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Calcium Modulation of Microvascular Sensitivity during Renovascular Hypertension (41194)

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Abstract. The effects of *in vivo* alterations in local calcium concentration on small vessel responses to norepinephrine were obtained for a first order arteriole and venule in the cremaster muscle of normotensive and one-kidney, one-clip Goldblatt hypertensive rats. The cremaster with intact circulation and innervation was suspended in a modified Krebs solution which contained either 1.3, 2.6, or 5.1 mM $CaCl_2$. Closed-circuit television microscopy was used to measure vessel diameters. The resting lumenal diameters of first-order arterioles in hypertensive rats were 31% smaller than lumenal diameters of the corresponding arterioles in normotensive rats. Concentration–response curves showed that the norepinephrine sensitivity (pD_2) of first-order arterioles in normotensive rats was significantly increased by a change in bath [Ca^{2+}] from 1.3 to 2.6 mM; however, the norepinephrine sensitivity of comparable arterioles in hypertensive rats was not affected by this change in bath [Ca^{2+}]. These data indicate that mechanisms for calcium modulation of vasoconstriction are attenuated for large arterioles in the cremaster muscle of one-kidney, one-clip Goldblatt hypertensive rats.

Many investigators have studied vascular reactivity in normotensive and hypertensive animals. In general, perfusion studies have suggested that vascular reactivity is increased in animals with hypertension (1–3). Early *in vitro* studies have suggested that aortic strips from rats with renal or DOCA hypertension develop the same or less contractile force in response to norepinephrine or epinephrine than do strips from normotensive rats (4, 5). More recent studies have indicated that there is an increased responsiveness to norepinephrine for both aortic (6) and femoral artery strips (7, 8) from renal hypertensive rats.

Several hypotheses have evolved to explain the apparent changes in vascular responsiveness of hypertensive animals. One of these hypotheses is based primarily on alterations in blood vessel structure. Folkow *et al.* (9) have postulated that either expansion of the extracellular matrix or swelling of vascular smooth muscle causes a chronic decrease in the internal radius of arteries, thereby altering the mechanical advantage for smooth muscle contraction in these vessels. Another hypothesis suggests that there is a general increase in vascular sensitivity to circulating and neuronally released catecholamines (1, 10).

Several investigators (11, 12) have postulated that changes in sensitivity of vascular smooth muscle could represent an alteration in cellular calcium regulation in hypertensive animals. Catecholamine-induced contraction is dependent on external calcium for *in vitro* strips from arterial (13, 14) and venous (15, 16) vessels. In general, increased external calcium potentiates the constriction of *in vitro* artery strips for both normotensive and renal hypertensive animals (14). However, calcium potentiation of contractile responses to both potassium chloride and epinephrine is significantly greater for strips from renal hypertensive animals (14). These results suggest that a change in the availability of extracellular calcium for vascular smooth muscle contraction in small precapillary resistance vessels (arterioles) could produce the increased peripheral resistance which is observed with renovascular hypertension.

At present, there are few direct observations of the *in vivo* microvasculature to demonstrate increased arteriolar sensitivity during renovascular hypertension. Of these

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few studies, none have addressed the possible role of an alteration in calcium regulation of the vascular smooth muscle in microvessels. Click *et al.* (17) used a Grollman model of hypertension (a figure-of-eight ligature around the kidney) to observe increased arteriolar sensitivity to norepinephrine in the hamster cheekpouch. In contrast, we (18) have shown that the norepinephrine sensitivity of *in vivo* arterioles in the cremaster muscle of the one-kidney, one-clip Goldblatt hypertensive rat is decreased. In our present studies, we have made direct observations of the *in vivo* microcirculation to obtain norepinephrine concentration–response curves at three levels of bath calcium for first-order arterioles and venules in the cremaster muscle of normotensive and renovascular hypertensive rats. These studies were designed to determine whether or not increased extracellular calcium would potentiate a norepinephrine-induced constriction of arterioles and whether or not this potentiation would be altered for arterioles in renovascular hypertensive rats.

