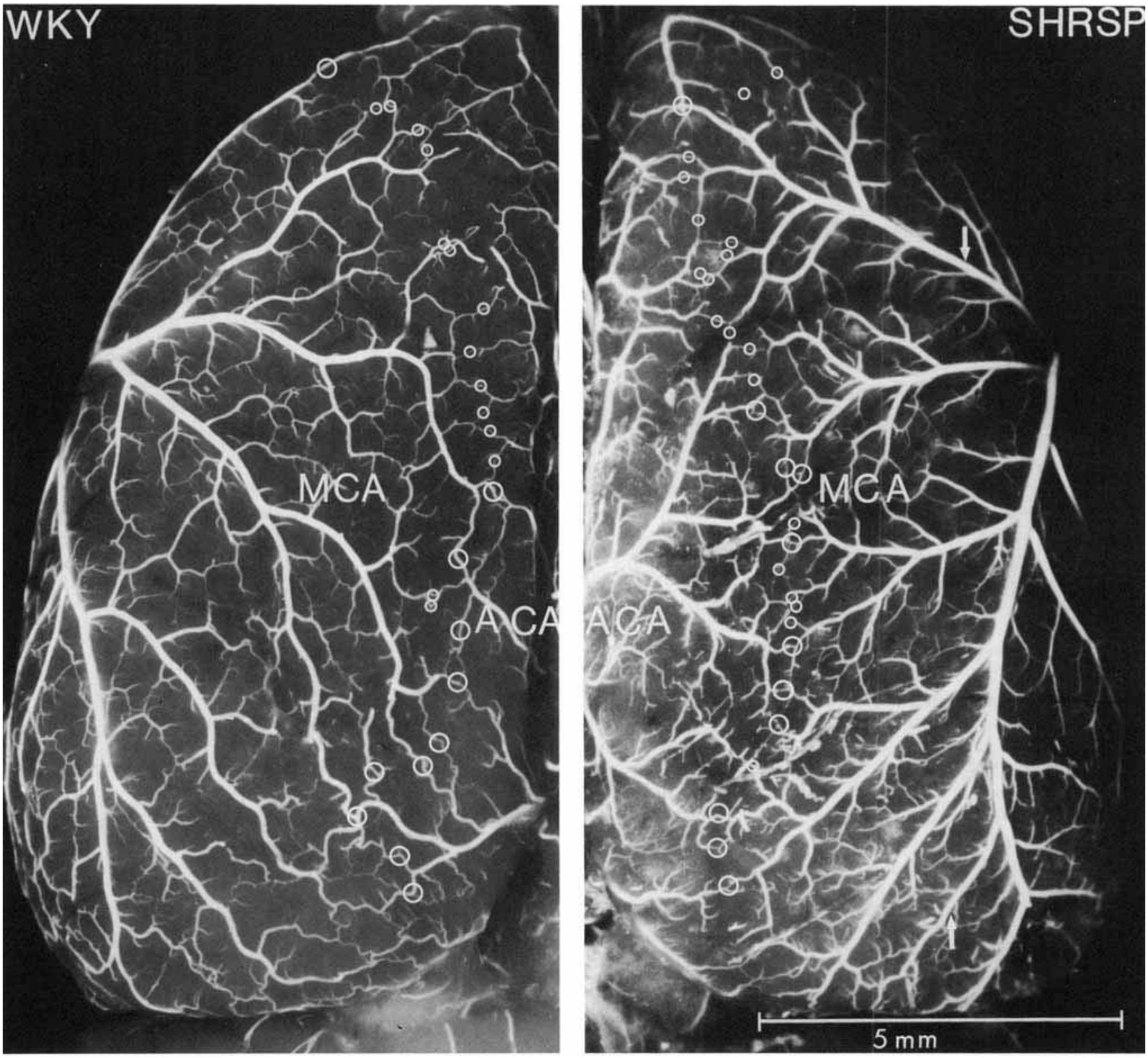

Fig. 1. Branches of the middle cerebral artery (MCA) anastomose (at circles) with branches of the anterior cerebral artery (ACA) on the dorsal aspect of a cerebral hemisphere in a 10-week-old Wistar Kyoto rat (WKY) and a 5-week-old stroke-prone spontaneously hypertensive rat (SHRSP). Anastomoses are distributed from the frontal region (top of figure) into the occipital region (bottom of figure).

