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Micro vascular Pressure, Flow, and Resistance in Spontaneously Hypertensive Rats

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SUMMARY Microvascular resistance (MVR), determined as the ratio of the second-order arteriolar blood pressure (servo-null method) to blood flow (dual-slit), was assessed in the cremaster muscle preparation of 7- to 8-week-old normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). The MVR was used as an index of resistance during 1) control conditions; 2) superfusion of graded concentrations of the catecholamine norepinephrine (NE; WKY n = 8, SHR n = 8); 3) superfusion with NE and in the presence of a fixed concentration of the vasodilator sodium nitroprusside (NP; 10^{-5} M); and 4) superfusion with the noncatecholamine phenylephrine (PE; WKY n = 8, SHR n = 9). The vasoconstrictor challenges were carried out to determine if there was any differential vascular sensitivity between the hypertensive and nonhypertensive rats to the exposure of an endogenous-like constrictor possessing a catechol nucleus as opposed to a strictly synthetic analog. The presence of NP was employed to assess the degree of vasoconstriction normally present under control conditions. The combination of NE and NP was used to test for a differential vasoconstrictor sensitivity, beginning from maximally dilated conditions. The MVR, assessed at the second-order arteriolar level, represents approximately 35% of the total resistance of the skeletal muscle and is intimately involved in maintaining proper end-organ perfusion pressure. The microvascular resistance of the SHR group was almost four times greater than that of the WKY group under control conditions. Maximum vasodilation with topical NP reduced the MVR in both groups, but the SHR microvascular resistance remained two times greater than that found in the WKY. The SHR had greater MVR responses following challenge with both NE and PE and also in the presence of NP during NE challenge. The conclusions are: 1) the microvascular resistance of the SHR is elevated under control conditions due to structural modifications of the vasculature and exacerbated following constrictor challenge as a result of heightened vasoconstrictor sensitivity; and 2) the elevated MVR in the SHR is not due to a simple arterial vasoconstriction that can be totally eliminated with vasodilation. (Hypertension 6: 877-886, 1984)

KEY WORDS • essential hypertension • vascular resistance • spontaneously hypertensive rat • microcirculation

THE complete etiology of essential hypertension is unknown, but it probably involves an interplay between cardiac and peripheral vascular factors.[1-3] Hypertension as exhibited by the spontaneously hypertensive rat (SHR) is associated with a relatively normal cardiac output (CO),[4-6] although the cardiac index has been found to be elevated.[6] This normal CO in both young and mature SHR mirrors the cardiac hemodynamics generally found in human patients with established essential hypertension.[7-9] Often, an increase in CO has been found in humans during the early, labile phase of hypertension. Although its significance is not clear at present, this early increase in CO is suspected to be the initial event in the pathogenesis of essential hypertension.[7-9] Peripheral vascular resistance, on the other hand, is elevated in both SHR and hypertensive humans.[5-7,10] The factors believed to affect vascular resistance are numerous and include medial encroachment into the arterial lumen and structural adaptations," sympathetic neural hyperactivity,[12] arterial rarefaction,[13-14] and altered humoral vascular sensitivity.[15-16]

Elevated arterial blood pressure responses to injected catecholamines have been well documented in human essential hypertension.[15-17-18] Results from vascular studies in the SHR, an animal model of essential hypertension, have been far less consistent. Some workers have reported equal or decreased responses to catecholamines,[16-19-M] while others have demonstrated increased responses.[21-23]

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Involvement of the microvasculature as a factor in the hypersensitivity of SHR arteries to catecholamines has not been proven. Hutchins et al.[24] reported no difference in cremaster arteriolar constriction between young SHR and normotensive rats after intraarterial injections of norepinephrine (NE). In contrast, Bohlen,[23] utilizing microiontophoretic application of NE directly on microvessels in the mature 18- to 20-week-old SHR, reported a definite heightened constriction of the arterioles in the cremaster preparation. Henrich and Hertel[26] examined microvessels in isolated mesenteries and those perfused with Tyrode's solution in 24- to 30-week-old SHR and normotensive WKY and Wistar control rats. They reported that SHR had a greater increase in perfusion pressure in response to NE than either of the normotensive animal groups, and they concluded that this elevated response was the result of a greater constriction of the small arterioles (21.2 /*xm* in diameter).

Most reports suggest a definite hypersensitivity of microvessels to catecholamines, as assessed by changes in arterial diameter. To date, few studies have demonstrated the effects of diameter changes on vascular resistance at the microvascular level,[27,28] and none have determined the microvascular resistance changes in response to injected or superfused catecholamines.

In the present study, we examined the resistive influence of this distal portion of the circulatory system, the microvasculature, under varying states. This section of the vasculature comprises approximately one-third of the skeletal muscle vascular resistance and is important in maintaining the appropriate blood pressure and flow in accordance with constantly changing systemic and local conditions. Specifically, the objectives of this study were to: 1) obtain quantitative data on the resistance of the cremaster muscle microvasculature in normotensive and spontaneously hypertensive rats; 2) monitor the change in resistance during superfusion with catecholamine and noncatecholamine alpha-adrenergic agonists; and 3) determine if the dose-response relationships are altered by pretreatment with a pharmacological vasodilator.

