Effects of Vasodilation on Plasma Distribution in SHR Cremaster Muscle Microvessels

CARLETON H. BAKER, PH.D., FRANK R. WILMOTH, PH.D., AND E. T. SUTTON, M.S.

SUMMARY Alterations in the structure, number, reactivity, contractility and sensitivity of resistance vessels of hypertensive animals have been reported. If the etiology of hypertension is due to one or a combination of these factors, it could logically be expected that the distribution of blood flow from the arterial to venous circulation through parallel microcirculatory circuits could be affected. The right cremaster muscles of pentobarbital anesthetized Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) (6-8 weeks old) were exposed and prepared for fluorescent videomicroscopy. The right iliac artery was cannulated with PE-10 tubing, the tip of which was placed at the aortic bifurcation for bolus injections of FITC-dextran (70,000 molecular weight) and arterial pressure measurement. Passage of the indicator through the microcirculation was recorded on videotape during control and during vasodilation by topical application of adenosine (0.2 M). Time-concentration curves were recorded by means of dual window videodensitometry upon replay of the tape. Arterial pressure averaged 85 ± 3 mm Hg in WKY rats and 110 ± 5 mm Hg in SHR. Arteriolar flow velocity varied directly with small arteriolar diameter. Dilation significantly reduced the venular appearance (τa), mean transit time (t), and curve width time (τc) in WKY and SHR. The τc was significantly more reduced in SHR than WKY. This would suggest that, in WKY, dilation may have opened some new parallel circuits but principally increased flow velocity through existing circuits. In SHR, new shorter and/or higher velocity circuits were opened as evidenced by the reduced τc with the longer and/or lower velocity circuits largely unaffected. It is concluded that in SHR there are vessels that are closed and thereby contribute to the elevated blood flow resistance that are dilated by adenosine.

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KEY WORDS • FITC-dextran • indicator dispersion • mean transit time • venules • arterioles • capillaries

It has been proposed by the work from numerous laboratories that the elevated peripheral resistance present in hypertension is due to one or probably a combination of factors. These factors are currently believed to include thickening of the media of the vascular wall,1 reduction in the number of terminal arterioles2-4 and capillaries,5 as well as increases in the following arterial and arteriolar smooth muscle parameters: reactivity, contractility,6 and sensitivity to neural and catecholamine stimulation.6,7 Increased number and size8,9 of some postcapillary venules and increased tone and contractility of these vessels10,11 have been reported.

It is well known that the flow of blood of skeletal muscle at rest is quite low (3-5 ml/min/100 g),12 reflecting a high vascular tone. It has also been reported that a wide range of metabolites released from actively contracting skeletal muscle will result in reduction of the microvascular tone, decreasing blood flow resistance, and consequently greatly increasing the blood flow.13 If the microvasculature of the hypertensive is markedly altered from that of the normotensive animal, it could be expected that blood flow and volume distribution are different in the hypertensive compared to normal animal, as we have previously shown.9 It could be further expected that the flow distribution would change in a different manner in the hypertensive animal, compared to normal, when the microvasculature is dilated.

One of the metabolites believed to be a major factor producing vasodilation in actively contracting skeletal muscle is adenosine.14,15 Since this is a naturally occurring vasodilator, we have chosen to apply it topically to produce local vasodilation in the cremaster muscles of spontaneously hypertensive rats (SHR) and the genetically related but normotensive WKY rats.

We have developed an indicator dilution technique utilizing vital microscopy and videodensitometry. Intraarterial injection of a bolus of blood containing labeled plasma passes sequentially through the series-coupled microvascular segments. The resulting time-concentration curves are recorded from the micro-
vessels of each segment and analyzed. We have previously reported that the passage of this indicator from the arterial to the venous segments of resting cremaster muscle is slower in SHR than WKY rats.9 The data in this report show that new parallel circuits to WKY from the arterial to the venous segments of resting and/or higher velocity circuits are opened, in contrast to SHR shorter cremaster muscle is slower in SHR than WKY rats.9

