Effect of hypoxia on percent of arteriolar and capillary beds perfused in the rat brain

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FRANCOIS-DAINVILLE, ERNEST, ELLEN BUCHWEITZ, AND HARVEY R. WEISS. *Effect of hypoxia on percent of arteriolar and capillary beds perfused in the rat brain.* J. Appl. Physiol. 60(1): 280–288, 1986.—The effects of moderate and severe hypoxia on quantitative regional morphometric indexes of the total and perfused arteriolar and capillary network were studied in the rat brain to determine whether diffusion distances were reduced in hypoxia. Fluorescein isothiocyanate (FITC)-labeled dextran was injected into the femoral vein of conscious control and hypoxic rats. After 20 s, the animal was decapitated and the head was frozen in liquid N_2. Sections from eight brain regions were photographed to detect the perfused microvessels and then stained for alkaline phosphatase to visualize the total vascular network. There were significant increases in percent perfused arteriolar and capillary morphology between the two groups of hypoxic animals and control animals. In control rats, the percent of capillaries perfused averaged 45.6 ± 0.6% (mean ± SE). In moderate hypoxia 63.4 ± 1.8% of the vessels were perfused and in severe hypoxia 89.4 ± 0.1% were perfused. The percentage of arterioles perfused changed similarly. There were no significant differences in any index of total or percent perfused arteriolar or capillary morphometry among the regions within any group. During severe hypoxia, a greater percentage of the capillary reserves was utilized. These results demonstrate a uniform response to hypoxia in the capillary and arteriolar network of the conscious rat brain.

brain; cerebral hypoxia; cerebral capillary morphometry; cerebral arteriolar morphometry; rat unanesthetized

ARTERIAL HYPOXEMIA CAUSES a lowering of cerebral tissue P_{O_2} and increases in the level of cytochrome and NAD$^+$-NADH reduction (8, 14, 16, 29). To maintain cerebral metabolism and high energy phosphate levels (13, 17, 23, 28) there must be a cardiovascular response. This cardiovascular response to hypoxia could involve both an increase in cerebral blood flow through currently perfused vessels and/or an increase in the number of perfused vessels, which results in a reduction in diffusion distances. It has been demonstrated that hypoxia increases cerebral blood flow (9, 10, 20, 21, 28), but whether the number of perfused vessels increases is not entirely clear. Weiss and Edelman (30) had reported an increase in the number of perfused cerebral capillaries during hypoxia using a qualitative technique. Similar increases in the utilization of available capillaries during hypoxia in brain and other organs using different methods have also been reported (4, 8, 19, 20). All cerebral capillaries appear to be perfused during asphyxia (27). It is not clear, however, whether the number of capillaries perfused can vary with the degree of hypoxia. While pial arterioles have been reported to dilate in response to hypoxia and hypercapnia (3, 15), there are no reports as to whether previously unperfused arterioles are recruited. While increases in cerebral blood flow are probably the brain's main response to hypoxia, reduction in diffusion distances can also provide additional O_2 transport. The purpose of the present report was to determine whether moderate and severe hypoxia produced a graded decrease in the number of perfused vessels in the arteriolar and capillary bed of the brain.

To compare various indexes of perfused and total arteriolar and capillary bed morphology in the rat brain, we have employed a method that simultaneously and quantitatively determines capillary and arteriolar volume, length, and surface area as well as number per square millimeter, and diameter (27). The method involves the systemic injection of a high molecular weight fluorescent labeled dextran which remains in the cerebral circulation and selectively labels the perfused arteriolar and capillary bed. The total arteriolar and capillary beds were identified through alkaline phosphatase staining of tissue isolated from specific brain regions.

METHODS

These studies were conducted on 21 Long-Evans rats of either sex ranging in weight from 350 to 500 g. The animals were anesthetized with ether and gently restrained with a loosely fitting plaster cast. A catheter was placed into a femoral artery and a femoral vein. The arterial catheter was connected to a Statham P23AA pressure transducer and records of arterial pressure and heart rates were obtained on a Beckman R-411 recorder. This catheter was also used to obtain a 0.3-ml anaerobic arterial blood sample. This sample was subsequently analyzed for P_{O_2}, P_{CO_2} and pH on a Radiometer BMS 3 blood gas analyzer. The venous catheter was used to inject fluorescein isothiocyanate-dextran (FITC-dextran), 70,000 mol wt. After surgical preparation, the ether inhalation was stopped and the animals subsequently regained consciousness. The experiments were performed three hours later.

The rats were divided into three experimental groups of seven animals each. One group served as controls; one group breathed 10% O_2-90% N_2 for 10 min; one group breathed 6% O_2-94% N_2 for 10 min. Mean arterial blood pressure and heart rate were recorded before and during treatment. Blood was sampled prior to and at the end of each experimental period.

The experimental protocol to determine microvascular morphometric indexes was identical in each group of animals. Approximately 100 $mg \cdot kg^{-1}$ of FITC-dextran (Sigma Chemical) was administered intravenously as a 0.5 ml bolus and flushed with 0.5 ml of saline. Twenty seconds after this injection, the rats' heads were guillotined and quickly dropped into liquid N_2. The rats' heads were stored at −70°C until analyzed.

