PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 453–458 (1980)

Comparisons of Cutaneous Microvessels from Spontaneously Hypertensive, Normotensive Wistar–Kyoto, and Normal Wistar Rats (40895)[1]

DAVID W. HAACK,* JOHN J. SCHAFFER,† AND JOHN G. SIMPSON‡

Department of Anatomy, University of Michigan Medical School, Ann Arbor, Michigan 48109; †University of St. Louis Medical School, St. Louis, Missouri 63103; and ‡Department of Pathology, University of Aberdeen, Aberdeen, AB9 27D Scotland

Abstract. Samples of abdominal skin from SHR, WKY, and NW rats were examined to determine: (a) if gross structural differences were apparent among the cutanous vascular beds of the three strains of rats, and (b) if the structural differences were generalized or localized to a specific level of the vascular tree. The most significant differences among the three strains of rats were at the level of the smallest (fourth-order) arterioles. The SHR had 30% fewer of the smallest arterioles than WKY and 38% fewer than NW. However, the smallest arterioles from SHR had diameters 22 and 26% larger than similar vessels from WKY and NW, respectively. Consequently, there were no significant differences in calculated resistance among vessels from the three strains of rats. No significant differences were found in the vessel branching angles among the three strains of rats either. It cannot be determined from these studies whether the genetic mechanism responsible for the differences in arteriolar numbers and diameters observed among these highly inbred strains of rats is related to the genetic mechanism responsible for hypertension in SHR.

Studies of vascular responsiveness in spontaneously hypertensive rats (SHR) suggest that the elevated peripheral resistance observed in SHR is due to a structural change in the walls of arteries and arterioles (1). The structural change is thought to be an increase in the thickness of the tunica media that reduces the luminal diameter of arterial vessels and consequently increases the resistance to flow (2, 3). However, Hutchins *et al.* (4, 5), studying the cremasteric muscle vascular bed, have reported that the elevated peripheral resistance of the SHR may also be due to a reduction in the number of small arterioles.

The present study was designed to determine if differences existed in the number or in the internal diameter of arterioles and veins among cutaneous vascular beds of SHR, normotensive Wistar–Kyoto (WKY), and normal Wistar (NW) rats.

Materials and methods. Eighteen male rats were used in this study. Six were SHR, 6 were WKY, and 6 were NW rats. The animals were obtained from a commercial supplier (Charles River Laboratories, Wilmington, Mass.) and from inbred colonies derived from stock supplied by NIH (Dr. Carl Hansen, Chief of Veterinary Services, NIH) and maintained in this laboratory. Animals were housed under uniform conditions, and received standard rat chow (Purina) and water *ad libitum*. The animals in each group ranged in age from 5 to 10 weeks with the average ages being 62, 54, and 48 days for the SHR, WKY, and NW, respectively. The mean weights of the rats were 190 ± 9 g for the SHR, 176 ± 8 g for WKY, and 212 ± 11 g for the NW. The differences in age or body weight among the three strains were not significant.

Systolic blood pressures were determined by photoplethsmography of the tail vessels of rats that had been warmed at 37°C for 10 min and then placed in a warmed restraining device. Three readings were taken from each animal and the mean of the readings was assumed to be the rat's systolic blood pressure. Blood pressures and body weights were determined immediately before tissue collection.

Each animal was anesthetized with ether, the hair was removed from its abdomen, and the animal was placed on its back on an electric heating pad. A sample of abdominal skin, extending 2 cm caudally from a point about 5 mm caudal to the xyphoid

[1] Supported by NIH Grant HL 18575.

0037-9727/80/080453-06$01.00/0

process and 1.5 cm to either side of the midline, was quickly removed, placed in 2% glutaraldehyde in 0.1 *M* cacodylate, pH 7.4, and pinned flat at approximately its normal shape and size. After 4 hr in fixative the tissue was dehydrated with a graded series of alcohols followed by propylene oxide and infiltrated with Epon. The skin sample was then clamped between two lantern slide cover glasses and incubated for 18 hr at 45°C and 24 hr at 60°C to polymerize the Epon. To prevent bonding of the Epon to the cover glasses an antistick cooking agent, Pam (Boyle–Midway, Inc., N.Y.), was sprayed onto each cover glass before it was used.