**Methods**

Ten WKY rats and 12 SHR (114–209 g; 6 to 7 weeks old) were anesthetized with sodium pentobarbital (5 mg/100 g) and supplemented as required. This age was necessary, since the cremaster muscle (a thin skeletal muscle) in older animals becomes too thick for good vessel imaging of the indicators (described below) as they flow through the microvessels. The animals were placed in a supine position on a heating pad and rectal temperatures maintained at 36°–37°C. The left femoral artery was exposed and cannulated with PE-10 tubing. The tip was placed in the distal end of the aorta for measurement of mean arterial pressure and for indicator injections. This system was filled with a heparinized saline solution. The cremaster muscle preparation was a modification of one by Baez.16 The right testicle was a modification of one by Baez.16 The right testicle sutured the muscle in a flat position over an optical port in the tissue bath, and this was then moved to an AO Microstar-20 microscope stage for observation. The bath solution was maintained at pH = 7.4, PO2 = 30–35 mm Hg, by bubbling a gas mixture of 95% nitrogen and 5% CO2 through the bath and checking it with a Radiometer gas analyzer. A scrotal temperature of 34°C was maintained in the bath by a heating coil.

A mercury light source with appropriate filters for epi-illumination fluorescence microscopy was used. A closed circuit television (CCTV) camera with silicon tube (COHU-SIT) was mounted on the microscope and the images recorded on a video tape recorder (VTR). Time in minutes, seconds, tenths and hundredths of a second was recorded from a time inserter connected in series with the VTR and CCTV monitor. The hundred-second switch was activated by the indicator injection and recorded on the VTR. Either ×10 or ×15 oculars and ×4 or ×10 objectives were used on the microscope.

A Vista Electronics model 306 video sampler (VS) was connected in series with the VTR and the monitor. The VS has two intensity sensitive windows, each one placed over a microvessel on the monitor. Outputs of the silicon camera tube and the VS windows were linear over the range of intensities used in this study. A photocell was placed over the injection marker on the face of the monitor, and the output of each of these recorded. Additional vessels could be sampled by repositioning the windows, with subsequent replaying of the video tape. The curves could then be compared on the same time base because the injection signals were common to all recordings. The heights of the recorded curves could not be compared quantitatively because of the variability in the depth of the vessels in the tissue and the different diameters of the vessels. However, the time-concentration profiles from a given vessel and the relationships between recording loci were extremely reproducible with successive injections.17 Vessel dimensions were measured as the calibrated distance between the two windows of the video sampler. This was accomplished by calibrating the digital readout of the video sampler utilizing a stage micrometer and separating the windows at 20, 50, and 100 μm at each level of magnification. Reproducibility of the measurement of vessel diameter was represented by 30 attempts at determining this parameter in an approximately 27 μm arteriole, yielding a mean value of 26.90 μm ± 0.96 sd.

The bolus injection indicator dilution technique requires constant perfusion pressure, flow rate, and resistance for the duration of the indicator passage. This bolus is injected into the terminal end of the aorta and then passes down the femoral artery to the small artery supplying the muscle. With careful preparation, the central artery (A,) and vein (V,) of the cremaster muscle with several orders of branching arterioles (A,, A2, A3) capillaries and venules (V,, V2, V3) can be isolated for observation. The tissue is perfused at natural flow (determined by the arterial pressure and the peripheral resistance). Therefore, the input function to the vascular area is the curve recorded from the A, arteriole, and the curve recorded in each series-coupled segment is both an output function resulting from the passage between segments and in input function for the next segment. The final output function is in the largest venule (V,). Changes in the curves from A, to V, are the results of the indicator dispersion due to blood flow and volume distribution through the microvessels, which is an integrated effect of all velocity gradients proximal to the segment where the measurement is being made.

The concentration-optical response relationship has been demonstrated to be linear for fluorescence by Nakamura and Wayland18 and Riva et al.19 The fluorescent indicator fluorescein isothiocyanate (FITC) is excited at 490 and the emission is at 520 nm. The FITC-dextran (70,000 MW, Pharmacia AB, Sweden) was used as the plasma label.

