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. (Hypertension 1990;15:415–419)

Microvascular alterations of resistance vessels contribute to a considerable degree to the increased peripheral resistance in spontaneous hypertension.1 Skeletal muscle vascular resistance in particular is responsible for the bulk of increased peripheral resistance.2 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.1,3 However, direct microvascular observations of various skeletal muscles in situ do not consistently demonstrate a reduced diameter of arteriolar resistance vessels.4–6 These studies suggest microvascular rarefaction 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.7 For the present study, we used the dorsal microcirculatory chamber for microscopic observations of the cutaneous maximus muscle in conscious rats.8 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.8 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 microbleed-
ings during recovery were observed; 2) no signs of
inflammation, such as increased vasodilation, neovas-
cularization or edema were found; and 3) no exces-
sive 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 objec-
tives 1.25; Numerical Aperture [N.A.], 0.04 and×2.5;
N.A., 0.08) served to document the entire microvas-
culature and its angioarchitecture. Arteriolar and
venular trees were selected. During a 3-minute
period, recordings were made of consecutive seg-
ments 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 record-
ings were analyzed for maximal and minimal diame-
ter, 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 per-
formed 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±24 beats/min).

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

![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.](image-url)
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- to 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 and circulating angiotensin II. Earlier studies have indicated that the reason for a greater constriction of large arterioles in SHR may be the more dense innervation 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 develop-
opment. Work is in progress now in our laboratories with different forms of antihypertensive treatment to investigate the primary or secondary nature of these vascular changes. The dorsal chamber may serve an important role in such studies as it allows the observation in the same preparation for a prolonged period of time.

Resting diameters of A3 and A4 vessels were not different in SHR when compared with WKY rats. This means that, at least in skeletal muscle, there is no contribution based on diameter reduction of the distal part of the microcirculation to the increased vascular resistance in SHR. This agrees with previous conclusions concerning the role of small arterioles in the control of resistance in SHR.\(^1\)\(^{10}\) Postcapillary venules were slightly dilated in SHR when compared with WKY rats. It is possible that postcapillary dilatation prevents an increased capillary filtration by changing the precapillary-to-postcapillary resistance ratio. Diameters of large venules (V1) were slightly less in SHR. Because the V1 vessels belong to the capacitance vessels, a decreased diameter may explain why SHR have a reduced regional blood volume.\(^2\)

Another aspect of the microcirculation addressed in this study was the occurrence of vasomotion (see Table 1). Vasomotion was limited to the smaller arterioles and occurred in approximately half of the vessels observed. This points to the good condition of our conscious animal preparation, as anesthesia and surgical manipulation profoundly depress vasomotion.\(^8\)\(^{18}\)\(^{19}\) The frequency of vasomotion we observed was between 5 and 8 cycles/min, which agrees with previous observations in small arterioles in the hamster skinfold preparation.\(^20\) Higher frequencies of vasomotion were reported in the skeletal muscle of decerebrated\(^21\) or pentobarbital-anesthetized rat,\(^22\) whereas much lower frequencies were reported in the cerebral microcirculation of awake rabbits.\(^23\)

Apart from an influence of anesthetics, the pattern of vasomotion may vary for different arterioles. Slaaf et al\(^24\) have shown that within the tenuissimus muscle of the rabbit a different cycle length can be observed for the most proximal part of a transverse arteriole and more distally located areas. Similar observations were made in the skin: frequencies of 1–4 cycles/min were reported in large arterioles, as compared with frequencies of 7–14 cycles/min in small arterioles.\(^20\)\(^{25}\)

Borders and Zweifach\(^26\) reported a decreased vasomotion amplitude in 7–9-week-old SHR spinothrapezius muscle. At the A3 level, we found an increased amplitude. The difference in these results may be related to the stage of development of spontaneous hypertension. We used rats with an established hypertension, a normal skeletal muscle blood flow, and an increased large arteriolar resistance. SHR 7–9 weeks old are still in a phase of development of hypertension characterized by an increased cardiac output and skeletal muscle perfusion and a near normal vascular resistance.\(^2\) Thus, the later increase in vasomotion amplitude may serve as a mechanism to allow adequate flow distribution beyond the level of increased vascular resistance. This hypothesis would plead against a major role of vasomotion in the control of vascular resistance, suggesting instead its contribution to tissue flow distribution. The stronger degree of vasomotion in SHR may lead to temporary and intermittent rarefaction of small arterioles. On the other hand, an increased amplitude of vasomotion may suggest a higher myogenic constrictor tone of the small arterioles.\(^27\) Further studies are needed to define the role of vasomotion in vascular control in spontaneous hypertension more precisely. The dorsal chamber may serve a role in these studies.

In summary, the present study shows a decreased large arteriolar diameter in conscious 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.

**References**

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