At the time of collection each tissue was assigned a number that was not decoded until after all the data had been collected and compiled for statistical analysis. Thus the identity of each tissue was hidden until the data were analysed.

Photographic montages of each sample were constructed from photomicrographs recorded on 35-mm film (Panatomic X, Kodak) at a magnification of 25 diameters and printed at a final magnification of 70 diameters. Each montage represented 2 cm^2 of tissue and depicted blood vessels lying in a plane parallel to the surface of the dermis exposed upon removal of the skin sample.

The vessels in each montage were classified according to their position in the branching heirarchy. The largest vessels, which were in the same anatomical position in each preparation, were named first-order arterioles or veins and the smallest were fourth order. The number and class of vessels at each level of branching was determined and the internal diameter of every vessel in the montage was measured. The identity of each vessel was verified by microscopic examination of the skin sample corresponding to the montage being studied. Arterioles had thick walls, followed rather straight courses through the tissue, and branched dichotomously to yield uniformly sized derivative vessels and therefore could be easily distinguished from veins which had thinner walls, followed more tortuous routes, and branched more acutely yielding various sizes of derivative vessels. Both vessels containing blood cells and vessels without blood cells were counted and measured. In addition the lengths of two arterioles per sample were measured at the second through fourth levels. To select vessels for the length measurements a first-order arteriole was chosen at random and the first derivative vessel at each successive level of branching was measured. Finally, the angle at which each derivative vessel left the parent vessel was measured by the method by Zamir (6).

Univariant analysis of variance followed by Scheffe's multiple comparisons procedure was used to determine the level of significance of the differences observed among the data obtained for the three strains of rats.

Results. The mean systolic blood pressures were 149 ± 4 mm Hg for the SHR, 115 ± 5 mm Hg for the WKY, and 124 ± 3 mm Hg for the NW. The pressures of the SHR were significantly different from those of either the WKY ($P < 0.01$) or NW ($P < 0.05$). However, the pressures of the WKY and NW were not significantly different.

Both SHR and WKY had significantly fewer ($P < 0.01$) third-order arterial vessels than NW (Table I). The difference between the SHR and WKY at this level of branching was not significant. At the level of the fourth-order arterioles, SHR had 30% fewer vessels than tissues from WKY ($P < 0.01$) and 38% fewer fourth-order arterioles than NW samples ($P < 0.01$). In addition WKY samples had 12% fewer fourth-order arterioles than NW ($P < 0.01$). Finally, SHR had 28% fewer fourth-order veins than NW ($P < 0.01$).

Mean internal diameters of second-, third-, and fourth-order arterioles from SHR were 13, 14, and 22% larger ($P < 0.05$), respectively, than similar vessels in WKY (Table II). Second-, third-, and fourth-order arterioles in skins from SHR were 13, 10, and 26% larger than respective vessels in samples from NW. However only the second- and fourth-order arterioles were significantly different. None of the mean arterial internal diameters in WKY tissues were significantly different from those in NW samples. The only significant difference in sizes of veins was between fourth-order veins of SHR and NW. the