Methods

Experimental Animals and Surgical Procedure

We obtained male Wistar-Kyoto (WKY) rats and SHR from the Charles River Breeding Laboratories (Wilmington, Massachusetts); they were derived from the Okamoto and Aoki strains.[29] All rats were 7 to 8 weeks old and weighed between 100 and 120 g. Each animal was initially anesthetized for the surgical procedure with Nembutal (5.0 mg/100 g body weight, i.p.). During the acute experimental procedures, the rats were maintained under anesthesia by a constant arterial infusion (0.008 ml/min) with a Harvard infusion pump (Harvard Instruments, Inc., South Natick, Massachusetts) of a heparinized (10 units/ml) anesthetic solution (Inactin 0.08 mg/min). The infusion maintained the animal at a constant level of anesthesia, kept the blood pressure cannula patent at all times, and replaced lost fluid volume due to respiration. Inactin was chosen as an anesthetic for the experimental phase of the research because: 1) it allowed the systemic blood pressure to be maintained at near awake pressure values; 2) it had minimal depressive effects on the heart rate and rate of respiration, which is not the case for Nembutal or chlorolose/urethane; and 3) it has an extremely wide margin of safety, which allowed the maintenance of a long-term, steady blood pressure at the level of surgical anesthesia. The depth of anesthesia was felt to be crucial, since Judy et al.[12] have shown that SHRs maintained at a level of light anesthesia exhibit sympathetic (renal) nerve activity very similar to that found in the awake SHR.

The technique used to prepare the cremaster muscle for observation with the microscope was adapted from the method reported by Baez.[30] The experimental setup, described previously,[27] employed an optically clear pedestal that was thermostatically controlled to maintain the tissue temperature at 34° ± 0.1 ° C, which is the in situ temperature of the cremaster muscle.[30] The muscle preparation was allowed to stabilize for 1 hour before any measurements were made. The criteria for rejecting the preparation for hemodynamic study were: 1) red blood cell extravasation (petechia) in the muscle tissue; 2) lack of vasodilation (10% or greater) in response to a topically applied 10^{-3} M concentration of adenosine; and 3) lack of vasoconstriction (20% or greater) to a topically applied 10^{-7} M concentration of NE or 5 x 10^{-6} M concentration of phenylephrine (PE) in the second-order artery under observation.

Cremaster Muscle Microvascular Anatomy

The arterial blood vessels of the cremaster muscle microvasculature were assigned to categories according to their branching order. The major feeding artery, the cremasteric artery, was designated first order. Succeeding branches were assigned consecutive order numbers from two through five. The fifth-order arteries in this preparation directly supplied the capillaries. This study dealt specifically with the microvascular input resistance (MVR) as measured at the level of the second-order artery in both the WKY and SHR groups. The MVR represents the vascular resistance distal to the site of measurement.

Experimental Techniques

Blood velocity measurements were made by using a modified dual-slit method[31] and an on-line cross-correlation technique.[32–34] For subsequent blood flow calculations, the vessel diameter was measured with an eyepiece micrometer. Accurate measurements could be made with the micrometer since these vessels exhibited relatively little vasomotion in the presence of the Inactin anesthetic. The accuracy of the red blood cell (RBC) velocity tracking device (Instrumentation for Physiology & Medicine, Inc., San Diego, California) was by calibration with a rotating disc placed in the optical path of the microscope system.

Microvessel blood pressures (hereafter referred to as micropressures) were measured with a W.P.I. M-900

Micropressure System (W.P.I.; New Haven, Connecticut), which was based on the servo-null concept.[35,38] In this procedure, the blood pressure cannula was a micropipette filled with a 2 M NaCl solution that was inserted into a vessel with the aid of a stage mounted micromanipulator. The micropipettes (W.P.I., 1 mm diameter Kwik-Fill borosilicate type) were pulled (tapered) on a Narishige dual magnet pipette-puller. The best results were obtained with the servo-null micropressure system when external tip diameters were between 1.5 and 2.5 /urn. In practice, the micropipettes, once properly filled, had an impedance of 1.5 to 2.5 megohms. A micromanipulator was redesigned to allow it to be stage-mounted and affixed in various positions around the mounting board for optimal placement of the micropipettes. Use of the stage-mounted micromanipulator enabled the microscope to be moved in any X-Y position or defocused without disturbing the placement of the micropipette tip in the muscle tissue.

A Leitz Ortholux trinocular microscope was used with a x 10 objective lens in a combination with x 16 ocular lenses. The trinocular tube, employed to pick up the paired modulated light signals for the RBC velocity tracker, was fitted with a x 20 ocular lens. The objective was modified by the addition of a dipping cone, which penetrated the surface of the moving superfusion solution and thus did not allow the observed image to be subjected to any optical aberrations resulting from the undulating liquid-air interface. The optical magnification of the objective and dipping cone immersed in the superfusion solution was found to be equal to the magnification of the dry objective alone (nonimmersed), and therefore an optical magnification correction factor was unnecessary.