Methods. Male Sprague–Dawley rats were used as normotensive control animals and for the surgical production of one-kidney, one-clip, Goldblatt hypertension. Our surgical procedure (18) involved unilateral nephrectomy and contralateral renal artery constriction (o. d. ~ 260 μm) at 3–4 weeks of age. These rats received a saline drinking solution (150 mM NaCl and 10 mM KCl) *ad libitum* after the day of surgery. Normotensive control rats had no surgery and were given tap water to drink.

Four weeks after the surgery, mean arterial blood pressure during light anesthesia with ether was measured through a cannula in the tail artery. A mean tail artery pressure greater than 130 mm Hg was used to define hypertensive animals for subsequent studies of the cremaster microvasculature. Approximately 50% of the rats survived our surgical procedure and about 50% of these demonstrated hypertension 4 weeks later at the time of tail artery cannulation. Direct observations of the *in vivo* microcirculation in the cremaster muscle of the hypertensive rats were made 2 to 3 days after tail artery cannulation.

For our acute microcirculatory studies, animals (weight range of 146–197 g) were anesthetized with an intraperitoneal injection of urethane (800 mg/kg) and α-chloralose (60 mg/kg). In our preparation of the rat cremaster (19), the right testicle was exposed by an incision in the scrotum, and the cremaster muscle which surrounded the right testicle was incised medially. This cremaster which still had intact innervation and circulation was then spread with sutures over an optical port in a 60-ml tissue bath which was filled with a modified Krebs solution.

The modified Krebs solution contained 1.3, 2.6, or 5.1 mM calcium chloride. Other constituents of the cremaster bath solution were $NaHCO_3$ (25.0 mM), dextrose (11.6 mM), KCl (4.7 mM), KH_2PO_4 (1.19 mM), and $MgSO_4 \cdot 7H_2O$ (1.19 mM). Appropriate amounts of sodium chloride (115, 113, or 109 mmole) were added to give a measured osmolality of 285–290 mOsm for all bath solutions. Throughout each experiment, CO_2 and N_2 gases were bubbled through the cremaster bath solution to give a pH of 7.2. Bath temperature was maintained at 32°. Rectal temperature was maintained at 36° by control of the current through a heating pad which was placed under the animal.

Measurements of lumenal diameters for the first-order arteriole and venule (major vessels supplying and draining the cremaster) were made on a calibrated television monitor at approximately 1500× magnification. Mean arterial pressure was measured via a cannula in the left femoral artery while heart rate was monitored with subcutaneous needle electrodes.

Three levels of bath calcium were used for both normotensive and hypertensive rats to give a total of six animal groups. For each rat, a complete norepinephrine concentration–response curve for a first-order arteriole and a curve for the adjacent first-order venule were determined at only one calcium level. To obtain these complete concentration–response curves, we observed the changes in arteriole and venule diameter for five to eight concentrations of norepinephrine bitartrate (Sigma). For each concentration of norepinephrine, there was a 5-min period for measurement of control

vessel diameters, and a 10-min period for measurement of vessel responses which followed the addition of norepinephrine to the cremaster bath. During a subsequent 20-min recovery period, the cremaster bath was drained and refilled five times with fresh Kreb's solution to allow the arteriole and venule to return to near control diameters.

A test concentration of 3×10^{-8} M norepinephrine was the first concentration which was used in all experiments. The vessel responses to this test dose were not included as data for the determination of the concentration–response curves, but the vessel responses to this initial concentration were used to select a concentration of 3×10^{-9}, 1×10^{-8}, or 3×10^{-8} M norepinephrine as the starting concentration for the concentration–response curve. This procedure insured that at least one concentration of norepinephrine would give a vessel response that was below the ED_{50} value for the arteriole and the venule in each experiment. Increasingly greater concentrations of norepinephrine (with approximately threefold increases in concentration) were applied to the cremaster until both the first-order arteriole and venule gave a large constriction which was approximately equal to (within 5%) or less than the constriction for the preceding concentration of norepinephrine.