The distance between two recording sites (A and B) in a given microvessel was determined as the calibrated distance between the two windows of the video sampler, as described above for vessel diameter measurements. The distance (mm) divided by the difference in the mean transit times of the curves (tA, B) recorded at the two sites gives the mean flow velocity (mm/sec) in this vessel. This technique is not readily applicable to venules as there generally is not sufficient distance between anastomosing capillaries and/or venules emptying into these vessels. In arterioles, the branching does not seem to affect the time-concentration curves when recorded downstream from the branch. A minimal distance of about 250 μm is necessary for meaningful calculations.
The mean transit time \( t \) is the average time required for the indicator particles to flow from the point of injection to the recording site and was calculated for each curve from the following equation:

\[
I = \sum_{0}^{t_E} \frac{(I(t) \times t)}{\Sigma I(t)}
\]

where \( t_E \) is the curve duration and \( I(t) \) is the curve height.

The quantitative conversion of optical measurement to concentration was not necessary since the relationship between the parameters was linear. For the calculation of \( t \), only the relative changes in curve height with time were necessary.

The dispersion of indicator through a microvascular network was assessed as follows. The size of the injection bolus was constant for a given experiment (0.005 ml). The duration of the injection was constant (0.5 second). Therefore, the dispersion of indicator that occurred throughout the vascular network was a function of the flow patterns through the vessels. All times reported are the cumulative times from the time of injection and reflect the integrated effects of all velocity gradients proximal to the segment where the measurement is being made. Changes in the appearance time \( t_E \), curve duration \( t_E \), peak time \( t_E \), as well as \( t \), were used in assessing indicator dispersion. The appearance time is the time for the fastest flowing indicator particles to arrive at the recording site from the point of injection. The curve duration is the time of the entire curve from time of injection to return to baseline. The peak time is the time of maximum concentration during the curve. Arteriolar and venular values were compared.

Time-concentration curves were obtained in first order arterioles \( A_1 \) and first order venules \( V_1 \) of each rat cremaster muscle microcirculation. The FITC-dextran injection volume was small, and thus the indicator was diluted in a relatively large volume. Therefore, the background fluorescence was low, and for successive injections the new baseline level was recorded before each injection. The indicator was injected three times during control and during adenosine application in each animal. The indicator curves exhibited very similar configurations with successive injections during control or during adenosine application. This would indicate that with the constant perfusion pressure there were no significant changes in resistance during each period of study. Since the injection signal was on the videotape and common for all curves for all vascular segments, the curves for the various segments were obtained by repeated replay of the tape.

Femoral arterial pressure was determined by means of a Statham P23DB pressure transducer. All pressures and time-concentration curves were recorded on an Electronics for Medicine recorder (Electronics for Medicine, White Plains, New York). Related values were statistically compared using a paired \( t \) test and reported as \( \pm \) SEM. Means were considered significantly different if the probability was 0.05 or less.

**Results**

**Time-Concentration Curves**

Figure 1 is a replot of mean values of FITC-dextran time-concentration curves from first-order arterioles and first-order venules in a WKY rat and a SHR at control and during topical application of adenosine (0.2 M). The control curves in both groups were similar to those previously described from our laboratory. In the WKY, the arteriolar diameter was 96 \( \mu \)m at control and 95 \( \mu \)m during adenosine. The companion venule was 117 \( \mu \)m at control and 112 \( \mu \)m during adenosine. The SHR arteriolar diameter was 95 \( \mu \)m at control and 93 \( \mu \)m during adenosine. The companion venule was 132 \( \mu \)m at control and 136 \( \mu \)m during adenosine. The WKY and SHR arteriolar curves after adenosine were shifted to the left (shorter times) with a more rapid rise to the peak. The WKY control venular curve had a long tail, and the curve was quite protracted in time. With adenosine dilation, the appearance time \( t_E \), peak time \( t_E \), and the duration \( t_E \) of the curve were reduced. The control SHR venular curve resembled the control WKY venular curve with the exception that the appearance time was about 3 seconds later in the SHR. With adenosine, the SHR and WKY venular curves appearance times decreased markedly as well as the periods of the curves from \( t_E \) to \( t_E \). The venular curve \( t_E \) decreased less in WKY than SHR. The \( t_E \) value was similar for both curves.