An anastomosis is the site of smallest internal diameter of a collatral or one-half the distance along the vessel with opposing branch angles (see Figs. 1, 3).

Coordinates (x,y) on the lumenal boundary (at 0 and 180 degrees) of a vessel were sampled with a Summagraphics Corporation Bit Pad Digitizer interfaced to a Commodore Microcomputer (Coyle, 1981). Vessel internal diameter was computed as the straight line distance between the 0 and 180 degree points. Diameters were calculated for the 10 largest branches of the anterior cerebral artery (ACA), the 10 largest rami of the MCA, and all the ACA-MCA anastomoses. The measured ACA branches were located between the midline and ACA-MCA anastomoses (see circles, Fig. 1). Mean distance from the midline to the measured MCA branches is indicated by the arrows with orientation parasagittal to the midline in Figure 1.

Statistical Procedures

Internal (lumenal) diameter values of the ACA and MCA branches and the ACA-MCA anastomoses were averaged for each hemisphere. The hemisphere values were averaged and stated as the mean ± sem. Tables 1 and 2 state the numbers of hemispheres included in each

TABLE 1. Parameters for rats

Variable	WKY Young	WKY Old	SHRSP Young	SHRSP Old
Age range (weeks)	8–10	50	5	40–69
No. hemispheres	4	6	4	4
Mean internal diameter largest ACA branches (μm)	88 ± 3	86 ± 2	93 ± 4	65 ± 3**
Mean internal diameter ACA-MCA anastomoses (μm)	55 ± 3	47 ± 1*	32 ± 2	32 ± 1
Mean internal diameter largest MCA branches (μm)	107 ± 3	102 ± 5	117 ± 7	81 ± 4**

*$p < 0.01$ young vs old.
**$p < 0.0001$ young vs old.

analysis. Two SHRSP hemispheres were unsuitable for measurements because of vessel damage or incomplete vessel filling. Data for the 23-week old rat was included in the correlation analysis, but the data was excluded from the comparison of vessel diameters in young (5–10 week) and old (40–69 week) rats because of the intermediate age. Vessel size comparisons were made with an analysis of variance (ANOVA) test. P-values under 0.05 were considered to be significant.

RESULTS

Locations and Relationships of Vessels

Branches of the azygos anterior cerebral artery (ACA) course from the longitudinal fissure to join rami of the middle cerebral artery (MCA) (Fig. 1). Mean distance from the midline to the measured MCA branch was 5.3 ± 0.2mm for WKY and 5.1 ± 0.3mm for SHRSP (Fig. 1). The general location and pattern of distribution of the ACA-MCA anastomoses on the dorsal aspect of the hemisphere are shown for WKY and SHRSP in Figure 1. High magnification photographs (see Fig. 3) were necessary to confirm the presence of the junctions and for internal diameter measurements of the vessels.

Number and Distribution of Anastomoses

Mean number of ACA-MCA anastomoses per hemisphere was 27 ± 1 for SHRSP and 26 ± 1 for WKY. Mean distance from the midline to the anastomoses was not appreciably different for SHRSP (2.1 ± 0.1 mm) and WKY (2.3 ± 0.0 mm). Density of anastomoses on the longitudinal axis was not grossly different for SHRSP and WKY.

Internal Diameter of Vessels in Young and Old Rats

The largest ACA and MCA branches in young SHRSP were not appreciably different in internal diameter from the largest ACA and MCA branches in young WKY (Table 1, Fig. 2A). The ACA-MCA anastomoses in SHRSP were significantly smaller ($p < 0.0001$) in lumenal diameter than the anastomoses in young and old WKY (Fig. 2A,B). The largest ACA and MCA branches in old SHRSP were significantly smaller ($p < 0.0001$) in diameter than the largest ACA and MCA branches in old WKY (Fig. 2B).

Vessel Configurations

In SHRSP the collateral arteriole that bridges distal rami of the ACA and MCA was often the last branch before the supply ramus became a cortical penetrating arteriole (Fig. 3B,C). Such collaterals in SHRSP were often less than one-half the internal diameter of the cortical penetrating arteriole. These vessels may present a high resistance bottleneck. Collateral vessels in WKY were larger in diameter than the cortical penetrating arterioles (Fig. 3A).