Hemodynamic Calculations

Arterial blood flow (nanoliters/sec unless otherwise noted) was calculated from Equation 1:

$$Q = \frac{TTD^2}{4} \frac{V}{1.6} \times 10^{-9} \qquad (1)$$

where: Q = microvessel blood flow; D = vessel diameter in micrometers; V_c = velocity as measured from the RBC velocity tracker in mm/sec; and V^l .6 equals mean RBC velocity.[36]

The microvascular input resistance (MVR), as defined and determined in this study, was the ratio of the measured second-order arteriole micropressure to simultaneously determined blood flow in the same arteriole. Implicit to the calculation was the assumption that the venous pressure was negligible. Since the measured parameters were dependent upon the summation of the individual downstream resistances, the MVR reflected the total resistance to blood flow distal to the site of measurement and was used as an index for comparison between the SHR and WKY rats during subsequent exposure to vasoactive compounds.

Experimental Protocols

This study involved the observation of the second-order artery that coursed through the medial aspect of the cremaster muscle. This portion of the muscle was thinner than the lateral aspect and allowed fine discrimination of small diameter changes. After insertion of a micropipette (for micropressure determination) into the second-order artery as close as physically possible to the parent vessel (first-order), simultaneous baseline values were established for vessel diameter, blood velocity, and micropressure over a minimum time period of 5 minutes. The preparation was then tested for proper acceptance criteria (described earlier in the surgical procedure section).

The MVR in the WKY and SHR groups was calculated to determine the resistance to blood flow due to that portion of the microvasculature distal to the site of observation. The MVR changes in response to topically applied graded concentrations of NE, an endogenous catecholamine alpha-adrenergic agonist, and PE, a noncatecholamine alpha-agonist, were used to evaluate the differences in vascular bed sensitivity. The NE challenge was also subsequently performed in the presence of the vasodilator sodium nitroprusside (NP), to determine if the differences in MVR were a function of the initial state of contraction of the vascular muscle bed.

Upon passing the acceptance criteria, the entire cremaster muscle preparation in the first group of SHR *(n* = 8) and WKY *(n* = 8) rats was superfused with NE in graded concentrations from 2 X 10^{-9} M to multiples (i.e., 5 x 10^{-9} M,1 x 10^{-8} M, 5 x 10-* M and so forth) until the blood flow became extremely irregular, starting and stopping in concert with the blood pressure pulse. Each preceding concentration was washed out with plain Krebs solution until the previous control values were reestablished before the next concentration of NE was given. In the second group of SHR *(n* = 9) and WKY *(n* = 8) rats the procedure was duplicated except that PE was substituted for NE. The initial concentration was 5 x 10^{-7} M. The agonists were allowed to flow continuously over the tissue (2 ml/min) until all three measured parameters either reached a plateau value or began to revert back toward control values. Excessively high concentrations of either agonist were not given, because the RBC velocity tracker did not properly measure the irregular blood flow that would result from intense vasoconstriction. Sescond, there was the problem of clouding of the tissue, which resulted from tissue exposure to excessively high concentrations of the constrictor substance. After the relative maximum was achieved for each of the vascular parameters measured in the second-order artery, the muscle bed was again washed out with Krebs solution until the individual measured parameters approximated their previous baseline.

In the first group of animals, the muscle preparation was then exposed to the rapidly-acting vasodilator 10^{-5} M sodium nitroprusside (NP) during a second challenge with NE, which began with an initial NE concentration of 10^{-7} M and followed the same procedure

previously described. In this study NP was found to be an especially useful vasodilator, since it maximally dilated all arteries in the cremaster preparation throughout that phase of the experiment without traumatizing the tissue or lowering the systemic blood pressure, and its vasodilatory effects were readily overcome with additional NE.

Accuracy of Measurements: Threshold Determinations

To assess the possible difference in vasoconstrictor threshold concentrations between the values for MVR in the SHR and WKY groups, a factor was needed to determine if the change in MVR (dR/R) that resulted from a particular constrictor concentration was indeed different from the control value. MVR is a function of micropressure, RBC velocity, and vessel diameter squared, and the accuracy of the measurement of each of these parameters is based upon individual calibration procedures. The micropressure system, calibrated with pressure heads of predetermined values, was found to be accurate to within 3% (dP/P) over its entire range. The RBC velocity tracking system, calibrated with the use of a rotating disk (of various known rates) placed in the optical light path, was within 8% (dV/V) of the actual values. The diameter measurements could be determined to within *Vi* of one division on the eyepiece micrometer. In terms of an average second-order arterial diameter (approximately 60 *i±m),* the accuracy corresponded to 2.5% (dD/D). Since the diameter is squared (D x D) to obtain the area of the vessel, twice the factor for the accuracy was needed to calculate the total accuracy of this measurement, which was 5%. A composite MVR accuracy factor was determined from Equation 2 for a worst-case situation:

$$\frac{dR}{R} = \frac{dP}{P} + \frac{dV}{V} + \frac{2dD}{D} \qquad (2)$$

The overall accuracy for the MVR determination was calculated to be within ± 16% of the average value (dR/R). Thus, for two values to be judged significantly different (for the purpose of determining the vasoconstrictor thresholds), there had to be a difference of twice the accuracy fetor, or 32%, since both the control value and the pharmacologically perturbed value for MVR had an individual accuracy factor associated with its measurements. The significance of the difference between the mean threshold values as well as the MVR differences at each constrictor concentration was analyzed by Student *t* test *(p* < 0.05).

Results

Norepinephrine: Catecholamine Vasoconstrictor Challenge

Table 1 shows the mean values (± SEM) for the second-order arterial vessel diameters, RBC velocities, and micropressures. The mean systemic blood pressures were 94.5 ± 2.2 mm Hg for the WKY group and 134.9 ± 4.8 for the SHR group *(p* < 0.001). Diameters of SHR ateries were smaller under control conditions and after NE challenge, except at the highest NE concentration when distal arterioles were so severely constricted that continuous blood flow was prevented in the muscle preparation. RBC velocities were also lower in the SHR, generally by 50% or less, than in the WKY group. Micropressures under control conditions were elevated 38% in SHR compared to WKY, yet the micropressure values in both groups represented a consistent 37% of the systemic blood pressure. During the course of NE challenge, micropressures increased to a larger degree in SHR and achieved a value 74% higher than in the WKY group.

Comparison of the calculated data (Figure 1) showed that under control conditions the MVR (mean ± SEM) of the second-order artery in the SHR group (1.50 ± 0.31 mm Hg sec/nl) was almost four times greater than in the WKY group (0.38 ± 0.06 mm Hg sec/nl). Supervision with NE increased the MVR in both groups, but the responses were significantly greater in SHR to all but the highest concentration of NE. The NE threshold values differed significantly between the two groups, 16.8 x 10^{-9} M NEfor WKY vs 5.1 x 10^{-9} M NE for SHR *(p* < 0.05).

Figure 2 compares the corresponding MVR data when referenced to the experimental animal's MVR value under control conditions. This normalization technique eliminates the multiplying effect due to an initial offset in the control MVR values during subsequent pharmacological perturbation. These data show that the SHR had a greater relative increase in MVR for all but the highest NE concentration.

Phenylephrine: Noncatecholamine Vasoconstrictor Challenge

Table 2 shows the mean values for diameters, RBC velocities, and micropressures in the control state dur-

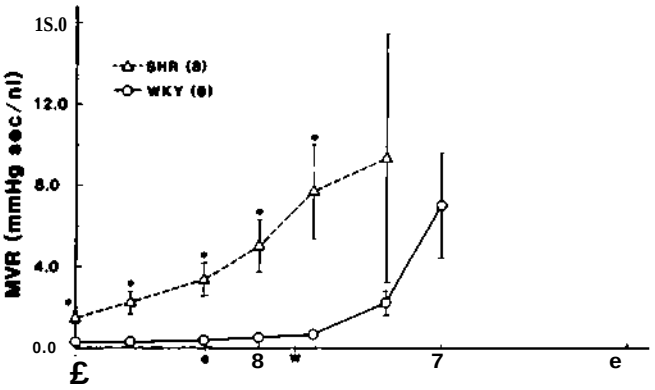

FIGURE I. *Second-order artery microvascuiature input resistance vs norepinephrine (NE) concentration. Control period (C) indicates no agonist was given at this time. Values represent means ± SEM. The NE threshold concentrations for the spontaneously hypertensive rats (SHR [S]) and Wistar-Kyoto (WKY IW] rats), are indicated on the.x-a.xis. Asterisks (*) indicate* p < *0.05; by Student* t *test.*

TABLE 1. *Second-Order Arterial Measurements Following Graded Norepinephrine Challenge*

Drug concentration	Arterial diameter (μm)		Blood velocity (mm/sec)		Micropressure (mm Hg)	
	WKY	SHR	WKY	SHR	WKY	SHR
Control	68.6	57.0	34.6	17.3	35.1	48.5
SEM	3.7	3.3	4.0	1.4	1.4	1.3
(n)	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		0.005		0.001	
2 x 10~^{9}M [NE]	68.6	54.6	34.6	15.8	35.1	51.6
SEM	3.7	2.5	4.0	3.0	1.4	2.0
(n)	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		0.005		0.001	
5 x 10~^{9}M[NE]	66.7	53.1	32.9	13.6	34.9	58.1
SEM	3.0	3.7	4.5	3.2	1.2	3.2
(n)	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		0.005		0.001	
1 x 10"^{8}M[NE]	64.9	51.9	29.1	11.1	36.1	62.8
SEM	3.5	3.7	4.9	2.8	1.4	3.0
(1)	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		0.010		0.001	
2 x 10"^{8}M[NE]	63.6	51.5	27.1	9.8	40.8	70.5
SEM	3.2	3.9	4.4	2.8	1.8	4.1
(n)	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		0.005		0.001	
5 x 10"^{8}M [NE]	55.4	51.9	17.3	9.9	48.4	74.6
SEM	3.8	5.9	4.2	3.4	2.6	4.3
(1)	(8)	(4)	(8)	(4)	(8)	(4)
p value	NS		NS		0.001	
1 x 10"^{7}M[NE]	46.5	—	8.4	—	51.2	—
SEM	4.8	—	2.2	—	3.7	—
(")	(6)	(0)	(6)	(0)	(6)	(0)
p value	NA		NA		NA	