All brains were exposed by cutting the head into wafers on a band saw at −20°C. The following regions were then isolated from the wafers and prepared for cutting on a microtome cryostat: cortex, hypothalamus, thalamus, lenticulate nuclei, hippocampus, cerebellum, pons, and medulla. The tissue sections were then mounted on a microtome specimen holder and coated with embedding medium for frozen tissue specimens (OCT compounds, Lab-Tek Prod). Sections of the tissue specimens (2 μm thick) were cut on a Slee automated microtome-cryostat set at −35°C and transferred to previously marked glass slides. The slides had been gently scratched with a diamond point to facilitate relocation. The sections were allowed to dry at room temperature for 1 h. Eight to 10 sections were obtained from each examined brain region. Each section was at least 200–300 μm from the previous one.

Photographs were obtained on a Zeiss fluorescent microscope equipped for automated photography. A ×40 planapochromat objective was used with a numerical aperture of 0.95 to examine the capillary network, and a ×10 objective was used to examine the arteriolar network. The slides were epi-illuminated with violet light from a 100W halogen source to excite the fluorescence of the FITC-dextran. A barrier filter was placed in the viewing field such that only wavelengths >495 nm were seen. This provided excellent viewing of the FITC-dextran filled vessels. A second photograph of the field was taken with normal lighting. This, together with the viewing coordinates obtained, helped to relocate the field. A photograph of the FITC-dextran and alkaline phosphatase-stained fields is shown in Fig. 1.

The slides were then stained for alkaline phosphatase (27). The slides were placed in buffered sucrose-formaldehyde solution for 1 min. The slides were washed twice in distilled water and then placed in freshly made, prewarmed incubation mixture for 30 min at 37°C. The incubation mixture consisted of 3.8 g/l sodium metaborate, 1.7 g/l magnesium sulfate, 1.3 g/l fast blue RR, and 0.4 g/l α-napthylphosphate dissolved in distilled water. The slides were then rinsed in distilled water, postfixed in buffered sucrose-formaldehyde solution and rerinsed. The region photographed with fluorescent light was relocated and a new photograph was obtained. This photograph identified the total microvascular bed. These photographs were then marked to show that portion of the capillary and arteriolar network which was perfused.

Various stereological determinations were obtained in all groups. Each field was counted twice, once for the total capillaries or arterioles and once for the perfused ones. The photographic negative was projected onto a Weibel stereological device with an appropriate grid. The fundamental principles of morphometric analysis have been reviewed (25, 26). These principles have been applied to determine parameters of interest concerning the capillary arteriolar network of the brain (1, 12).

We determined the volume fraction (V_V in mm^3/mm^3) of capillaries and arterioles by a point counting technique; $V_V = P_c/P_t$, where P_c and P_t are the number of test points falling within a profile of a capillary or arteriole and the total number of test points in the grid, respectively. We selected the number of test points so that the probable error in V_V would be <±5% for capillaries and ±7.5% for arterioles (26). The surface-to-volume ratio (S_V in mm^2/mm^3) was estimated from the number of intersections of capillaries or arterioles with a series of test lines (25). S_V was determined by the equation $S_V = 2I/L_t$, where I is the number of intersections of capillaries or arterioles with a series of test lines whose total length is L_t. The number per square millimeter was obtained by counting the number of microvessel profiles in the test area.

We have determined the average diameter (D) of capillaries and arterioles through measurement of the minimum diameter of any vessel cut in transverse section, as long as the maximum diameter was no more than 1.5 times that of the minimum. All vessels with minimum diameters above 12 μm were eliminated from all counting procedures for capillaries, and the vessels with diameters in the range of 19–50 μm were counted for arterioles. This technique allows accurate estimates of the diameter of total and perfused microvessels; it will determine edge to edge diameter, including the lumen and two endothelial walls.

A factorial analysis of variance with repeated measures design was used to determine whether differences in the measured parameters existed between control animals and hypoxic animals, and among the various brain regions. The statistical significance of the differences was determined by the Duncan post hoc procedure. A value of $P < 0.05$ was accepted as significant. All values are presented as means ± SE.

RESULTS

The blood gas and systemic hemodynamic parameters in control and hypoxic Long-Evans rats are presented in Table 1. In the control group, and prior to the hypoxic state in the experimental groups, arterial blood gases and pH were not significantly different. During hypoxia, arterial O_2 tension (Pa_{CO_2}) was significantly lower in the hypoxic rats compared with the control rats. During moderate hypoxia, Pa_{O_2} was ~55% of the control value and in the severely hypoxic group it was 40% of control. Arterial CO_2 tension (Pa_{CO_2}) was significantly lower in