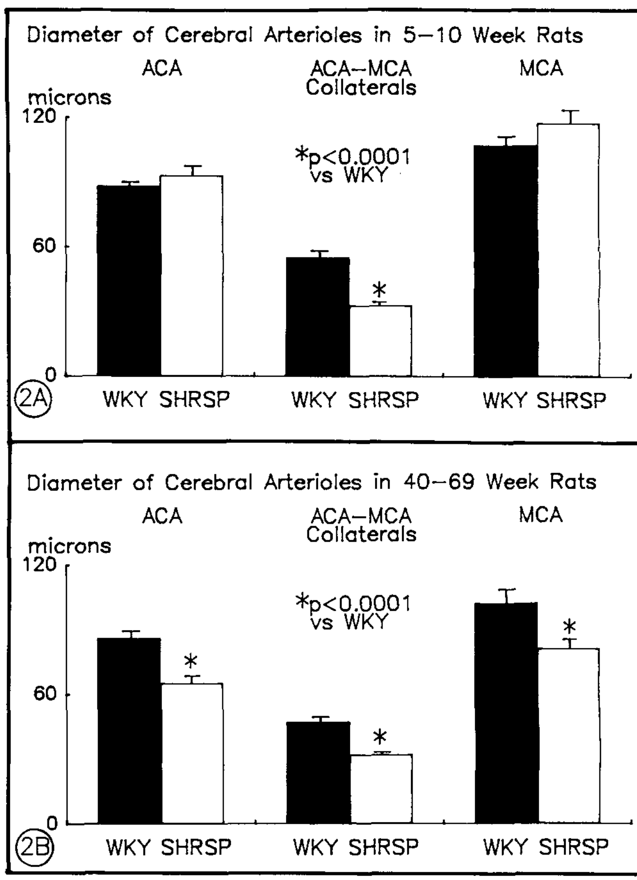

Fig. 2. Large diameter branches of the anterior (ACA) and middle (MCA) cerebral arteries, respectively, do not differ appreciably in internal diameter in young WKY and young SHRSP (**2A**), but do differ in size in old WKY and SHRSP (**2B**). Anastomosing ACA-MCA collaterals are smaller in internal diameter in SHRSP than WKY in both age groups (2A and 2B).