Dash (—) indicates value not recorded. NS = not significant; NA = not applicable; WKY = Wistar-Kyoto rat; SHR = spontaneously hypertensive rat; SEM = standard error of the mean.

ing PE challenge. The mean systemic blood pressure was 86.9 ± 2.6 mm Hg in the WKY group and 133.2 ± 2.7 mm Hg in the SHR group ($p < 0.001$). Arterial diameters were again smaller in the SHR, but although the RBC velocity was lower in the SHR, it was not significantly different in this comparison. The micropressure was elevated 39% in SHR compared to WKY, which was the same as was found in the previous group of SHR and WKY rats. The micropressure expressed as a percentage of the mean systemic blood pressure was not significantly different between the two groups and averaged approximately 43% under control conditions in the second-order artery. After the PE challenge, the SHR micropressure increased to a level 75% greater than that found in the WKY.

The control second-order MVR for the SHR group (0.95 ± 0.15 mm Hg sec/nl) was approximately four

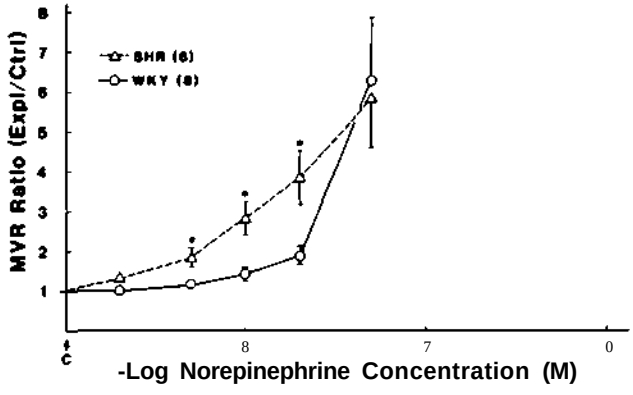

FIGURE *2. Second-order artery microvascular input resistance ratio (change in resistance relative to control resistance) vs norepinephrine concentration.*

TABLE 2. *Second-Order Arterial Measurements Following Graded Phenylephrine Challenge*

Drue concentration	Arterial diameter *(jxm)*		Blood velocity (mm/sec)		Micropressure (mm Hg)	
	WKY	SHR	WKY	SHR	WKY	SHR
Control	74.8	60.6	29.5	26.1	39.3	54.5
SEM	2.3	2.1	2.8	3.1	1.7	3.6
(i)	(8)	(9)	(8)	(9)	(8)	(9)
p value	0.001		NS		0.005	
5 x 10"^{7} M [PE]	74.4	54.8	25.5	17.8	39.0	62.2
SEM	2.5	2.3	3.8	2.2	1.7	4.8
(n)	(8)	(9)	(8)	(9)	(8)	(9)
p value	0.001		NS		0.005	
10"^{6} M [PE]	72.9	49.3	24.5	16.3	43.0	68.0
SEM	2.8	3.1	4.3	1.9	2.5	4.7
(")	(8)	(9)	(8)	(9)	(8)	(9)
p value	0.001		NS		0.001	
2 x 10"^{6}M[PE]	67.4	45.8	19.5	9.4	42.4	74.1
SEM	1.9	2.4	4.2	1.4	1.8	4.2
(n)	(8)	(9)	(8)	(9)	(8)	(9)
p value	0.001		0.005		0.001	
5 x 10~^{6}M[PE]	53.9	36.5	16 5	4.9	51.8	83.2
SEM	2.2	2.5	4.4	0.8	2.1	3.5
(«)	(8)	(9)	(8)	(9)	(8)	(9)
p value	0.001		0.001		0.001	
10~^{3} M [PE]	47.4	—	11.9	—	54.9	—
SEM	2.1	—	3.3	—	2.7	—
(1)	(7)	(0)	(7)	(0)	(7)	(0)
p value	NA		NA		NA	
2 x 10"^{3} M [PE]	41.2	—	8.7	—	54.9	—
SEM	2.3	—	2.3	—	2.4	—
(1)	(7)	(0)	(7)	(0)	(7)	(0)
p value	NA		NA		NA	

Dash (—) indicates value not recorded; NS = not significant; NA = not applicable.