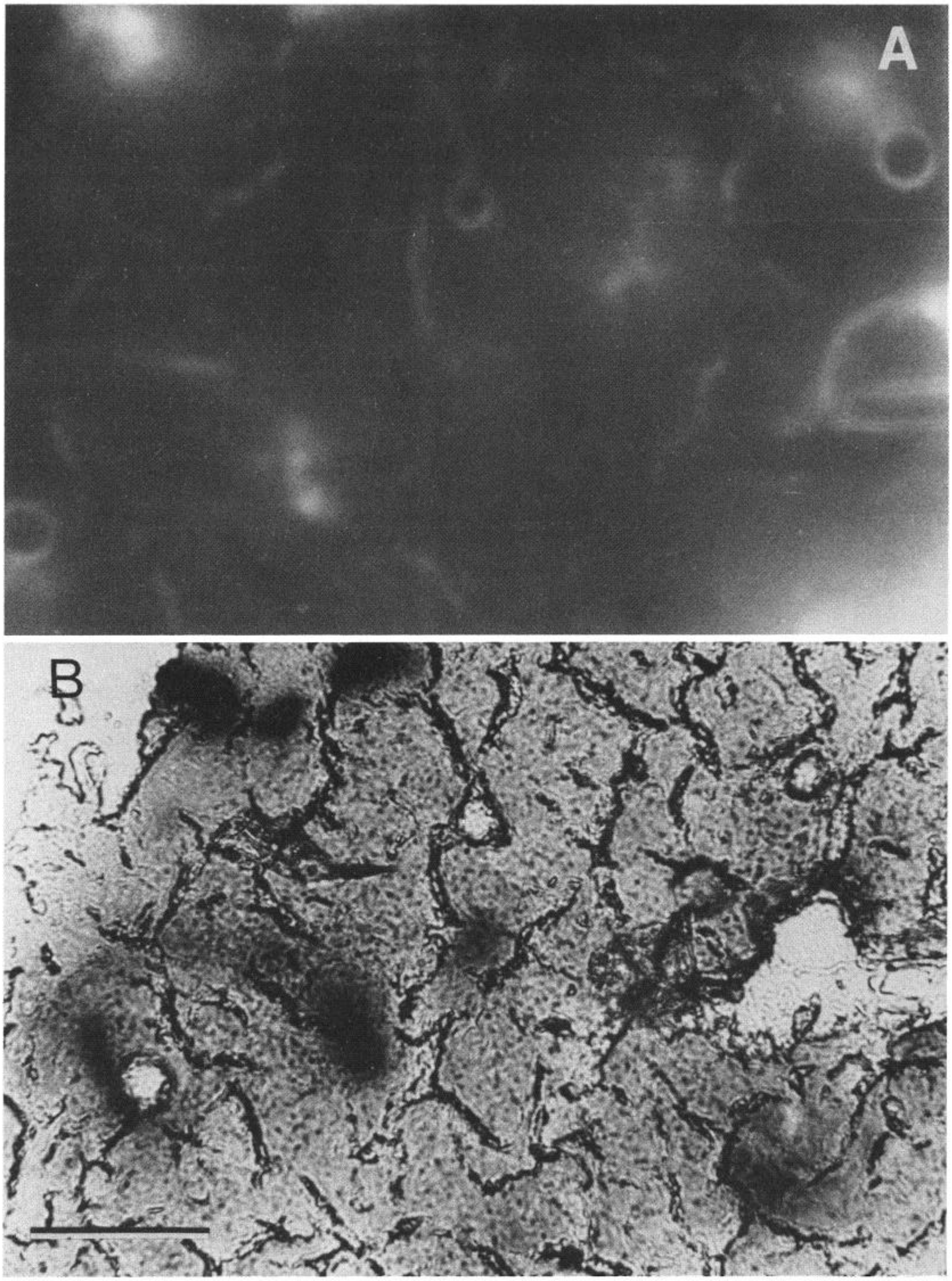

FIG. 1. Photomicrographs of thalamus. Light areas in *A*: microvessels containing FITC-dextran; *B*: same field after microvessel endothelia (dark areas) were stained for alkaline phosphatase. *Bar*, 50 μm.

the severely hypoxic group of animals compared with the control or moderately hypoxic groups, and was 17.6 ± 1.8 Torr. Pa_{CO_2} was decreased from its prehypoxic value in both experimental groups, while pH was not altered. Systemic blood pressure was not significantly different in control and experimental groups in the prehypoxic state. After hypoxia was induced, there were also no significant differences in blood pressure. Heart rate was significantly higher in the severely hypoxic group prior to induction of hypoxia compared with the other groups. No other heart rate differences were significant.

Microvessel volume per cubic millimeter. Average total capillary and arteriolar volume per cubic millimeter were not significantly different when the three groups were compared (Table 2). Average total arteriolar V_V was ~1/10 that of the capillary network. Regional values for the total and perfused capillary and arteriolar V_V are presented in Tables 3 and 4. There were no significant regional differences in the anatomical distribution of the capillary or arteriolar bed within any group.

There were significant increases in average percent perfused arteriolar and capillary morphometry between the two groups of hypoxic animals and the control animals (Table 2). These average percent perfused capillary values were all significantly different from each other. Regional values for the percentage of the capillary network which was perfused are shown in Fig. 2. The percent perfused capillary V_V was not significantly different within any of the experimental groups when regional comparisons were performed. With decreasing Pa_{O_2}, most regions exhibited a significant increase in the percentage of the capillary bed which was perfused (Fig. 2). The average percentage of arterioles perfused in each group of animals was similar to the percentage of capillaries perfused (Table 2). The severely hypoxic value was significantly different from the control and moderate hypoxic ones. Figure 1 also presents the regional percent perfused V_V for the arteriolar network. There were no significant regional differences in this parameter in any group. The percentage of the arteriolar network which was perfused increased significantly in most regions with decreasing Pa_{O_2}.

Microvessel surface area per cubic millimeter. The average total surface area per cubic millimeter of the capillary and arteriolar bed for the three experimental groups is presented in Table 2. There were no significant differences in this parameter between groups for the arteriolar or capillary bed. Regional differences in this anatomic parameter were also lacking in within group comparisons (Tables 3 and 4). Data for the S_V of the perfused portion of this bed are also presented in the above tables.

