Microvascular Alterations in Adult Conscious Spontaneously Hypertensive Rats

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The dorsal skin flap technique was used to study skeletal muscle microcirculation in conscious 10–12-week-old spontaneously hypertensive rats and normotensive Wistar-Kyoto control rats. Videorecordings were made for off-line analysis of consecutive segments of the vascular bed. Resting diameters were significantly smaller in spontaneously hypertensive rats than in Wistar-Kyoto rats at the first-order (—28%) and second-order arteriolar (—21%) levels. Precapillary third-order and fourth-order arterioles of spontaneously hypertensive rats had normal diameters, whereas postcapillary small venule diameters were slightly larger in spontaneously hypertensive rats. Thirty percent and 41% of the spontaneously hypertensive rat and Wistar-Kyoto rat third-order arteriolar vessels and 63 and 45% of the fourth-order arteriolar vessels exhibited vasomotion. Vasomotion amplitude, but not frequency, was significantly higher in spontaneously hypertensive rats than in Wistar-Kyoto rats. It is concluded that, in the established phase of spontaneous hypertension in the rat, a decreased diameter of large arterioles is the major mechanism underlying the increased vascular resistance in cutaneous skeletal muscle.

(Microvascular alterations of resistance vessels contribute to a considerable degree to the increased peripheral resistance in spontaneous hypertension. Skeletal muscle vascular resistance in particular is responsible for the bulk of increased peripheral resistance. The primary mechanism for increasing the resistance in a microvessel is the decrease of its diameter. There is growing evidence, on the basis of indirect observations, that larger arterioles of spontaneously hypertensive rats (SHR) exhibit smaller diameters and are the major contributors to the increased vascular resistance. However, direct microvascular observations of various skeletal muscles in situ do not consistently demonstrate a reduced diameter of arteriolar resistance vessels. These studies suggest microvascular rarification as an alternate mechanism for increased vascular resistance.

The information concerning changes of the microvasculature of the SHR as compared with Wistar-Kyoto (WKY) rats is mainly obtained by intravital microscopic observations of acute preparations requiring surgical manipulation with general anesthetics. These interventions may disturb normal physiology, vascular smooth muscle reactivity, and induce vasodilation. Furthermore, the use of different anesthetics or combinations of anesthetics complicates comparison of experimental data. For the present study, we used the dorsal microcirculatory chamber for microscopic observations of the cutaneous maximus muscle in conscious rats. With this preparation, we compared the microcirculation in conscious adult SHR and WKY rats.

Methods

SHR and WKY rats (Charles River, Portage, Michigan), 10–12 weeks old, were provided with a dorsal microcirculatory chamber and an arterial catheter as described by Smith et al. In short, under pentobarbital (50 mg/kg i.p.) anesthesia, the hair at the back was removed by means of a chemical depilatory agent. An area approximately 6 cm in length and width beginning about 1 cm below the spine of the scapula was prepared on the dorsal side of the rat. The rat was placed on a surgery board and covered with a sterile drape to prevent contamination of the surgical field. The skin was punched, and a cut was made along the edge of the outline. The skin was carefully dissected from the underlying muscle. Then the chamber, consisting of two thermoneutral poly-
carbonate halves, was implanted. A small piece of cutaneous maximus muscle was prepared to fit in the chamber. After surgery, the rats were kept warm by placing them on a heating pad until they were fully conscious.

At least 1 week was allowed for full recovery. The preparation was considered to be good when 1) no major bleedings during surgery and no microbleedings during recovery were observed; 2) no signs of inflammation, such as increased vasodilation, neovascularization or edema were found; and 3) no excessive leucocyte rolling and sticking at venular walls was present. On the experimental day, rats were placed in the rodent restrainer for in vivo microscopy. Details about the microscope, which was rotated 90°, and recording system can be found elsewhere.8 Rats were loosely restrained. The restrainer minimized motion but did not impede respiration. The chamber was fixed to the restrainer, and the window was cleared with distilled water using a cotton wool stick. The restrainer was mounted on the vertical stage of the microscope with the rat in a ventral side-down position. In areas with optimal optical clarity, video recordings with low magnification (Zeiss, Plan objectives 1.25; Numerical Aperture [N.A.], 0.04 and ×2.5; N.A., 0.08) served to document the entire microvasculature and its angioarchitecture. Arteriolar and venular trees were selected. During a 3-minute period, recordings were made of consecutive segments of the vascular tree for off-line diameter measurements with a Zeiss Plan ×10; N.A., 0.22. The types of vessels were grouped to the functional branching order and classified alphanumerically. Thus, primary perfusing arterioles were designated as the first-order arterioles (A1) with their branches designated second-order (A2). The branches of second-order and third-order arterioles are referred to as A3 and A4, respectively. The same vessel classification was used for the venous side (V1–V4). Diameters were measured off-line by image splitting with a shearing monitor.9 Total optical magnification for vessel diameter measurement was ×40.