TABLE 1. NUMBER OF VESSELS

Branching order (°)	Strain	Number[a] (mean ± SE)	Comparisons	
A. Arteries				
1	SHR	3.0 ± 0.3	SHR vs WKY	NS
	WKY	2.8 ± 0.4	SHR vs NW	NS
	NW	3.2 ± 0.3	WKY vs NW	NS
2	SHR	7.2 ± 0.8	SHR vs WKY	NS
	WKY	7.2 ± 0.4	SHR vs NW	NS
	NW	7.0 ± 0.7	WKY vs NW	NS
3	SHR	21.2 ± 0.9	SHR vs WKY	NS
	WKY	22.3 ± 0.6	SHR vs NW	$P < 0.01$
	NW	28.0 ± 0.8	WKY vs NW	$P < 0.01$
4	SHR	47.2 ± 1.9	SHR vs WKY	$P < 0.01$
	WKY	67.0 ± 1.8	SHR vs NW	$P < 0.01$
	NW	76.5 ± 1.8	WKY vs NW	$P < 0.01$
B. Veins				
1	SHR	3.3 ± 0.3	SHR vs WKY	NS
	WKY	3.3 ± 0.2	SHR vs NW	NS
	NW	4.3 ± 0.4	WKY vs NW	NS
2	SHR	13.0 ± 0.9	SHR vs WKY	NS
	WKY	12.8 ± 1.2	SHR vs NW	NS
	NW	13.2 ± 1.3	WKY vs NW	NS
3	SHR	77.0 ± 3.8	SHR vs WKY	NS
	WKY	78.8 ± 3.8	SHR vs NW	NS
	NW	80.7 ± 2.7	WKY vs NW	NS
4	SHR	273.2 ± 21.1	SHR vs WKY	NS
	WKY	299.3 ± 11.2	SHR vs NW	$P < 0.01$
	NW	376.2 ± 9.4	WKY vs NW	$P < 0.01$

[a] n = six animals/strain.

smallest veins in SHR samples were 9% larger than similar vessels in NW samples ($P < 0.05$).

Mean branching angles of both arterial and venous vessels in tissues from SHR were greater than respective angles measured in either NW or WKY samples. However, none of the differences in mean branching angles among the three strains of rats were significant.

The differences in vessel length among the three strains of rats did not vary in a consistant manner from level to level (Table III). In addition, no significant differences in vessel length were observed among the three strains of rats.

To determine whether the difference in the number and size of arterioles between control and hypertensive rats affects hemodynamics in respective vascular beds, the resistance at each level of branching was calculated using the formula: $R = 8\eta l (1)/\pi r (N)$ where l = vessel length (in cm) at a given level, $\eta = 0.03$ dyne-sec-cm^{-2}, r = mean radius of vessels (in cm) at a given level, and N = the number of vessels at that level (7).

The mean resistance calculated for fourth-order arterioles in SHR samples was about 53 and 43% of the mean resistances calculated for fourth-order arterioles in WKY and NW skin samples, respectively. However, none of the differences in mean calculated resistance among the three strains of rats were significant.

Discussion. Although structural differences may exist between blood vessels from control and hypertensive animals (1, 8), the precise nature of the differences and their location in the vascular tree are not clearly known. One structural change that has been observed is an increased area or mass of the tunica media of arteries from hypertensive animals (2, 9, 10). The increased mass is thought to reduce the internal diameter of the artery and consequently increase the resistance to flow. However, there are several limitations to the concept

TABLE II. BLOOD VESSEL DIAMETERS

Order (°)	Strain	Diameter (μm) (mean ± SE)[a]	Comparisons	
A. Arteries				
1	SHR	113.1 ± 5.9	SHR vs WKY	NS
	WKY	98.0 ± 8.4	SHR vs NW	NS
	NW	100.1 ± 4.0	WKY vs NW	NS
2	SHR	76.6 ± 2.2	SHR vs WKY	$P < 0.05$
	WKY	66.4 ± 2.8	SHR vs NW	$P < 0.05$
	NW	66.3 ± 2.3	WKY vs NW	NS
3	SHR	48.4 ± 1.9	SHR vs WKY	$P < 0.05$
	WKY	41.7 ± 1.4	SHR vs NW	NS
	NW	43.3 ± 5.0	WKY vs NW	NS
4	SHR	23.0 ± 1.2	SHR vs WKY	$P < 0.05$
	WKY	18.7 ± 0.7	SHR vs NW	$P < 0.01$
	NW	17.4 ± 0.4	WKY vs NW	NS
B. Veins				
1	SHR	223.3 ± 15.3	SHR vs WKY	NS
	WKY	244.9 ± 8.4	SHR vs NW	NS
	NW	219.5 ± 8.4	WKY vs NW	NS
2	SHR	128.2 ± 3.9	SHR vs WKY	NS
	WKY	138.4 ± 2.7	SHR vs NW	NS
	NW	124.8 ± 4.4	WKY vs NW	NS
3	SHR	57.6 ± 0.7	SHR vs WKY	NS
	WKY	57.6 ± 1.0	SHR vs NW	NS
	NW	59.2 ± 0.7	WKY vs NW	NS
4	SHR	26.4 ± 0.5	SHR vs WKY	NS
	WKY	24.7 ± 0.1	SHR vs NW	$P < 0.05$
	NW	24.1 ± 0.5	WKY vs NW	NS