The percent perfused arteriolar and capillary S_V increased significantly with increases in the degree of hypoxia (Table 2). These values were significantly different from each other for the capillary bed, and the severely hypoxic group value was higher than control for the arterioles. Regional values for the capillary and arteriolar bed are presented in Fig. 3. There were no significant regional differences in percent perfused S_V in any group. With increasing severity of hypoxia, the percentage of the microvascular bed which was perfused increased in most regions (Fig. 3).

Microvessel number per square millimeter. There was no significant difference in the average total Na between the control or hypoxic groups (Table 2). Regional differences in this parameter were also lacking in within group comparisons (Tables 3 and 4). The perfused portion of the microvascular bed is also shown in these tables.

TABLE 1. *Systemic hemodynamic and blood gas parameters in control rats vs. hypoxic rats*

	Control Rats	Moderately Hypoxic Rats (10% O_2)		Severely Hypoxic Rats (6% O_2)	
	Control	Control	Hypoxic	Control	Hypoxic
Blood pressure					
Systolic, Torr	120±5	121±14	107±18	129±10	110±9
Diastolic, Torr	103±8	100±9	85±16	124±10	105±9
Heart Rate, beats·min^{-1}	341±31	387±15	324±44	462±11*	449±6
Pa_{O_2}, Torr	94.6±3.3	102.7±6.9	52.1±1.5*‡	114.7±5.9	37.4±1.7*‡
Pa_{CO_2}, Torr	38.7±1.9	43.2±2.0	35.0±2.2*‡	36.2±1.7	17.7±1.9†‡
pH	7.42±0.01	7.34±0.02	7.35±0.03	7.37±0.03	7.35±0.06

Values are means ± SE. *, Significantly different from control group; †, significantly different from both control and moderate hypoxic groups; ‡, different from prehypoxic value.

TABLE 2. *Average morphometric parameters of the total and perfused capillary and arteriolar network of control vs. hypoxic rat brain*

	Control Rats			Moderately Hypoxic Rats (10% O_2)			Severely Hypoxic Rats (6% O_2)		
	Total	Perfused	% Perfused	Total	Perfused	% Perfused	Total	Perfused	% Perfused
V_V									
Cap, mm^3/mm^3	0.0399 ±0.0021	0.0204 ±0.0013	50.29 ±1.55	0.0576 ±0.0028	0.0405 ±0.0026	69.04 ±1.97	0.067 ±0.0121	0.0514 ±0.0020	92.20 ±1.93
Art, mm^3/mm^3	0.0079 ±0.0006	0.0043 ±0.0003	57.13 ±2.69	0.0061 ±0.0007	0.0047 ±0.0006	70.55 ±3.33	0.0065 ±0.0005	0.0057 ±0.0004	88.36 ±1.37
S_V									
Cap, mm^2/mm^3	18.08 ±0.87	9.05 ±0.65	48.75 ±2.07	28.64 ±1.35	19.84 ±1.25	68.21 ±2.09	49.09 ±22.16	24.27 ±1.22	88.98 ±2.26
Art, mm^2/mm^3	0.89 ±0.08	0.46 ±0.04	57.60 ±3.33	0.76 ±0.09	0.59 ±0.09	71.70 ±3.21	0.80 ±0.06	0.68 ±0.05	85.86 ±1.98
Na									
Cap, no./mm^2	430.7 ±27.1	205.7 ±20.0	45.64 ±1.66	565.7 ±21.9	366.3 ±21.9	63.41 ±1.89	448.2 ±14.9	401.9 ±15.2	89.44 ±1.13
Art, no./mm^3	9.3 ±0.8	4.4 ±0.4	50.79 ±2.58	6.8 ±0.7	4.9 ±0.6	66.10 ±3.40	5.5 ±0.3	4.6 ±0.3	86.23 ±1.67
Diam									
Cap, μm	7.02 ±0.13	7.48 ±0.23		6.34 ±0.14	6.50 ±0.08		6.55 ±0.06	6.68 ±0.07	
Art, μm	29.79 ±0.45	31.75 ±0.74		27.61 ±0.49	28.11 ±0.65		33.93 ±0.72	34.76 ±0.83	

Values are mean ± SE. V_V, microvessel volume/mm^3; S_V, microvessel surface area/mm^3; Na, microvessel no./mm^2; Art, arterioles; Cap, capillaries.

TABLE 3. *Average morphometric parameters of total and perfused capillary network of different regions in control rat brain vs. same regions in hypoxic rat brain*