The raw data for arteriole and venule diameters were smoothed with a 3-point digital computer filter (20). The smoothed vessel data were normalized by expressing each data point as a percentage of the average value during the control period which immediately preceded the application of each concentration of drug. The largest responses at each concentration of norepinephrine were used to construct a concentration–response curve for each vessel. The concentration (ED_{50}) of norepinephrine which produced one-half of the maximal constriction was graphically determined for each of the individual concentration–response curves. This value was converted to a pD_2 value ($pD_2 = -\log ED_{50}$) as a measure of vessel sensitivity to norepinephrine (21, 22).

Statistical comparisons among animal groups were made by an analysis of variance with interaction terms (23). When a significant difference was found by analysis of variance, Duncan's New Multiple Range Test with correction for unequal sample size (24) was used to identify significant differences among the various pairs of animal groups. For all tests, statistical significance was defined at the 5% probability level.

Results. The mean arterial blood pressure during urethane and α-chloralose anesthesia was 130 ± 2 mm Hg for all (N = 34) of the renovascular hypertensive rats in comparison to 95 ± 2 mm Hg for all (N = 25) of the normotensive Sprague–Dawley rats. Average heart rate for all of the hypertensive rats (367 ± 11 bpm) was similar to that for all of the normotensive rats (383 ± 11 bpm). No noticeable changes in heart rate or blood pressure were observed following the addition of any of the concentrations of norepinephrine to the cremaster bath.

The responses of a representative arteriole in the cremaster to topical applications of norepinephrine are illustrated in Fig. 1. After addition of norepinephrine to the bath, there was usually a short delay, followed by a rapid decrease in vascular diameter to give a maximal vessel constriction before the end of the 10-min drug period. Supramaximal concentrations of norepinephrine did not give complete lumenal closure for any first-order arterioles in this study.

Table I shows the arteriole and venule data for control vessel diameters, for minimal vessel diameters which were obtained with application of very high concentrations of norepinephrine, and for vessel sensitivity (pD_2 values) to norepinephrine. These data for the six animal groups were initially analyzed by a two-way analysis of variance to determine the parameters which were significantly affected by hypertension and those which were affected by changes in bath calcium levels. Analysis of variance indicated that hypertension had a significant effect on control arteriole diameter, on the minimal arteriole diameter at high norepinephrine concentrations, and on arteriolar sensitivity to norepinephrine. Bath calcium concentration had a significant ef-

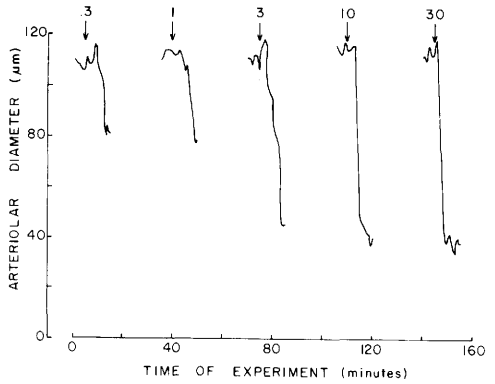

FIG. 1. The experimental protocol for the response of a first-order arteriole to norepinephrine (M × 10^{-7}) in the rat cremaster muscle. These data are from one microvascular experiment on a normotensive Sprague–Dawley rat with a cremaster bath [Ca^{2+}] of 2.6 mM. For each concentration of norepinephrine (indicated at the top of the figure), there was a 5-min control period, addition of norepinephrine to the bath (indicated by arrows), and a 10-min response period. During each 20-min recovery period the bath was changed with fresh Krebs solution to wash out the norepinephrine and to allow vessel diameter to return toward the control value.

fect on arteriolar sensitivity to norepinephrine. Changes in bath calcium did not have a statistically significant effect on control arteriolar diameter or on the minimal arteriolar diameter at high norepinephrine concentrations. Neither hypertension nor changes in bath calcium had a statistically significant effect on control venule diameter, on minimal venule diameter at high norepinephrine concentrations, or on venule sensitivity to norepinephrine.