For vessels showing vasomotion, diameter recordings were analyzed for maximal and minimal diameter, mean vessel diameter (maximal plus minimal/2), vasomotion amplitude, relative amplitude (ratio of vasomotion amplitude and mean vascular diameter), and frequency of vasomotion. The total observation period of each rat lasted 90 minutes. Mean arterial pressure was measured by the tail-artery method with a 150 PC flow-thru pressure sensor (Micro Switch, Honeywell, Freeport, Illinois).

All data are presented as mean±SEM. Data of SHR and WKY rats were compared, using an analysis of variance for repeated measurements. Differences were considered statistically significant if p<0.05.

Results

All surgical procedures were successfully performed in five SHR and seven WKY rats allowing an analysis of eight to 39 microvessels. The SHR had a markedly (p<0.001) higher mean arterial pressure (141±2 mm Hg) compared with WKY rats (110±2 mm Hg). No statistically significant differences in heart rate were found between SHR (312±14 beats/min) and WKY rats (304±4 beats/min).

The general network anatomy was similar for SHR and WKY rats. In Figure 1, mean arteriolar diameter

FIGURE 1. Bar graph showing average diameter (DIAM) of each category of arteriolar and venular vessels. Data are expressed as mean±SEM. Numbers above bars indicate number of vessels analyzed in five spontaneously hypertensive rats (SHR) or seven Wistar-Kyoto (WKY) rats, and the asterisk denotes a statistically significant difference between SHR and WKY rats (*p<0.05; **p<0.001). A1, A2, A3, A4, first-, second-, third-, fourth-order arterioles; V1, V2, V3, V4, first-, second-, third-, fourth-order venules.

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The present study was performed in a recently described model for investigation of the microcirculation in awake rats. The healing period of at least 1 week after implantation of the dorsal chamber allows the rats to recover and leads to a stable microcirculatory preparation with no signs of microbleeding. After this period, vasomotion was present in a considerable number of small arterioles, indicating the good condition of the preparation.

Our studies show a decrease in diameter in the A1 and A2 vessels in SHR (see Figure 1). These vessels are the primary sites of increased resistance in hypertension. Previous studies, using anesthetized rats, indicated a similar decrease in large arteriolar diameter in SHR intestine and brain. Furthermore, on the basis of microvascular pressure-flow relation studies, Roy and Mayrovitz concluded that there were decreased luminal diameters in the cremaster muscle of 7–8-week-old SHR. However, in other studies on SHR skeletal muscle microcirculation, large arterioles were found to have a diameter equal or even slightly higher than normal.

Several factors could be involved in the discrepancies between these findings. The first is the use of anesthesia or other experimental maneuvers. We have recently shown that pentobarbital and, to a lesser degree, chloralose-urethane depress reactivity of the muscular microcirculation to stimulation by the sympathetic nervous system or the increased sympathetic tone. Thus, by using an anesthetic that depresses sympathetic function a diameter difference between SHR and WKY rats may be lost.

A second reason for discrepancies in the observations on the degree of narrowing of arteriolar lumen may be the nature of the vascular bed investigated. Thus, skeletal muscle could be relatively insensitive to factors that cause narrowing of the arteriolar lumen. Previous work by Meininger and coworkers and Joshua et al shows that a lack of skeletal muscle arteriolar narrowing is certainly not a feature of all models of hypertension. These authors found a significant decrease in large, but not in small, arteriolar diameter in skeletal muscle of rats with renovascular and deoxycorticosterone-salt hypertension. Our data support these findings for the SHR and indicate that large arteriole lumen narrowing is a general feature of experimental hypertension.

On the basis of work published thus far, it is impossible to conclude whether the large arteriole lumen narrowing is a secondary phenomenon to the existence of hypertension or is essential to its developmen...
vasomotion amplitude in 7-9-week-old SHR were reported in large arterioles, as compared with WKY rats. It is possible that postcapillary venules were slightly dilated in SHR when compared with WKY rats. Because skeletal muscle is responsible for the bulk of increased vascular resistance in spontaneous hypertension in rats, our data support the view that an increased peripheral resistance in adult SHR is the consequence of a smaller diameter of resistance-sized arterioles.

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