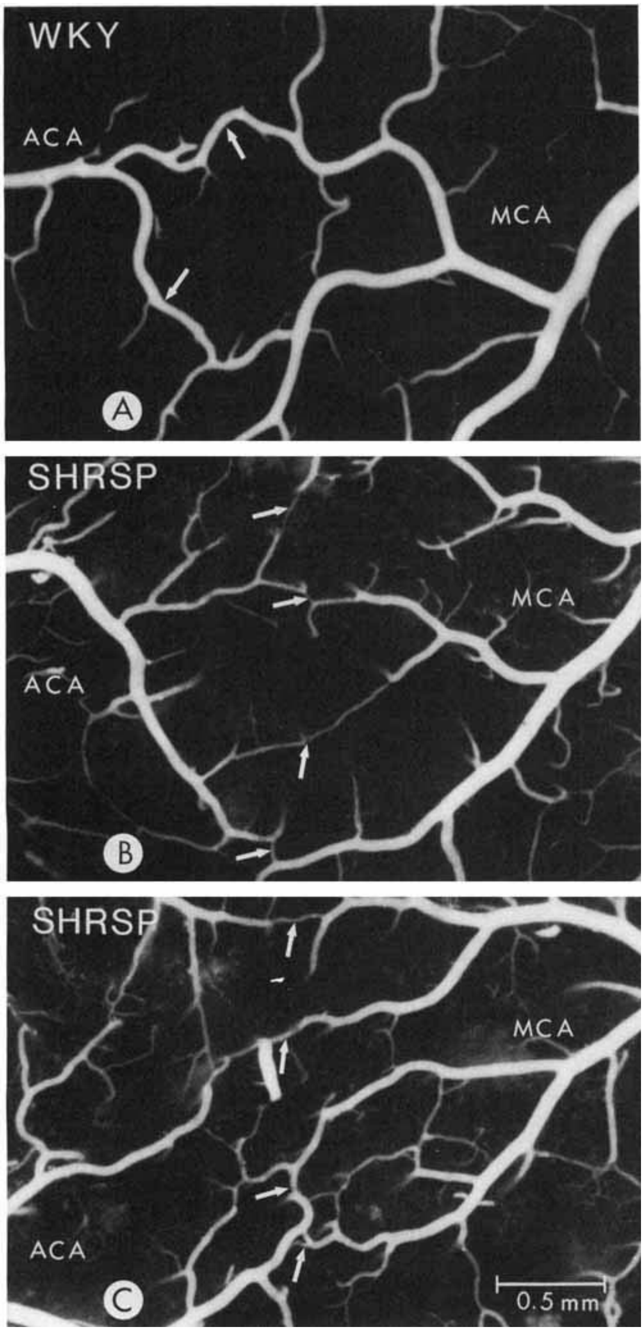

Fig. 3. End-to-end anastomosing branches of the ACA and MCA in an 8-week-old WKY are larger in internal diameter than cortical penetrating branches (A). In a 5-week-old SHRSP the ACA-MCA anastomoses (arrows) are often less than 50 percent of the internal diameter of the supply vessels (B), and the collaterals are smaller in diameter than cortical penetrating arterioles (B and C).

Vessel Size and Age Relationships

There were significant negative correlations between age and 1) size of the largest ACA and MCA branches in SHRSP, and 2) diameter of the ACA-MCA anastomoses in WKY (Table 2). There was no significant linear

TABLE 2. Correlation values for vessel size versus age

Vessel location	n	WKY	n	SHRSP
ACA	10	−.1806	10	−.8391**
ACA-MCA anastomoses	10	−.7820**	10	−.3120
MCA	10	−.2312	10	−.7080*

*$p < 0.05$ correlation greater than zero.
**$p < 0.01$ correlation greater than zero.

correlation of lumenal size with age for the largest ACA and MCA branches of WKY and the ACA-MCA anastomoses of SHRSP. Thus, the larger cerebral rami in SHRSP and the collateral arterioles in WKY are smaller in diameter in older animals than younger rats.

DISCUSSION

This study establishes three major findings. 1. The anastomoses that join distal branches of the anterior and middle cerebral arteries are no greater in number in WKY than SHRSP, but the ACA-MCA anastomoses are smaller in internal diameter in SHRSP than WKY. This finding suggests that in fully relaxed collateral arterioles, the resistance is greater in SHRSP than WKY. 2. The large diameter cerebral branches in SHRSP decrease in internal diameter with aging. 3. The collaterals in WKY decrease in internal diameter with aging. The findings suggest that the resistance of these vascular segments when fully relaxed is greater in old animals than in young ones.

Consideration of Methods

Vessels fixed in formalin are not easily compared for maximum diameters using currently available methods. Papaverine was used to standardize all vessels at maximal dilatation and to minimize resistance for latex filling. Passive vessels are smaller than fully distended vessels. Complete distension of latex-filled vessels depends on the elastic properties of the vessel wall, the viscosity of the latex, and the transmural pressure. Because of high viscosity of the latex compared to blood and the lack of vascular tone, aortic pressures meaningful for perfusion by blood may be particularly misleading if applied during injection of the latex.

Underfilling of the vascular tree is detected by observing media ending abruptly in a vessel and the discontinuous pattern is evidence of a more distal unfilled vascular segment (Coyle and Jokelainen, 1982). Sudden changes in vessel diameter at a nonbranch site is evidence of incomplete distension. Overdistension occurs after the most distal pial arterioles are filled and results in vessel rupture with leakage of the latex. Thus, microscopic observation through the closed cranial window during latex injection monitored vessel filling in the most distal arterial branches to insure adequate filling and to prevent vessel bursting. The finding that the largest cerebral arterioles were not appreciably different in internal diameter in young SHRSP and young WKY is evidence that the vessels were equally distended in the two strains of rats.

Relationships to Findings by Others

In vivo study of 18- to 21-week-old rats revealed that passive diameters of MCA branches (1A-4A) in SHR were not significantly different in size from those of WKY, providing the animals were at their natural arterial blood pressure, and the suggestion was made that arteriolar distensibility is decreased in SHR (Harper and Bohlen, 1984). There was no significant difference in passive size of large diameter pial arterioles studied in vivo in 3–4 month old SHRSP (104 ± 6 μm) and WKY (109 ± 3 μm) (Baumbach et al., 1986). In 6–8-month-old SHRSP, passive diameters of the large pial arterioles were less in SHRSP (94 ± 5 μm) than WKY (105 ± 4 μm) and stress-strain analysis indicated that the distensibility was increased in large diameter pial branches of SHRSP with established hypertension despite vascular hypertrophy (Baumbach et al., 1986). Thus, the in vivo finding of smaller diameter arterioles in old versus young SHRSP corroborates the negative correlation of large branch size with aging in formalin-fixed tissue of the current study. The ACA-MCA anastomoses being of smaller diameter in SHRSP than WKY suggests either the collaterals were not fully distended or that fully distended collaterals are smaller in diameter in SHRSP than in WKY, possibly as a result of vessel hypertrophy or changes secondary to hypertension (Hart et al., 1980; Nordborg and Johansson, 1980; Nordborg et al., 1985). Neither possibility was ruled out.