For the arteriolar parameters which were statistically affected by hypertension or by changes in bath calcium according to an analysis of variance, Duncan's New Multiple Range Test was used to make statistical comparisons between specific pairs of animal groups. Control arteriolar diameters (Table I) were significantly smaller for comparison of hypertensive rats to normotensive rats at each level of bath calcium. Minimal lumenal diameters of first-order arterioles at high norepinephrine concentrations appeared to be smaller for hypertensive rats at each bath calcium level; however, there was a statistically significant difference between hypertensive and normotensive rats only at the 2.6 mM bath calcium level.

Arteriolar sensitivity (pD_2 value) to norepinephrine was significantly affected both by the presence of hypertension and by changes in bath calcium. Arterioles in hypertensive rats were significantly less sensitive to norepinephrine at the 2.6 mM bath calcium level than were arterioles in normotensive rats; but the pD_2 values for normotensive and hypertensive rats were similar at the 1.3 and 5.1 mM bath calcium levels. In normotensive rats (Table 1), ar-

TABLE I. RESPONSE OF FIRST-ORDER ARTERIOLES AND VENULES TO NOREPINEPHRINE IN THE CREMASTER MUSCLE OF NORMOTENSIVE AND HYPERTENSIVE RATS DURING EXPOSURE OF THE CREMASTER TO DIFFERENT CALCIUM CONCENTRATIONS[a]

Bath [Ca^{2+}] (mM)	N	Control vessel diameter (μm)		Minimal vessel diameter (μm)		Sensitivity (pD_2)	
		Arteriole	Venule	Arteriole	Venule	Arteriole	Venule
				Normotensive rats			
1.3	9	102 ± 6[b]	162 ± 12	34 ± 2	96 ± 7	5.95 ± .10	6.85 ± .06
2.6	8	117 ± 5	166 ± 8	39 ± 4	94 ± 7	6.91 ± .15	7.20 ± .09
5.1	8	109 ± 2	169 ± 10	41 ± 6	95 ± 5	6.41 ± .14	7.02 ± .10
				Hypertensive rats			
1.3	9	73 ± 4[c]	190 ± 8	31 ± 2	116 ± 6	5.88 ± .07	7.08 ± .11
2.6	11	73 ± 3	168 ± 9	29 ± 2	88 ± 5	6.17 ± .15	7.24 ± .09
5.1	14	78 ± 4	187 ± 8	34 ± 2	97 ± 9	6.25 ± .17	7.08 ± .12

[a] Data are expressed as mean ± SEM.
[b] The mean arteriolar diameter for the 25 normotensive animals was 109 ± μm.
[c] The mean arteriolar diameter for the 34 hypertensive animals was 75 ± 3 μm, which was significantly less than the mean arteriolar diameter for normotensive animals.

terioles were significantly more sensitive to norepinephrine for the 2.6 mM bath calcium than were arterioles which were exposed to the 1.3 or 5.1 mM bath calcium levels. In hypertensive rats, there was a trend for increased arteriolar sensitivity with increased bath calcium levels; but, this trend could not be statistically demonstrated by an analysis of variance or by a linear regression analysis.

Discussion. Our normotensive rats had an increased arteriolar sensitivity to norepinephrine at a cremaster bath calcium concentration of 2.6 mM calcium compared to a bath concentration of 1.3 mM calcium. This potentiated norepinephrine response for arterioles in the 2.6 mM bath calcium could represent an effect of an increase in the net flux of calcium into vascular smooth muscle cells. Several investigators (25, 26) have suggested that norepinephrine gives an increase in vascular smooth muscle cell permeability to calcium in addition to an increase in the release of sequestered intracellular calcium. *In vitro* studies (13, 14) have also indicated that there is a potentiation of contraction responses of vascular strips with elevations in external calcium.