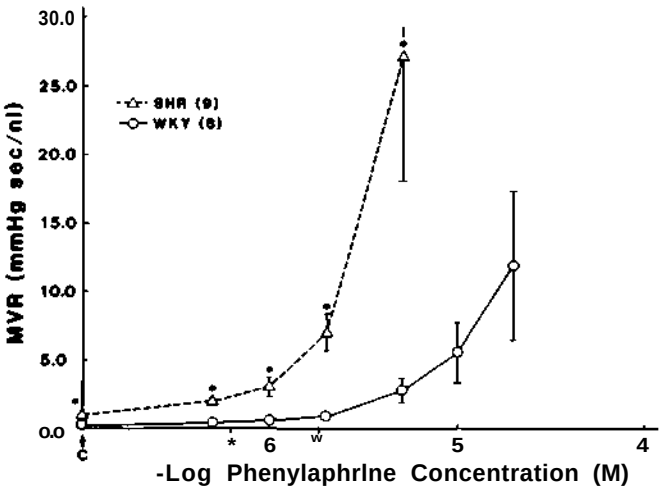

FIGURE 3. *Second-order artery microvascular input resistance vs phenylephrine concentration.*

times greater than for the WKY group (0.22 ± 0.03), which was similar to the results presented for the NE data. Use of the noncatecholamine PE as the vasoconstrictor substance during graded superfusion produced results similar to those of NE with the exception of the time to the peak response (approximately 5 minutes for NE vs 10 minutes for PE). Figure 3 illustrates that, in the presence of all PE concentrations tested, the SHR group showed a hypersensitivity to the vasoconstrictor both in terms of absolute values as well as normalized values (Figure 4). The calculated PE threshold concentrations were again different for the two groups (18.1 X 10-^{7}MPEforWKYvs6.7 x 10'^{7} M PEfor **SHR** ($p < 0.05$).

Norepinephrine Challenge in the Presence of Sodium Nitroprusside

Table 3 gives the mean values for diameters, velocities, and micropressures under control conditions after

TABLE 3. *Second-Order Arterial Measurements Following Graded Norepinephrine Challenge in the Presence of!0~5 M Nitroprusside*

Drug concentration	Arterial diameter (jtm)		Blood velocity (mm/sec)		Micropressure (mm Hg)	
	WKY •	SHR	WKY	SHR	WKY	SHR
Control	68.6	57.7	26.8	15.3	31.4	46.5
SEM	3.6	3.2	3.6	2.9	0.9	1.7
(«)	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		0.050		0.001	
10"5 M [NP]	77.1	67.4	28.5	22.9	30.0	38.4
SEM	3.1	2.5	3.2	2.9	0.7	2.3
(")	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		NS		0.001	
2 x 10-8 M[NE]	76.0	67.0	26.7	17.8	30.0	42.0
SEM	2.6	2.6	3.7	3 0	0.7	1.5
(1)	(8)	(8)	(8)	(8)	(8)	(8)
p value	0.050		NS		O 001	
5 x 10"8M[NE]	75.6	67.0	23.3	14.4	35.0	53.1
SEM	3.5	3.3	3.0	2.6	2.2	4.2
(1)	(8)	(8)	(8)	(8)	(8)	(8)
p value	NS		0.050		0.001	
1 x 10"7M[NE]	70.1	65.5	21.4	12.7	42.1	60.5
SEM	2.9	3.7	2.8	2.0	1.9	3.2
(1)	(8)	(8)	(8)	(8)	(8)	(8)
p value	NS		0.050		0.001	
2 x 10"7M[NE]	66.3	61.6	17.9	8.4	42.3	68.1
SEM	3.0	3.7	2.3	3.9	2.0	4.1
(1)	(8)	(8)	(8)	(8)	(8)	(8)
p value	NS		0.005		0.001	
5 x 10~7M[NE]	61.6	62.8	12.2	5.3	45.4	80.3
SEM	3.5	2.0	1.9	1.4	3.0	3.8
(«)	(7)	(4)	(7)	(4)	(7)	(4)
p value	NS		0.050		0.001	

NP = nitroprusside.

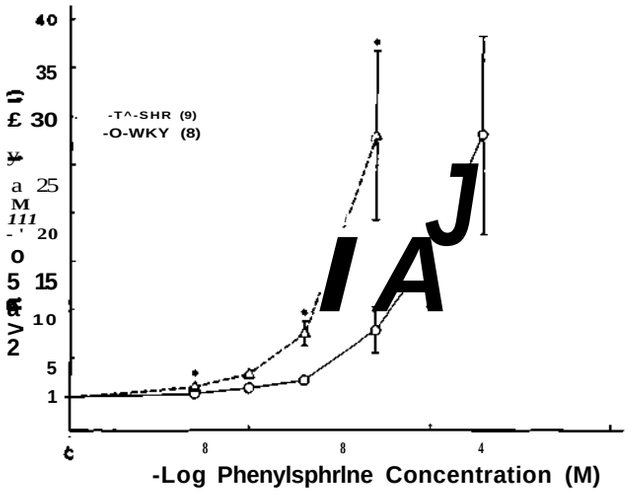

FIGURE 4. *Second-order artery microvascular input resistance ratio vs phenylephrine concentration.*