	Control Rats		Moderately Hypoxic Rats (10% O_2)		Severely Hypoxic Rats (6% O_2)	
	Total	Perfused	Total	Perfused	Total	Perfused
V_V						
Cor	0.0402±0.0083	0.0179±0.0039	0.0683±0.0089	0.0460±0.0085	0.0615±0.0035	0.0587±0.0037
Cere	0.0520±0.0066	0.0279±0.0034	0.0449±0.0066	0.0337±0.0062	0.0515±0.0063	0.0478±0.0064
Med	0.0452±0.0063	0.0269±0.0045	0.0539±0.0069	0.0388±0.0080	0.0493±0.0073	0.0457±0.0073
Pons	0.0370±0.0043	0.0173±0.0028	0.0535±0.0068	0.0375±0.0094	0.0567±0.0079	0.0521±0.0081
Hippo	0.0331±0.0040	0.0152±0.0029	0.0567±0.0077	0.0410±0.0058	0.0529±0.0051	0.0473±0.0045
Thal	0.0352±0.0029	0.0193±0.0015	0.0666±0.0128	0.0463±0.0111	0.0629±0.0067	0.0578±0.0062
Hypo	0.0374±0.0072	0.0180±0.0039	0.0500±0.0014	0.0357±0.0028	0.0529±0.0048	0.0507±0.0047
LN	0.0391±0.0071	0.0206±0.0047	0.0599±0.0041	0.0431±0.0039	0.0602±0.0969	0.0506±0.0043
S_V						
Cor	17.04±3.50	7.17±1.69	34.18±3.98	20.64±4.74	29.50±3.98	25.43±2.53
Cere	21.62±3.43	11.78±2.01	25.46±3.25	17.63±3.21	26.69±5.44	24.66±5.15
Med	21.32±2.84	13.09±2.78	27.92±3.84	20.21±3.78	20.94±17.76	18.85±3.65
Pons	18.45±2.31	8.74±1.59	26.55±4.44	18.03±4.88	26.12±3.54	24.45±3.58
Hippo	15.21±2.01	6.62±1.52	30.12±3.26	22.21±2.58	25.73±2.71	22.66±2.64
Thal	13.01±1.03	9.67±0.84	30.55±5.86	21.71±4.73	30.46±4.91	28.69±4.67
Hypo	15.06±2.44	7.10±1.57	23.18±1.77	17.05±2.04	26.71±2.32	25.04±2.45
LN	16.73±1.38	7.92±1.18	28.96±2.07	20.15±1.87	26.57±2.09	24.36±2.65
Na						
Cor	446.41±73.51	183.19±42.92	637.53±102.97	400.41±98.28	463.41±42.09	435.67±38.87
Cere	508.66±81.92	244.40±46.73	535.56±49.75	343.45±58.96	469.67±54.56	416.50±57.41
Med	509.84±128.43	301.16±120.78	555.89±59.81	379.48±68.68	424.22±39.29	376.51±36.18
Pons	425.37±78.11	164.37±31.32	551.17±59.28	361.56±79.38	478.20±45.08	426.38±49.02
Hippo	353.39±74.95	154.31±41.83	591.00±77.71	379.89±57.14	439.23±48.33	378.07±45.77
Thal	421.96±44.23	208.62±32.28	560.79±55.31	343.43±59.03	519.84±52.55	468.93±53.03
Hypo	339.02±47.15	164.90±37.43	507.50±32.08	340.49±41.95	385.17±27.07	353.99±33.56
LN	443.34±70.47	221.04±48.56	563.18±45.23	371.15±39.08	406.09±20.47	359.38±22.60

Values are means ± SE; V_V represents capillary volume/mm^3; S_V represents capillary surface area/mm^3; Na represents capillary no./mm^2; COR, cortex; Cere, cerebellum; Med, medulla; Pons, pons; Hippo, hippocampus; Thal, thalamus; Hypo, hypothalamus; LN, lenticulate nuclei.

The percent perfused N_a (average capillary number) increased significantly with increasing degrees of hypoxia (Table 2). The values were significantly different from each other for both the arteriolar and capillary beds. There were no significant differences between regions in comparisons of percent perfused N_a for any group for both arterioles and capillaries. There were significant differences in percent perfused capillary N_a between the control and the hypoxic groups such that percent perfused N_a was greater in the hypoxic group. Similar results were seen in the arteriolar bed, such that the hypoxic groups had a greater percent perfused N_a.

Microvessel diameter. The diameters of the arterioles averaged 30.41 ± 0.23 μm and the diameter of the capillaries averaged 6.55 ± 0.07 μm. No significant differences were observed between the examined groups in either total or perfused diameters (Table 2). Furthermore, there were no regional differences in this parameter within or between any experimental group. The histograms (Figs. 4 and 5) represent the distribution of measured diameters of 2,030 capillaries and 586 arterioles for the total microvascular bed. These distributions do not follow the normal distribution. The perfused diameters also follow a similar nonnormal distribution.

DISCUSSION

The method utilized in this study stimultaneously and quantitatively determined morphometric indices of both the perfused and total capillary and arteriolar network of the brain on a regional basis. The method requires an accurate labeling of the perfused capillary and arteriolar bed, minimal changes in the vascular bed during labeling, and estimation of tissue shrinkage. The limitations of this method have been discussed in detail (27). We have used an FITC-labeled dextran to mark the perfused vasculature. The injection itself does not affect the number or diameter of the capillaries or arterioles in the perfused vascular bed. Our method of obtaining the tissue sample, which requires severance of the blood supply, similarly does not affect capillary or arteriolar number or diameter. We have chosen a plasma label to mark a greater proportion of the blood, since microvascular hematocrit is even lower than large vessel hematocrit. It has been shown that dextrans, even of the high molecular weight used in the present study, leak out of the vascular system with time. For this reason, the fluorescent photographs were only used to identify those vessels which were perfused. Quantitative morphometric data were obtained from the photograph of the alkaline phosphatase-stained vessels according to standard principles of stereology (25, 26). Some of the larger vessels called capillaries in the present report may be arteriolar or venular capillaries or actual small arterioles or venules. The number of observed vessels of this size (Fig. 4) are quite

TABLE 4. *Average morphometric parameters total and perfused arteriolar network of different regions in control rat brain vs. same regions in hypoxic rat brain*

