Neural and Local Control of Arterioles in SHR

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SUMMARY This study sought to determine if neural influences and/or alterations in arteriolar responses to oxygen could contribute to an elevated microvascular resistance in spontaneously hypertensive rats (SHR). Diameters of third-order arterioles (3A) and fourth-order arterioles (4A) were measured in the cremaster muscle of 12- to 15-week-old SHR and normotensive Wistar-Kyoto (WKY) controls anesthetized with pentobarbital. The preparation was suffused with physiological salt solution (PSS) equilibrated with various concentrations of oxygen (0% O₂, 5% O₂, or 10% O₂) with and without local neural blockade with 10⁻⁷ M tetrodotoxin (TTX). Total active tone was assessed with 1(H M adenosine. SHR 3A (but not 4A) exhibited a smaller resting diameter than WKY, and larger dilations in response to TTX and adenosine. When suffusion solution PO₂ was elevated in the presence or absence of TTX, SHR arterioles constricted more than did those of WKY, and SHR 4A exhibited a higher incidence of complete closure. Therefore, both neural influences and local vascular control mechanisms may contribute to an elevated microvascular resistance in SHR. (Hypertension 6: 530-535, 1984)

KEY WORDS. • microcirculation • metabolic autoregulatory mechanisms • tetrodotoxin • myogenic autoregulatory mechanisms • hypertension • oxygen • cremaster muscle

A n increase in total peripheral resistance (TPR) is a common denominator in all forms of hypertension. This maintained increase in TPR in hypertension could be due either to structural changes in vessel walls, to decreases in the number of arterioles open for flow, or to continuously enhanced activity of the vascular smooth muscle (VSM) effector cells. These changes could contribute to the elevated TPR either by constriction of flowing arterioles or by active closure or "functional rarefaction" of the existing vessels open to flow.

Increased active VSM tone in hypertension could result from an elevated neural excitatory input, an enhanced intrinsic (myogenic) activity of the VSM cells, and/or an increased VSM sensitivity or reactivity to vasoactive agents and intrinsic stimuli. In addition, a recent study has suggested that oxygen availability may be important for the maintenance of an elevated vascular resistance in spontaneously hypertensive rats (SHR). However, in rats with renovascular hypertension, arteriolar sensitivity to oxygen may be either increased or decreased.

The object of our present study was to employ direct observation of the skeletal muscle microcirculation of SHR and age-matched normotensive Wistar-Kyoto (WKY) controls to determine: 1) if part of the elevated microvascular resistance in hypertensive animals might result from increased active VSM tone in arteriolar resistance vessels; 2) if any existing increase in arteriolar tone might result from an elevated neural excitatory input; and 3) if the response of skeletal muscle arterioles to changes in O₂ availability might be altered in SHR and therefore potentially contribute to an increased microvascular resistance in the hypertensive animals. The results of the study indicated that active neurogenic tone is elevated in the resistance vessels of SHR and that arteriolar constrictor responses to increased oxygen availability are enhanced in SHR. Therefore, both neural influences and local vascular control (i.e., autoregulatory) mechanisms could potentially contribute to an elevated microvascular resistance in SHR.

Methods

General Procedures
We studied 91 12- to 15-week-old male SHR (293 ± 4 g) and 70 age-matched WKY control rats (301 ± 4 g) from Taconic Farms, Inc., Germantown, New York. Rats were anesthetized with 60 mg/kg sodium pentobarbital i.p. The trachea was cannulated to ensure a patent airway, a femoral vein was cannulated for...
the administration of small doses of supplemental anesthesia as necessary, and a carotid artery was cannulated for the measurement of arterial pressure with a Statham P23ID arterial pressure transducer. The mean arterial pressure of the rats employed in this study was 156 ± 6 mm Hg (mean ± se) in SHR and 109 ± 4 mm Hg (mean ± se) in WKY.

After the initial surgery was completed, a suffused open cremaster muscle was prepared by a modification of the method of Baez. The animal chamber was then placed on the stage of a Leitz microscope, the preparation was transilluminated, and the cremaster muscle was suffused with a physiological salt solution (PSS) that was kept at a constant temperature (35°C). The PSS was bicarbonate-buffered (pH 7.35) and contained the following (mM): NaCl, 130; KCl, 4.7; NaH₂PO₄, 1.18; MgSO₄, 1.17; CaCl₂, 1.6; NaHCO₃, 14.9; and disodium EDTA, 0.026. The PO₂, PCO₂, and pH of the suffusate were maintained by equilibrating the PSS with gas mixtures containing 5% CO₂, various percentages of O₂, and N₂ as a filler gas. Equilibration with atmospheric air was prevented by the use of gas impermeable tubing, short delivery lines, and a fairly rapid suffusion rate (approximately 3 ml/min) devoid of stagnant eddies.