vasodilation with 10^{-5} M sodium nitroprusside (NP), and during NE challenge in the presence of the dilator. The NP stimulated dilation of the arteries in both groups, but the SHR vessels still remained smaller than those of the WKY group. The percentage of arterial vasodilation in either group did not differ significantly (WKY = 13%; SHR = 18%; NS). The RBC velocity increased to a greater extent in SHR (70% vs 9% in WKY;/? < 0.05). The micropressure fell more in SHR (18% vs 4% in *WKY;p* < 0.001), but not to normotensive levels.

After maximal vasodilation, the difference in MVR between the two groups was reduced compared to control conditions (Figure 5); the MVR was still approximately two times higher in SHR, however (WKY = 0.26 ± 0.04 mm Hg sec/nl; SHR = 0.53 ± 0.07). Thus, in the presence of NP, the MVR decrease was greater in SHR (60%) than in WKY (31%), but this greater decrease found in SHR was not enough to over-

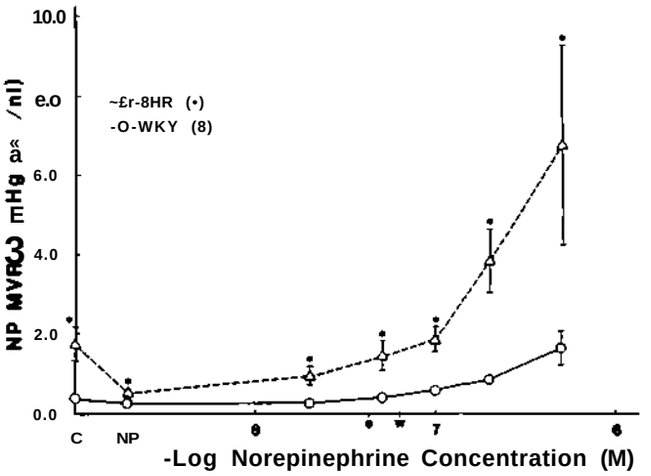

FIGURE 5. *Second-order artery microvascular input resistance (in the presence of I0~^{s} M sodium nitroprusside) vs norepinephrine concentration. NP indicates initial superfusion of nitroprusside.*

come the difference observed under control conditions. The MVR was also greater in SHR for the entire range of superfused NE concentrations tested. The NE threshold concentrations were not significantly lower in SHR (3.8 x 1O"^{8}M NE) compared to WKY (5.8 x 1O~^{8} M NE). The relative difference in MVR response to the graded concentrations of NE (Figure 6) indicated that, in the majority of concentrations tested, the SHR demonstrated a hypersensitivity to NE in the presence of a fixed concentration of NP when compared to the initial MVR values.

Discussion

Elevated total peripheral resistance (TPR) is characteristic of SHR hypertension and is well documented.[3,3,6]- "· ^ This elevated TPR, which represents the summation of the individual changes in organ vascular resistance, is not necessarily the result of a ubiquitous resistance elevation in all of these organs. Hiley and Yates[41] have shown that although the CO was equivalent in SHR and normotensive WKY control rats, the blood flow to the skeletal muscle in SHR was decreased by 50%. These findings are in good agreement with the results of Roy and Mayrovitz,[27] who compared microvascular blood flow in the cremaster muscle. Additionally, Hiley and Yates[41] also showed that the distribution of the CO in the SHR to the liver (244% greater) and gastrointestinal tract (66% less) was different from the CO in normotensive rats. In view of this variation in end-organ vascular resistance found in SHR relative to WKY, we undertook to determine the extent of the elevated microvascular resistance in the SHR skeletal (cremaster) muscle compared to the normotensive counterpart. We found in the present study that the MVR assessed at the second-order arterial level was approximately four times greater in the SHR under control conditions. This elevated MVR, which we observed at the distal part of the SHR vascular resistive circuit, was mainly a result of consistently smaller arterial diameters and elevated micropressures.

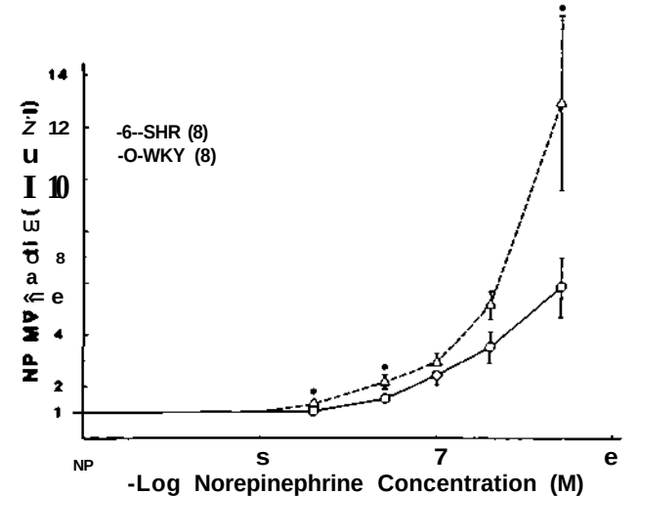

FIGURE 6. *Second-order artery microvascular input resistance ratio relative to dilated control (in the presence of I0^{5} sodium nitroprusside) vs norepinephrine concentration.*