	Control Rats		Moderately Hypoxic Rats (10% O_2)		Severely Hypoxic Rats (6% O_2)	
	Total	Perfused	Total	Perfused	Total	Perfused
V_V						
Cor	0.0062±0.0014	0.0033±0.0009	0.0060±0.0022	0.0042±0.0020	0.0042±0.0008	0.0038±0.0007
Cere	0.0102±0.0025	0.0054±0.0013	0.0054±0.0027	0.0045±0.0027	0.0054±0.0011	0.0050±0.0010
Med	0.0081±0.0011	0.0050±0.0010	0.0073±0.0020	0.0056±0.0020	0.0061±0.0018	0.0054±0.0015
Pons	0.0063±0.0020	0.0034±0.0012	0.0066±0.0022	0.0050±0.0020	0.0062±0.0010	0.0052±0.0009
Hippo	0.0089±0.0022	0.0046±0.0009	0.0052±0.0011	0.0041±0.0009	0.0064±0.0013	0.0054±0.0011
Thal	0.0061±0.0014	0.0033±0.0007	0.0089±0.0027	0.0071±0.0026	0.0072±0.0005	0.0061±0.0008
Hypo	0.0082±0.0019	0.0043±0.0007	0.0047±0.0006	0.0029±0.0005	0.0074±0.0004	0.0068±0.0011
LN	0.0092±0.0020	0.0049±0.0010	0.0046±0.0013	0.0037±0.0011	0.0090±0.0026	0.0077±0.0019
S_V						
Cor	0.633±0.181	0.333±0.078	0.743±0.246	0.530±0.300	0.680±0.131	0.573±0.119
Cere	1.165±0.347	0.515±0.172	0.692±0.364	0.586±0.354	0.726±0.172	0.666±0.161
Med	0.892±0.111	0.496±0.061	0.971±0.227	0.738±0.320	0.721±0.155	0.608±0.134
Pons	0.896±0.286	0.458±0.144	0.658±0.241	0.500±0.204	0.691±0.186	0.573±0.157
Hippo	0.902±0.291	0.466±0.109	0.674±0.207	0.506±0.166	0.821±0.163	0.716±0.145
Thal	0.805±0.273	0.470±0.167	1.158±0.403	0.914±0.352	0.791±0.057	0.650±0.086
Hypo	0.846±0.269	0.461±0.171	0.517±0.122	0.367±0.113	0.751±0.057	0.700±0.067
LN	0.985±0.206	0.486±0.106	0.665±0.145	0.523±0.199	1.250±0.324	0.936±0.246
Na						
Cor	7.85±1.73	3.68±1.04	7.40±2.62	4.79±2.70	4.35±0.86	3.75±0.69
Cere	11.75±3.14	5.82±1.63	5.53±2.04	4.20±2.05	4.86±1.11	4.31±0.92
Med	8.11±1.11	3.95±0.50	8.53±2.14	5.88±1.99	1.99±0.67	4.22±0.54
Pons	8.43±2.23	4.26±1.46	7.42±2.31	5.05±1.97	5.78±1.00	4.84±0.76
Hippo	10.45±2.73	4.26±1.17	5.65±0.96	4.52±0.87	5.60±0.10	4.44±0.74
Thal	7.68±2.21	3.84±1.05	9.90±2.92	7.40±2.72	7.11±0.67	5.51±0.74
Hypo	10.13±2.37	4.75±0.98	5.29±0.54	3.46±0.40	5.17±0.52	4.52±0.28
LN	10.07±1.79	5.00±0.98	4.96±1.07	3.68±0.93	6.46±1.35	5.60±1.07

Values are means ± SE; V_V, arteriolar volume/mm³; S_V, arteriolar surface area/mm³; Na, arteriolar no./mm²; Cor, cortex; Cere, cerebellum; Med, medulla; Pons, pons; Hippo, hippocampus; Thal, thalamus; Hypo, hypothalamus; LN, lenticulate nuclei.

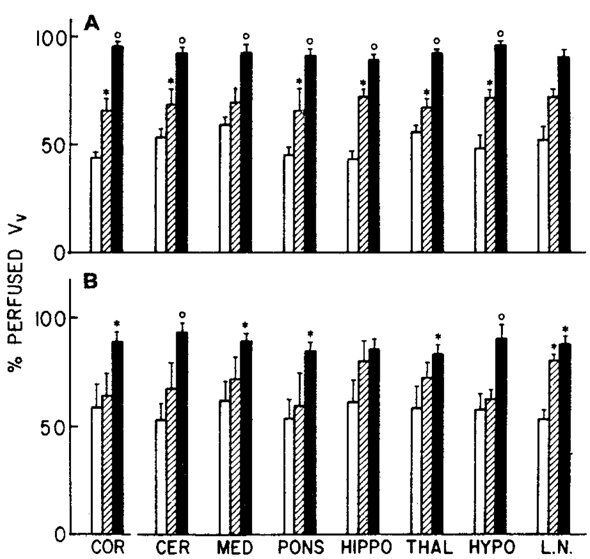

FIG. 2. Percentage of capillary (*A*) and arteriolar (*B*) volume per mm³ brain, V_V, which is perfused in control (*clear*) and rats exposed to 2 levels of hypoxia (*striped*, 10% O_2; *dark*, 6% O_2) for the various brain regions examined. Cor, cortex; Cer, cerebellum; Med, medulla; Pons, pons; Hippo, hippocampus; Thal, thalamus; Hypo, hypothalamus; L.N., lenticulate nuclei. *, Different from control; ° different from control and 10% O_2 groups.