[a] n = six animals/strain.

of hypertrophy of the tunica media reducing the lumenal diameter as being a cause of hypertension. First, the mass or area of the tunica media may increase without affecting the size of the lumen (2, 8, 11). Second, hypertrophy of the tunica media may not occur in vessels less than 200 μm in diameter (9) thus the resistance vessels may not change. Third, many of the studies, both morphological and physiological, were limited to larger arteries (femorals, carotids, and aortae) and therefore may not accurately reflect the responses of resistance vessels to the factors that produce hypertension. Finally, recent *in vivo* studies of the microvasculature have found little or no difference in the thickness of arteriolar walls between hypertensive and control animals (12–14).

TABLE III. VESSEL LENGTHS OF ARTERIES

Order (°)	Strain[a]	Length[b] (mm ± SE)
2	SHR	3.25 ± 0.96
	WKY	2.81 ± 0.69
	NW	3.83 ± 0.83
3	SHR	3.07 ± 0.82
	WKY	2.40 ± 0.49
	NW	2.17 ± 0.37
4	SHR	0.99 ± 0.18
	WKY	1.16 ± 0.24
	NW	1.23 ± 0.27

[a] Six animals/strain.
[b] Two vessels/animal.

The present study did not address the problem of hypertrophy of the tunica media but was intended to determine whether or not gross structural differences were apparent among the cutaneous vascular beds of spontaneously hypertensive rats and control rats of the Wistar–Kyoto and Wistar strains. An additional goal was to determine if any differences between control and hypertensive vascular beds were localized to a specific region of the bed or were generalized.

The present investigation revealed two

salient points. First, the greatest differences among cutaneous vascular beds from three strains of rats, SHR, WKY, and NW, occurred at the level of the fourth-order arterioles. This difference was expressed as a reduction in number but an increase in diameter of the smallest arterioles. Second, the differences observed between cutaneous vessels from the normotensive WKY and NW were nearly as great as those found between similar vessels from the hypertensive SHR and the normotensive WKY. The data on blood vessel diameters must be interpreted with caution because the vessels had been removed from the influence of blood pressure, sympathetic nervous activity, and circulating hormones before diameter measurements were taken. However, these data confirm results presented for the cremaster muscle (4, 12) and mesentery (13).

The skin was selected for this study because, although it may not serve as a major regulator of peripheral vascular resistance, the cutaneous vascular bed does receive about 9% of the total cardiac output (15). In addition, cutaneous vasculature is readily accessible and relatively easy to prepare for microscopy. Finally, the skin vasculature is not exposed to the wide range of local metabolic demands that are placed on the vasculature of skeletal muscle (16). Blood vessels in skin are primarily affected by central neural or humoral cardiovascular regulatory mechanisms, or genetic factors.

One difference exists between the present study of cutaneous vessels and the work of Hutchins *et al.* (4) on vessels in cremaster muscles. In the present study the number of fourth-order veins was not significantly different between SHR and WKY. However, Hutchins *et al.* (4) reported a significant increase in the number of fourth-order veins in SHR as compared with WKY. The reason for this difference is not clear but may reflect differences in the angioarchitecture of the two tissues or differences in the responses of vessels in the two tissues to hypertension.

Hallback *et al.* (17) reported that an acute 35% reduction in the number of maximally dilated 50-μm arterioles did not significantly change the "threshold" norepinephrine dose, the steepness of resistance curves, or the maximum pressor responses to norepinephrine. They therefore concluded that arteriolar rarification was not a major determinant of SHR hemodynamics in which steepness of the resistance curve and maximum pressor responses are increased. However, it is not clear how closely this acute, mechanical rarification parallels the more chronic, developmental rarification found in SHR (18).