The microcirculation was viewed on a closed circuit television system. Internal diameters of third-order (3A) and fourth-order (4A) arterioles (classification of Hutchins and Darnell) were measured on the monitor with a moveable reference line system (accuracy ± 1 μm) operated through a Model 321 Colorado Video Analyzer (Colorado Video, Inc., Boulder, Colorado) and connected to a Grass Model 7 polygraph (Grass Instrument Company, Quincy, Massachusetts). When possible, arteriolar diameters were measured near the bifurcation of a 3A and 4A arteriole, with the diameter of the 3A being measured just proximal to the bifurcation and that of the 4A being measured on a straight section of arteriole slightly removed from the bifurcation. The diameter measurement recorded for each experimental point in an animal reflected the average of three to six individual measurements at each vessel site. In cases where vasomotion was evident, the recorded diameter measurement reflected the midpoint of the average maximum and minimum values occurring during the vasomotion cycle. Vessels chosen for study had inside walls that were clearly visible and did not exhibit stagnant or near-stagnant flow, adhering leukocytes, segmental constrictions, or other obvious indications of trauma.

Assessment of Arteriolar Tone and Oxygen Responses

After the animal chamber had been placed on the stage of the microscope, the preparation was allowed to equilibrate for 30 to 60 minutes while being suffused with PSS equilibrated with 0% O₂—5% CO₂—95% N₂. During 0% O₂ suffusion, the O₂ supply to the tissue was derived from the vasculature rather than the suffusion solution. After the equilibration period, overall active vascular smooth muscle tone in the arterioles was assessed by measuring the dilation occurring in response to topical application of a 10⁻⁴ M solution of adenosine with a Pasteur pipette.

After the arteriole returned to control diameter, the PO₂ of the suffusion solution was increased for 20 minutes by equilibrating it with either 5% O₂ or 10% O₂. Under these conditions, the approximate PO₂ of the suffusate could be calculated from the fractional concentration of O₂ according to Dalton’s law, while mean tissue PO₂, as measured in hamster cremaster muscle by Klitzman et al., was approximately 15 mm Hg during 0% O₂ suffusion, 25 mm Hg during 5% O₂ suffusion, and 35 to 40 mm Hg during 10% O₂ suffusion. Arteriolar responses to alterations in O₂ availability in SHR and WKY were assessed by comparing the absolute magnitude of constriction (μm) in arterioles during elevation of suffusion solution PO₂ and the fraction of all observed arterioles that were open during 0% O₂ suffusion but that exhibited complete closure when suffusion solution PO₂ was increased by changing the gas equilibration mixture from 0% O₂ to either 5% O₂ or 10% O₂.

At the end of the test period, 0% O₂ suffusion was restored, and the preparation was allowed to reequilibrate for 20 minutes. In some experiments, the response to a different level of increased O₂ availability was tested after the reequilibration period under 0% O₂ suffusion. In other experiments, the amount of active neurogenic tone in the vessels was assessed by suffusion with 10⁻⁷ g/ml of the specific neuronal blocking agent tetrodotoxin (TTX). This agent has previously been demonstrated to have no direct effect upon VSM tone or arteriolar responses to increased oxygen availability.

In the TTX experiments, TTX was added to the PSS in the reservoir to achieve a final concentration of 10⁻⁷ g/ml. The cremaster muscle was then continuously suffused with PSS containing the blocker. After 10 minutes of 0% O₂ suffusion with TTX, the amount of nonneural active tone in the vessels was assessed by measuring the response to topical application of 10⁻⁴ M adenosine, as described above. In one series of experiments, arteriolar responses to alterations in oxygen availability were tested during continuous suffusion with TTX. In each experimental protocol, the duration of the various test suffusions (increased PO₂, TTX, adenosine) was adequate to attain a steady-state diameter in all the vessels that were observed.