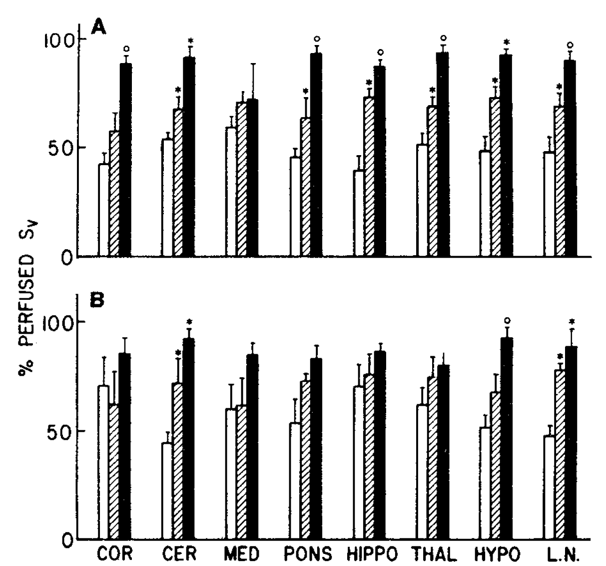

FIG. 3. Percentage of capillary (*A*) and arteriolar (*B*) surface area per mm³ brain (S_V), which is perfused in control (*clear*) and rats exposed to 2 levels of hypoxia (*striped*, 10% O_2, *dark*, 6% O_2) for various brain regions examined. For abbreviations, see Fig. 2. *, Different from control; °, different from control and 10% O_2 groups.

small, and differential changes in their perfusion could not account for our results. Alkaline phosphatase stains the endothelium of all arterioles and capillaries. Venules may also be stained. Two criteria were used to differentiate arterioles from venules: vessel wall thickness (presence or absence of muscular media) and the lesser staining of venules.

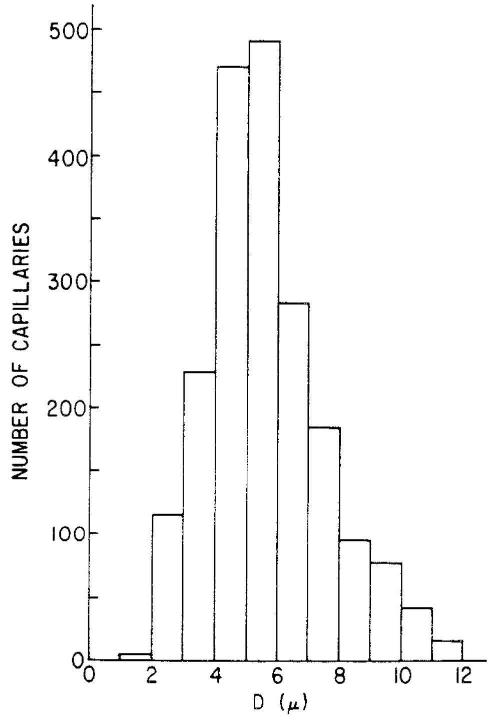

FIG. 4. Histogram of all measured diameters (*D*) of capillaries from various studied brain regions.

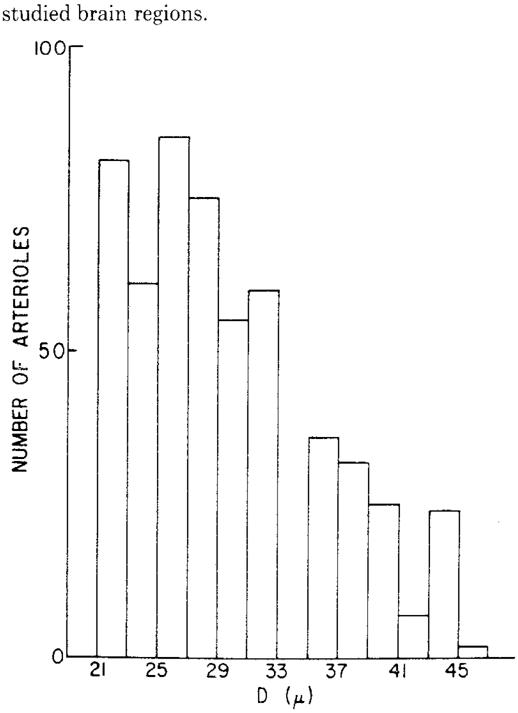

FIG. 5. Histogram of all measured diameters (*D*) of arterioles from various studied brain regions.

The purpose of this study was to compare various indices of perfused capillary and arteriolar morphometry of selected regions of the control, moderately, and severely hypoxic rat brains to determine whether a graded increase in the percent perfused microvessels occurred. Hypoxia causes an increase in cerebral blood flow, but whether there is a concomitant reduction in diffusion distances is not certain. We hypothesized that these indices of perfused capillary and arteriolar morphometry would be increased in a graded manner in hypoxic rat brain and also that there might be some regional differences. The effect of asphyxia is to significantly increase the number of perfused capillaries. Approximately 90% of the microvasculature was perfused after 2 min of asphyxia in rat (27). It has previously been assumed that after longer periods of asphyxia, 100% of the capillary bed was perfused (5, 30).