Previously, concern has been expressed that some of the differences observed between SHR and control rats were due to genetic characteristics unrelated to the mechanisms producing hypertension (19). At least one study has indicated that there is a basis for this concern (20). In addition, a longitudinal study of arterioles in SHR and WKY has shown that the difference in numbers of artioles between these two strains develops after the onset of hypertension (18). The reduction in numbers of arterioles may occur in response to hypertension or may represent a mechanism by which hypertension is maintained. However WKY tissues have significantly fewer arterioles than tissues from NW rats but blood pressures of the two strains do not differ significantly. In addition, calculations of resistance suggest that the resistance in fourth-order arteries from SHR may be less than similar vessels from either WKY or NW. Therefore, the differences observed among the three strains of rats used in the present study may be due to genetic factors unrelated to the causes of hypertension.

The data obtained from the cutaneous vessels of rats are consistent with recent data from combined morphologic and hemodynamic studies of the vascular beds in cremaster muscles (12) and mesentery (13). The consistency of these results from three functionally different vascular beds indicates that there is a generalized alteration of the arteriolar architecture in SHR.

1. Folkow, B., Hallback, M., Lundgren, Y., and Weiss, L., Acta Physiol. Scand. 79, 373 (1970).
2. Friedman, S. M., Nakashima, M., and Mar, M., Microvasc. Res. 3, 416 (1971).
3. Lundgren, Y., Acta Physiol. Scand. 408 (Suppl.), 1 (1974).

4. Hutchins, P. M., and Darnell, A. E., Circ. Res. **34, 35** (Suppl. 1), 1–161 (1974).
5. Hutchins, P. M., Dusseau, J. W., Marr, M. C., and Greene, A. W., *in* "Microvascular Aspects of Spontaneous Hypertension" (H. Henrich, ed.) University Park Press, in press.
6. Zamir, M., J. Theor. Biol. **62**, 227 (1976).
7. Berne, R. M., and Levy, M. N., "Cardiovascular Physiology," 3rd ed., p. 59. Mosby, St. Louis (1977).
8. Hansen, T. R., Abrams, G. D., and Bohr, D. F., Circ. Res. **34–35** (Suppl. 1), 1–101 (1974).
9. Furuyama, M., Tohoku J. Exp. Med. **76**, 388 (1962).
10. Mulvaney, M. J., Hansen, P. K., and Aalkjaer, C., Circ. Res. **43**, 854 (1978).
11. Folkow, B., Hallback, M., Lundgren, Y., Sivertsson, R., and Weiss, L., Circ. Res. **32–33** (Suppl. 1), 1–2 (1973).
12. Bohlen, H. G., and Lobach, D., Blood Vessels **15**, 322 (1978).
13. Henrich, H., Hertel, R., and Assman, R., Pflugers Arch. **375**, 153 (1978).
14. Bohlen, H. G., Amer. J. Physiol: Heart Circ. Physiol. **5**, H157 (1979).
15. Ganong, W. F., "Review of Medical Physiology," p. 453. Lange, Los Altos, Calif. (1977).
16. Sparks, H. V., *in* "Peripheral Circulation" (P. C. Johnson, ed.), p. 193. Wiley, New York (1978).
17. Hallback, M., Gothberg, G., Lundin, S., Ricksten, S.-E., and Folkow, B., Acta Physiol. Scand. **97**, 233 (1976).
18. Hutchins, P., Marr, M., and Dusseau, J., Fed. Proc. **36**, 953 (1977).
19. Undenfriend, S., Bumpus, F. M., Foster, H. L., Freis, E. D., Hansen, C. T., Louenberg, W. M., and Yamorie, Y., ILAR News **19**, G-3 (1976).
20. Nickerson, P. A., Amer. J. Pathol. **84**, 545 (1976).

Received October 29, 1979. P.S.E.B.M. 1980, Vol. 164.