Statistical Tests and Data Analysis

In each series of experiments, the null hypothesis tested was that there was no difference in the response of a given vessel branching order of SHR and WKY to a given test. Differences between the means of two groups were assessed by Student’s t test for unpaired data, while differences between multiple group means were assessed by analysis of variance with a subsequent multiple range test. Differences in the frequency of arteriolar closure in response to increased O₂ availability were assessed by contingency table analysis with a chi-square test. A difference of p < 0.05 was considered to be statistically significant.
Results

Arteriolar Diameters and Responses to Adenosine and Tetrodotoxin

The mean diameter (± se) of SHR 3As (26 ± 0.9 μm, n = 82) was slightly but significantly less than that of WKY (29 ± 0.6 μm, n = 92) (p < 0.05), while the mean diameter of 4A was similar in SHR (15 ± 0.6 μm, n = 85) and WKY (15 ± 0.4 μm, n = 117). Relative to WKY, SHR 3A exhibited a significantly larger dilation in response to adenosine (Figure 1) and TTX (Figure 2), while 4A of SHR and WKY exhibited similar responses to both these agents. There were no significant differences in the dilation of 3A or 4A of SHR and WKY in response to adenosine application during TTX suffusion (Figure 3).

Arteriolar Responses to Alterations in O₂ Availability

Figure 4 compares the constriction of 3A and 4A of SHR and WKY during elevation of suffusion solution PO₂ in the presence and absence of neural blockade with TTX. Arterioles of SHR appeared to be more sensitive to increases in O₂ availability than those of WKY, as evidenced by the significantly larger constrictions that generally occurred when the PO₂ of the suffusion solution was elevated by changing the gas equilibration mixture from 0% O₂ to either 5% O₂ or 10% O₂. This increased incidence of closure in 4A of SHR in response to oxygen was observed both in the presence and absence of neural blockade with TTX.

TTX. Relative to WKY, 4A of SHR exhibited a significantly higher incidence of complete closure when the PO₂ of the suffusion solution was elevated by changing from 0% O₂ to either 5% O₂ or 10% O₂. This increased incidence of closure in 4A of SHR in response to oxygen was observed both in the presence and absence of neural blockade with TTX.

Figure 5 compares the incidence of complete closure of 4A of SHR and WKY in response to elevation of suffusion solution PO₂ in the presence and absence of TTX. Relative to WKY, 4A of SHR exhibited a significantly higher incidence of complete closure when the PO₂ of the suffusion solution was elevated by changing from 0% O₂ to either 5% O₂ or 10% O₂. This increased incidence of closure in 4A of SHR in response to oxygen was observed both in the presence and absence of neural blockade with TTX.

FIGURE 1. Dilation (μm) of third-order (3A) and fourth-order (4A) arterioles of the cremaster muscle of 12- to 15-week-old SHR (stippled bars) and WKY controls (open bars) in response to topical application of 10⁻⁴ M adenosine solution. Data are expressed as mean increase (± se) from control diameter measured immediately before application of adenosine. Numbers above bars indicate number of vessel sites in 13 to 23 animals, and the asterisk denotes a significant difference (p < 0.05) between SHR and WKY.

FIGURE 2. Response of third-order (3A) and fourth-order (4A) arterioles of SHR (stippled bars) and WKY (open bars) to a 10-minute suffusion with the specific neural-blocking agent tetrodotoxin (10⁻⁷ g/ml). Data are expressed as mean change in microns (± se) from control diameter measured immediately prior to tetrodotoxin suffusion. Numbers represent the number of arterioles observed in 17 to 26 animals, and the asterisk denotes a significant difference (p < 0.05) between SHR and WKY.

FIGURE 3. Dilation (μm) of third-order (3A) and fourth-order (4A) arterioles of SHR (stippled bars) and WKY (open bars) in response to topical application of 10⁻⁴ M adenosine during neural blockade with 10⁻⁷ g/ml tetrodotoxin. Numbers above bars represent number of vessels observed in 31 to 40 animals. There were no significant differences in the response of SHR and WKY to adenosine application during neural blockade.
Discussion

In the present study, we observed a small but significant reduction in the diameter of 3A (but not 4A) of SHR relative to those of WKY. SHR 3A also exhibited larger dilations than those of WKY in response to adenosine and TTX. These results suggest that active VSM tone is elevated in flowing 3A resistance vessels of SHR, but not in the smaller 4A that may be more involved in controlling the distribution of flow within the tissue. The larger dilation of 3A of SHR in response to neural blockade with TTX and the equal dilation of SHR and WKY vessels in response to adenosine in the presence of TTX suggest that the elevated VSM tone in 3A of the hypertensive animals during 0% O₂ suffusion is primarily neurogenic.