Closing Comments

The significant negative correlation of ACA-MCA anastomosis size with age of WKY suggests that the resistance of the dorsal collaterals increases in aging WKY. Of 12 WKY, 5–7 months of age, none had infarction after MCA occlusion, and 1 month later blood flow to the territory of the occluded MCA was virtually normal (Coyle and Heistad, 1987). If collateral circulation to the territory of the occluded MCA becomes compromised in WKY, then the change must occur after 28 weeks of age.

There being no correlation of ACA-MCA anastomosis size with age for SHRSP suggests that the collaterals do not increase minimal resistance after 5 weeks of age. This notion is supported by findings that infarction size after MCA occlusion is no greater in 7–8-month-old SHRSP with established hypertension than in 5-week-old SHRSP (Coyle and Heistad, 1987; Coyle and Jokelainen, 1983). Thus, after 5 weeks of age resistance increases in fully relaxed vessels of SHRSP may occur in larger diameter pial surface arterioles, but not in the dorsal collaterals.

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LITERATURE CITED

Baumbach, G.L., P.B. Dobrin, M.N. Hart, and D.D. Heistad (1986) Cerebral vascular mechanics in early and established hypertension. Fed. Proc. *45:*289.

Bederson, J.B., L.H. Pitts, M. Tsuji, M.C. Nishimura, R.L. Davis, and H. Bartkowski (1986) Rat middle cerebral artery occlusion: Evaluation of the model and development of a neurologic examination. Stroke *17:*472–476.

Chen, S.T., C.Y., Hsu, E.L. Hogan, H. Maricq, and J.D. Balentine (1986) A model of focal ischemic stroke in the rat: Reproducible extensive cortical infarction. Stroke *17:*738–743.

Coyle, P. (1981) PET interface to BIT PAD. Micro- The 6502/6809 Journal. *38:*83–85.

Coyle, P. (1982) Middle cerebral artery occlusion in the young rat. Stroke *13:*855–859.

Coyle, P. (1984) Diameter and length changes in cerebral collaterals after middle cerebral artery occlusion in the young rat. Anat. Rec. *210:*357–364.

Coyle, P. (1985) Interruption of the middle cerebral artery in 10-day-old rat alters normal development of distal collaterals. Anat. Rec. *212:*179–182.

Coyle, P. (1986) Different susceptibilities to cerebral infarction in spontaneously hypertensive (SHR) and normotensive Sprague-Dawley rats. Stroke *17:*520–525.

Coyle, P., and P.T. Jokelainen (1982) Dorsal cerebral arterial collaterals of the rat. Anat. Rec. *203:*397–404.

Coyle, P., and P.T. Jokelainen (1983) Differential outcome to middle cerebral artery occlusion in spontaneously hypertensive stroke-prone rats (SHRSP) and Wistar Kyoto (WKY) rats. Stroke *14:*605–611.

Coyle, P., and D.D. Heistad (1986) Blood flow through cerebral collateral vessels in hypertensive and normotensive rats. Hypertension *8* [Suppl II]:II-67–II-71.

Coyle, P., and D.D. Heistad (1987) Blood flow through cerebral collateral vessels one month after middle cerebral artery occlusion. Stroke *18:*No. 2 (in press).

Harper, S.L., and H.G. Bohlen (1984) Microvascular adaptation in cerebral cortex of adult spontaneously hypertensive rats. Hypertension *6:*408–419.

Hart, M.N., Heistad, D.D., and M.J. Brody (1980) Effect of chronic hypertension and sympathetic denervation on wall/lumen ratio of cerebral vessels. Hypertension *2:*419–423.

Nordborg, C., and B.B. Johansson (1980) Morphometric study on cerebral vessels in spontaneously hypertensive rats. Stroke *11:*266–270.

Nordborg, C., K. Fredriksson, and B.B. Johansson (1985) The morphometry of consecutive segments in cerebral arteries of normotensive and spontaneously hypertensive rats. Stroke *16:*313–320.