**Hemodynamics**

Figure 2 shows that the control femoral arterial pressure averaged 85 \( \pm \) 3 mm Hg in WKY and 110 \( \pm \) 5 mm Hg in SHR. The two groups were significantly different \( (p < 0.01) \). Local exposure of the cremaster muscles to adenosine did not significantly alter these values. However, there was a significant \( (p < 0.05) \) increase in arteriolar blood flow velocity in both groups, which seemed to be the result of significant dilation \( (p < 0.05) \) of the A1 arterioles (fig. 3). Arteriolar blood flow velocity for SHR was not significantly different from WKY either before or during adenosine. Adenosine had no effect on the A1 and A2 arterioles and the V1, V2, and V3 venules.

**Time-Concentration Curve Parameters**

Table 1 presents the mean values from the FITC-dextran time-concentration curve recorded from the A1 arteriole and the V1 venule in SHR and WKY during the control period and during topical adenosine administration. Changes in the parameters of the arteriolar curves either between the groups of animals or due to the adenosine were minimal, presumably due to proximity to the injection site. With dilation, however, the \( t_E \) was reduced significantly \( (p < 0.05) \) in both groups. The venular curves were markedly altered by the vasodilation; figure 4 shows the reduction in each parameter. There were significant differences in the venular values both between animal groups as well as due to the effects of adenosine. The reduction in \( t_E \) with adenosine was significant in both WKY and SHR, with the SHR values decreasing significantly more than the...
WKY (p < 0.05). The $t_p$ decreased significantly in both WKY and SHR (p < 0.05) and by similar amounts. The reductions in $t_E$ were parallel to the changes in $t_p$. Both groups had significant changes, with the SHR values decreasing significantly more than the WKY values (p < 0.05). The changes in $t$ were significant in both groups, with the SHR values decreasing significantly (p < 0.05) more than the WKY values. The $t_a-t_D$, which represents the segments of the time-concentration curves when indicator concentration was increasing, decreased significantly in both groups (p < 0.05). The $t_A-t_D$, which represents the segments of the time-concentration curves when indicator concentration was decreasing, did decrease significantly in SHR, but not in WKY.

Figure 5 presents the changes in the dispersion ratio ($t/D$) of the venular time-concentration curves. There was no change in the WKY values, but the SHR values were significantly decreased (p < 0.05). SHR values also were decreased significantly more than WKY values (p < 0.05).

Discussion

The distribution of blood flow and volume in the microcirculation is dependent not only upon the normal architecture but also on alterations due to a pathological process such as hypertension. There have been numerous reports of microvessels altered by hypertension, resulting in elevated peripheral resistance. It is
**Figure 2.** Arterial pressures and arteriolar blood flow velocities before and during adenosine application in WKY and SHR rats cremaster muscle. (Brackets indicate $\bar{x} \pm$ SEM).

**Figure 3.** Arteriolar and venular diameters before and during adenosine application in WKY and SHR rat cremaster muscles (Brackets indicate $\bar{x} \pm$ SEM).
<table>
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<td></td>
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<td>( t_a )</td>
<td>( t_p )</td>
<td>( t_E )</td>
<td>( \bar{t} )</td>
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### Adenosine

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*Values are means ± SEM.
*\( p < 0.05 \) using paired t test, compared to adenosine value.

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**Figure 4.** Reduction in venular time-concentration curve parameters in SHR and WKY rats with adenosine application. (Brackets indicate \( \bar{x} \) ± SEM).
conceivable that these vascular alterations could result in blood flow distribution being differentially altered in the normotensive vs the hypertensive preparation. Flow and volume distribution would also be expected to change in a different manner in the hypertensive animal as compared to the normotensive animal when the microvasculature is diluted. In this study, the microcirculation of the cremaster muscles of normotensive WKY and SHR were compared before and during vasodilation with adenosine.