Our observation that 3A of SHR exhibit a smaller resting diameter than those of WKY is consistent with the findings of Roy and Mayrovitz, who also reported smaller arteriolar diameters in the cremaster muscle of SHR relative to WKY. However, these studies contrast with earlier ones that reported no significant difference in cremasteric arteriolar diameters of SHR and WKY. The reason for these differences is unclear, as is the explanation for the rather large variation in reported diameters of cremasteric arterioles in various studies.

FIGURE 4. Constriction (µm) of third-order (3A) (upper panel) and fourth-order (4A) (lower panel) arterioles of SHR (stippled bars) and WKY (open bars) in response to elevation of the O₂ content of the physiological salt solution (PSS) suffusing the tissue. The left side of each panel represents the O₂ response during suffusion with PSS alone, and the right side represents the response during neural blockade with 10⁻⁷ g/ml tetrodotoxin (PSS + TTX). Data are expressed as mean decrease in microns (± se) from control diameter measured during 0% O₂ suffusion. Numbers below bars indicate number of vessels observed in 7 to 29 animals. Asterisk denotes a significant difference (p < 0.05) between SHR and WKY at a given suffusion solution PO₂ (see text for details).

FIGURE 5. Incidence of complete closure of fourth-order (4A) arterioles of SHR (stippled bars) and WKY (open bars) in response to elevation of the O₂ content of the physiological salt solution (PSS) suffusing the tissue. Top panel summarizes closure in response to O₂ during suffusion with PSS alone and bottom panel represents closure in response to O₂ during neural blockade with 10⁻⁷ g/ml tetrodotoxin (PSS + TTX). Ordinate represents the percentage of observed arterioles that were open during 0% O₂ suffusion but closed in response to 5% O₂ or 10% O₂ suffusion. Numbers above bars indicate the number of arterioles that closed during 5% O₂ or 10% O₂ suffusion vs the number of open arterioles studied during 0% O₂ suffusion in seven to 29 animals. Asterisks denote a significant difference (p < 0.05) between SHR and WKY at a given suffusion solution PO₂ (see text for details).
As previously noted,\textsuperscript{7} lumen diameters can vary considerably in arterioles classified on the basis of branching order. One possible source of variation in resting arteriolar diameter may be the use of covered preparations in some studies\textsuperscript{1, 21} and suffusion solutions in others.\textsuperscript{2, 22} For example, the resting diameters of cremaster 3A of SHR and WKY in our experiments are larger than those reported in some studies employing covered preparations,\textsuperscript{3, 21} but are very similar to those reported for 3A of hypertensive\textsuperscript{2} and normotensive\textsuperscript{2, 22} rat cremaster muscles bathed with PSS equilibrated with 0% O\textsubscript{2}. In addition, Roy and Mayrovitz\textsuperscript{7} noted that lumen diameter can vary considerably along the length of an individual vessel of a given branching order. They concluded that such variations could be responsible not only for many of the differences in published diameters of individual branching orders, but also for the lack of difference in arteriolar diameter between SHR and WKY which had been previously reported in some studies.

The larger dilation of 3A that we observed in SHR in response to adenosine is consistent with the study of Roy and Mayrovitz,\textsuperscript{7} who also reported a greater dilation of SHR arterioles in response to adenosine. Our results contrast with those of earlier studies,\textsuperscript{21} however, which employed surgical denervation to eliminate neurogenic tone and sodium nitroprusside to maximally dilate cremaster arterioles of SHR and WKY. The contrasting results of these studies could reflect differences in experimental procedure, such as the use of rats of different ages (see below) or the use of different methods to eliminate neurogenic or overall VSM tone. For example, surgical denervation does not preclude the possible contribution of accessory innervation to arteriolar tone, whereas TTX produces complete blockade of neurogenic tone without affecting VSM directly.\textsuperscript{19} In addition, the use of sodium nitroprusside as a dilator may obscure differences in the amount of active VSM tone in microvessels of SHR and WKY, since this agent produces a profound systemic hypotensive response that appears to be greater in SHR than in WKY. In preliminary experiments, we found that topical application of nitroprusside to the cremaster muscle produced a significantly greater ($p < 0.01$) pressure decrease in SHR (94 ± 7 mm Hg, $n = 5$) than in WKY (52 ± 9 mm Hg, $n = 7$). If a large part of this pressure decrease is transmitted to the arterioles (which seems likely), it could result in a greater tendency for passive collapse of the relaxed vessels that experience the greatest pressure reduction, namely, those of SHR. In contrast, adenosine had no measurable effect on systemic arterial pressure in the present study or in the study of Roy and Mayrovitz,\textsuperscript{7} who also demonstrated a greater dilation in cremaster arterioles of SHR relative to WKY.