Direct Apha-Receptor Stimulation: Catecholamine Versus Noncatecholamine Agonists

Why MVR becomes elevated is unknown, but it is often thought to be caused by either a structural modification of the arterial vessels or a greater arterial response to circulating endogenous vasoconstrictors (such as epinephrine and NE). It is possible that there is an increased sensitivity in the venular smooth muscle and that this sensitivity is usually not influential due to the lower micropressures present in that portion of the vasculature and the greater abundance of vessels in the venous network. The purported hypersensitivity of the SHR microvasculature to vasoconstrictor substances is not well substantiated.[24-a-42] The variability may be the result of an age factor, since the rats used in this study were still in the developmental stage of hypertension. However, Mulvany et al.[43] have shown in isolated mesenteric arterial vessels (150 /Am in diameter) that the EDj„ values of NE, a measure of vascular sensitivity, did not vary within groups of SHR and WKY rats at 6, 12, and 24 weeks of age. Thus, it was of vital interest to determine if there were differences in the sensitivity of arteries to vasoconstrictors and if the elevated vascular resistance could be altered by pharmacologically changing local conditions.

In the present study, we found that the catecholamine NE and the noncatecholamine PE both elicited hypersensitivity in the SHR (as determined by greater relative changes in MVR) in the majority of concentrations tested, and especially in the lower, more physiological, range. Although we could not compare the agonists in terms of maximal response, we found that both agonists showed the same relative relationship for threshold concentrations. The threshold for both NE and PE was approximately three times greater in WKY

than SHR. The most notable difference between the two agonists was the time-to-peak response, which was about two times greater for PE than for NE, possibly due to the more endogenous-like nature of the NE and perhaps the greater affinity of the alpha-adrenergic receptor for NE.[44]

Non-Alpha Adrenergic Receptor Antagonism of **Norepinephrine Stimulation**

Although elevated responses to several alpha-adrenergic agonists have been documented in SHR, the question remains as to whether these greater MVR responses are due to an anatomical difference, such as an altered vessel lumen-to-wall ratio as proposed by Folkow et al.,[45] or to a physiological difference, such as an increased myofibrillar overlap due to enhanced sympathetic neural input. Since Bohlen and Lobach[46] studied medial hypertrophy extensively in SHR and found no difference, it was our intent to concentrate on the concept of an elevated contractile resting state that might subsequently affect the response to alpha-adrenergic stimulation. We induced direct vascular smooth muscle vasodilation with nitroprusside, which is not mediated through the alpha-adrenergic receptor, and found that the difference in microvascular input resistance between WKY and SHR was reduced considerably, but not totally eliminated. In a previous study[27] we also showed that most of the smal ler arterial diameters throughout the SHR microvasculature could be equalized by topical application of the vasodilator adenosine. The question left was whether the high degree of constriction normally present in the SHR control state has any effect on the final response to an exogenously applied alpha-agonist. We decided that one way to test such a possibility was to dilate the vasculature in the least injurious way and to repeat the vasoconstrictor challenge. We used the minimum concentration of nitroprusside (10^{-5} M) required to achieve full vasodilation of all arteries, and we were able to readily reverse the effects by washing with Krebs solution. We felt that the use of this pharmacological agent was the only feasible method of totally eliminating the effects of both neural input and local metabolic conditions that influence the cremaster microvasculature without severely altering the local cellular or superfusate environment.

If the vasodilation had eliminated any special initial contractile state present in the SHR arteries, the change in the NE-mediated MVR values should have been equalized among the WKY and SHR groups. This was not the case. Although the corresponding resistance curves did approach each other in the presence of nitroprusside (Figure 5), the values were never equalized. To further reduce any bias in the methodology due to initial offset of the MVR values, we also compared the dose-response curves for the SHR and WKY groups following normalization of the individual data relative to the baseline dilated values. Even upon normalization of the data (Figure 6), the SHR still showed greater changes in MVR in response to the majority of the NE concentrations tested. Thus, it would appear that the resting tone of the arterial smooth muscle did not have an influence upon the hypersensitivity to the superfused vasoconstrictor.

In summary, we found that the SHR displayed: 1) an elevated microvascular input resistance in the cremaster muscle microvasculature; 2) increased resistance responses to alpha-adrenergic agonist stimulation and decreased agonist thresholds, regardless of whether a catecholamine (NE) or noncatecholamine (PE) was used as the pharmacological agonist; and 3) heightened vasoconstrictor responses that were not due to the initial contractile state of the vascular smooth muscle.

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