The topical application of adenosine to the cremaster muscle vasculature of the two groups of rats did not change the arterial pressure of either group, indicating that the microvascular responses to adenosine were limited to the smaller arterioles, the input functions from the microcirculation, were quite protracted in time (tE up to 40 seconds) in both groups. These curves were different from those recorded from the venules of the mesenteric microcirculation22-25 in that the latter curves were only a few seconds in duration (tE up to 10 seconds). This would seem to indicate that there may be a greater cross-sectional area or capacity of the cremaster muscle post-arteriolar vasculature than of mesentery.

The extended period of the venous time-concentration curves is one of the most striking results in this report. As we have previously reported,9 the major vascular segments contributing to the extension of the curves are the capillaries and venules, and this is so for both SHR and WKY. There is a prolonged time for passage of the indicators through the capillaries and venules, indicating a marked increase in vascular cross-sectional areas and a resulting reduction in blood flow velocity. It is conceivable, however, that this explanation may be overly simplified. Branching effects, changing viscosity, and altered pressure gradients may also be contributing in some manner.

There are several hypotheses about the differences between the microvessels of SHR and WKY cremaster muscles. It has been reported that there is a reduction in vessel lumen, particularly of the larger arterioles (A1 and A2), due to thickening of the media of the vessel wall in the SHR. It is difficult to understand how this factor would explain our data since the A1 and A2 were not dilated by the adenosine in either WKY or SHR. Only the smaller groups of arterioles dilated in both WKY and SHR. Since the value for the series-coupled resistance in the A1 and A2 vessels would be unchanged, the differing flow distribution in SHR vs WKY would have to be explained on the basis of differences between WKY and SHR microvasculature downstream from the larger arterioles.

When the cremaster muscles were exposed to the adenosine there were several significant changes in the venular time-concentration curves. There were reductions in all of the dispersion parameters in both the SHR and WKY venules. However, tE, tE, t, and a/f decreased more in SHR than in WKY preparations. The exceptions were similar reductions of tE and tE-t, in
the WKY and SHR curves. An attempt was made to determine the amount of dispersion of the blood flow due to the amount of randomization imposed by the system. In this case this would be due to the branching vasculature and the spectrum of flow velocities through the vessels. If one assumes that a bolus injection of indicator would be dispersed the same as the blood flow, then the dispersion of the indicator should be reflected by the ratio T_I where, the smaller the value of the ratio, the greater would be the dispersion. The dispersion of the FITC-dextran as determined by the ratio t_I was unchanged in the arterioles of the SHR.

The minimal increase in dispersion (reduced t_I) observed in WKY after adenosine could be accounted for by increased velocity through existing circuits 26 without an increase in the number of parallel circuits opened to flow. One can examine the segments of the time-concentration curves using the concept that t_I represents the shortest and fastest circuits, t_e-t_i the mid-length and/or mid-velocity circuits, and t_e-t_a the longest and/or slowest circuit. There is a moderate but significant decrease in the venular appearance times (t_e) and a much greater reduction in the venular curve duration (t_e) principally due to reduction in the early part of the time-concentration curves (t_e-t_a). The SHR group responded to adenosine vasodilation by the opening of new shorter and/or higher velocity circuits with little effect on the longer and/or lower velocity circuits. This is supported by the significantly greater reductions in SHR of t_e, t_a, t_o, and t_I than in WKY. In addition, SHR t_e-t_o was as significantly decreased as the WKY values, and t_e-t_a was significantly reduced only in the SHR. The venular dispersion was significantly increased, as evidenced by a reduction in the ratio of t_I in the SHR. Since the flow velocities and vessel diameters were similar in WKY and SHR, it could be postulated that some of the indicator had passed through the newly opened shorter and higher velocity circuits in the SHR consequent to vasodilatation, resulting in the venular curve exhibiting a much greater reduction in t_e than in t. This was indeed the case with our data.

The data suggest that the flow distribution between SHR and WKY is different, as we have previously reported, and also that the flow distribution changes in SHR are significantly different from WKY when the microvasculature is dilated. The data would also indicate that in the cremaster muscle of SHR there are vascular circuits that are not normally open but are recruited when exposed to adenosine dilatation. It is conceivable that these closed circuits contribute to the elevated resistance found in SHR.

Acknowledgments
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References
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