It is clear that the primary response of the cardiovascular system to cerebral hypoxia is an increase in cerebral blood flow. This response is a threshold phenomenon reported in numerous articles and reviews (9, 10, 20, 21, 28) to begin at a Pa_{O_2} of ~50 Torr. There are some reports of regional differences in the cerebral flow response to hypoxia (2, 21), while others find no regional difference in the flow increase (28). Some of these differences may be related to anesthesia. Blood pressure under all experimental conditions was sufficient to maintain cerebral flow. The systemic hemodynamic parameters measured in the control rats utilized in this study are within physiologically normal ranges for the conscious rat. We have found no significant differences in either blood pressure or heart rate with the hypoxic rats. Arterial carbon dioxide levels were lower in the severe hypoxia group of animals, probably a reflection of a hyperventilation response to hypoxic stress (2).

Increase changes in cerebral blood flow induced by hypoxia can be brought about by arteriolar dilation or an increase in the number of perfused arterioles. The brain also has the additional mechanism of decreasing diffusion distance by perfusing previous unperfused capillaries (capillary recruitment). The ability to alter flow or diffusion distance depends, at least in part, on the anatomic arrangement of the cerebral microvasculature. Our data on the size and anatomic characteristics of the capillary bed were in the same range as others. For example, average capillary length per cubic millimeter, in brain, has been reported in the range of 800–1,100 (1, 6) which is similar to the present report. Reports of average capillary number, were also similar (6, 7). As in our previous report (27), we found no regional anatomic differences between large brain areas, e.g., cortex vs. medulla. Looking at a smaller scale, there have been reports of anatomic differences, e.g., different laminae vs. nuclei (1, 6, 12). We also found a much smaller arteriolar network, that was uniformly distributed across various brain regions.

Hypoxia lowers cerebral tissue PO_2 and causes a reduction in the level of oxygenation of cytochromes, etc. (8, 14, 16). Hypoxia also leads to a uniform regional reduction in O_2 saturation of small cerebral veins (28). It has also been reported that high-energy phosphates, ATP, ADP, AMP and phosphocreatine are not altered by hypoxia, either in the whole brain (11) or regionally (17). There are, however, reports of regional circulatory and metabolic changes caused by hypoxia in the brain (22, 24).

During hypoxia, there was a significant and uniform increase in the percent of the arteriolar network which was perfused with increasing degrees of hypoxemia. This means that not only did the reported increases in cerebral blood flow with hypoxia (9, 10, 19, 28) occur in already perfused microvessels, but there was recruitment of previously unperfused microvessels. This is a graded increase requiring rather severe hypoxia for maximal perfusion of the arteriolar bed. We can find no previous report of an increase, with hypoxia, in the number of perfused arterioles. During hypoxia, there is evidence for both uniform (28) and nonuniform increases (21, 24) in regional cerebral blood flow. There were no significant regional differences in the percentage of the arteriolar network perfused under control or either hypoxic condition in the present study. These relatively uniform increases in the percentage of the arteriolar network perfused with hypoxia do not directly address the issue of whether the cerebral blood flow response to hypoxia is uniform but do show in conscious rats that if it is not, flow rate, and not the distribution of that flow, would be regionally different.

A similar significant and uniform increase in the percentage of the capillary network perfused also occurred with decreasing Pa_{O_2}. Approximately 46% of the capillary network was perfused under control conditions and this increased to ~90% with the most severe hypoxic condition tested. There was a stepped increase in the percentage of perfused microvessels with hypoxia. Similar increases in perfused capillary density with hypoxia had been suggested previously using an indirect estimate (30). One possible explanation for the lack of perfusion of a greater number of microvessels with moderate hypoxia is that the animals become hypocapnic. This hypocapnia is a normal consequence of the hyperventilation response to hypoxia found in the conscious rat. CO_2 has a strong dilator effect on cerebral microvessels (3).

One possible explanation for the increased percentage of the microvascular bed containing FITC-dextran is that cerebral blood flow is increased and this causes the capillaries to fill more quickly with the dye. The report of Conway and Weiss (5), however, argues against this possibility. In that report, cerebral vasodilation was produced with papavarine. There was no increase in the estimate of the perfused capillary bed indicating that vasodilation alone does not necessarily reduce diffusion distances.

There were no significant regional differences in the percentage of the capillary bed perfused under control conditions. This agrees with our previous report (26). With increasing levels of hypoxia, we continued to find a lack of regional difference in the percentage of the microvascular bed perfused. In an earlier study (30) using an indirect estimate, we found no regional differences with 10% O_2 inhalation but with more severe hypoxia, CO or hypoxic-hypercapnia, the forebrain vasodilated to a greater extent than the hindbrain. Evidence exists both for and against regional differences in the cerebral flow response to hypoxia (21, 24, 28). The present report indicates that diffusion distances decrease and the surface area of the capillary network increase similarly in all observed brain regions.

In summary, no regional differences were found in the total arteriolar and capillary network of the brain. During hypoxia, a greater percentage of the arteriolar and capillary reserves was utilized. There was an increase both in the number of vessels perfused and in the vessel surface area available for extraction of O_2. The increases in the proportion of the capillary and arteriolar network which were perfused during hypoxia were uniform, indicating a lack of selective cerebral regional vulnerability, at least in terms of perfusion of the cerebral capillary and arteriolar network.

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