The apparent elevation of active neurogenic tone in skeletal muscle resistance vessels of SHR in the present experiments is consistent with the results of previous studies, which suggest that an increased sympathetic efferent activity exists in SHR,\textsuperscript{23, 24} and this elevation is also consistent with studies that demonstrate a greater adrenergically mediated VSM depolarization in small mesenteric veins of 12- to 15-week-old SHR relative to WKY.\textsuperscript{6-10} Since neurogenic excitatory influences appear to make their maximum contribution to VSM membrane depolarization in SHR at this age,\textsuperscript{9} the use of 12- to 15-week-old animals in the present study may provide the most likely explanation for the contrasting results of our experiments and those of Bohlen and Lobach.\textsuperscript{21} In the latter study, one group of rats was in the early (5- to 7-week) stage of hypertension while the other rats were in a stage later than the animals used in our study (16 to 18 weeks). Measurements of vascular smooth muscle transmembrane potential in mesenteric veins of 4- to 6-week-old animals have not demonstrated depolarization in SHR relative to WKY,\textsuperscript{9} which indicates that neurogenic excitatory influences have not reached their full expression in very young animals. In contrast, other evidence suggests that elevated neural traffic may become replaced in older animals as structural changes in vessel walls\textsuperscript{1} (and possibly anatomical rarefaction)\textsuperscript{9} begin to maintain the increased peripheral vascular resistance and blood pressure.

In contrast to 3A vessels, 4A of SHR did not exhibit a smaller resting diameter or a larger dilation in response to TTX or adenosine relative to those of WKY. This difference between 3A and 4A may reflect a relative absence of neural innervation in the 4A or an escape from a continually elevated level of adrenergic activation in the hypertensive animals. The latter hypothesis is plausible in light of the report of Marshall\textsuperscript{23} that terminal arterioles of the rat spineotrapezius muscle fail to maintain their constriction during electrical activation of the vasomotor nerves, while larger arterioles remain constricted throughout the stimulation period.

In addition to demonstrating an elevated neurogenic VSM tone in 3A resistance vessels of SHR, the present study indicates that arterioles of SHR exhibit a greater constriction than those of WKY in response to increased $O_2$ availability. SHR 4A also exhibit a significantly higher incidence of complete closure when suffusion solution $PO_2$ is elevated. The increased response of 3A and 4A to $O_2$ in SHR does not depend upon neural activity, since it also occurs in the presence of TTX.

The potential importance of oxygen in contributing to an elevated TPR in SHR has been suggested by Walsh and Tobia,\textsuperscript{14} who reported that $O_2$ must be available for subclavian vascular resistance to be elevated in young (8-week-old) cord-transsected SHR, and that repeated blood flow increases appear to produce an enhanced autoregulatory constrictor response in young cord-transsected SHR relative to WKY. As noted above, the equal response of SHR and WKY arterioles to adenosine applied during 0% $O_2$ suffusion in the presence of TTX suggests that neural influences (rather than local vascular control mechanisms) are the primary source of the elevated VSM tone in SHR during low $O_2$ suffusion. However, the enhanced response of SHR vessels to increased $O_2$ availability (both in the
presence and absence of TTX) suggests that arteriolar closure of the hypertensive animals will increase vascular resistance more than those of WKY when O₂ availability is increased. Therefore, under conditions where tissue PO₂ does not tend to be maintained at a reduced level by low O₂ suffusion, local vascular control mechanisms could potentially contribute to the elevated vascular resistance in SHR.

Bohlen and co-workers have demonstrated that arteriolar pressure is elevated at all branching orders in the cremaster muscle of SHR. In light of this observation, Prewitt et al. suggested that the increased microvascular perfusion pressure in SHR might result in blood flow in excess of the metabolic needs of the tissue and lead to an autoregulatory-related increase in vascular resistance. Although previous studies have demonstrated a higher incidence of arteriolar closure mediated by increased neural activity or hyperresponsiveness to norepinephrine in SHR, our experiments suggest that factors related to O₂ availability can also contribute to functional rarefaction of arterioles in SHR. The present study therefore provides evidence in favor of the hypothesis that local vascular control mechanisms could contribute not only to an enhanced vasoconstrictor tone in hypertensive animals but also to the active closure of skeletal muscle arterioles due to tissue overperfusion.

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