We observed a decrease in norepinephrine sensitivity of arterioles of normotensive rats when the calcium concentration of our bathing medium was elevated to 5.1 mM calcium. Holloway and Bohr (14) observed a fall in the maximal tension which was developed by arterial strips in response to KCl when bath calcium was elevated above 4.1 mM. They postulated that the reduction in constrictor responses could be due to a membrane stabilization effect of high levels of external calcium on the vascular smooth muscle cell to inhibit membrane excitation and to depress contraction.

The norepinephrine sensitivity of venules in our normotensive rats appeared to be greater than the norepinephrine sensitivity for adjacent arterioles. In addition, the norepinephrine sensitivity of venules in our normotensive rats did not vary significantly with alterations in cremaster bath calcium. This might indicate that venules have less dependence on external calcium as a source of activator calcium. Alternatively, this might suggest that venules are more sensitive than the adjacent arterioles to norepinephrine. Thus, the absence of a bath calcium concentration effect on venule sensitivity could mean that our lowest bath calcium concentration (1.3 mM) was at or above the level that is required for maximal norepinephrine sensitivity of venous smooth muscle.

Our present *in vivo* study shows that, in our renovascular hypertensive animals, arteriolar sensitivity to norepinephrine is not significantly influenced by increases in extracellular calcium. In addition, the sensitivity of first-order arterioles is the same or decreased in renovascular hypertensive rats in comparison to normotensive rats. In contrast, several *in vitro* studies have demonstrated increased norepinephrine reactivity (6–8) and greater enhancement of vessel responsiveness with increases in bath calcium concentration (13, 14) for femoral artery strips from hypertensive animals. The reason for these differences between our *in vivo* findings and the *in vitro* results of others is unknown. It is possible that these differences might reflect basic differences between measurements of tension development (essentially an isometric contraction) for *in vitro* strip studies and measurements of diameter changes (essentially muscle shortening with isotonic contraction) for *in vivo* microvascular studies. Alternatively, these differences might reflect basic differences between larger vessels which are used for *in vitro* strip studies and much smaller microvessels which are observed for *in vivo* microvascular studies.

In our present study, minimum arteriolar diameters which were achieved by application of high concentrations of norepinephrine were significantly smaller for the three hypertensive groups (32 $\pm$ 1 μm) than for the three normotensive groups (37 $\pm$ 1 μm). When maximal responses were expressed as a percentage of resting arteriolar diameter, the maximum arteriolar response to norepinephrine for hypertensive rats (58 $\pm$ 1%) was significantly less than for normotensive rats (64 $\pm$ 2%) to suggest a decreased contractility in hypertensive animals. Smaller minimal lumenal diameters with norepinephrine and decreased vascu-

lar contractility could be due to a structural alteration in the vessel wall of hypertensive animals. An increase in the nonelastic components or "waterlogging" of the vascular wall (9) could account for much of the attenuated arteriolar responses to norepinephrine and to increases in external calcium. In some preliminary studies where we have applied several vasodilators (isoproterenol, nitroprusside, and adenosine) to the cremaster muscle, we have found that first-order arterioles of hypertensive rats cannot be dilated, suggesting a structural rather than an acute reduction in vessel diameter in these hypertensive rats.

Several recent studies have indicated a decrease in venous compliance for dogs (27, 28) and rats (29) with a one-kidney, one-clip hypertension. Based on observations of increased water and sodium content in the wall of vein, Simon *et al.* (30) have postulated that a venous wall "edema" produces a decreased venous compliance in one-kidney, one-clip hypertensive rats. Our data do not support this concept of venous wall edema as a generalized phenomena throughout the venous tree in one-kidney, one-clip hypertensive rats. Control lumenal diameters and the minimal lumenal diameters that the cremaster venules could attain in response to high concentrations of norepinephrine were similar in our hypertensive and normotensive rats. These data along with our norepinephrine sensitivity data (pD_2) for venules at different bath calcium levels indicate that the first-order venules were unaltered in the cremaster muscle of our one-kidney hypertensive rats. The possibility exists that compliance changes during this form of hypertension could be restricted to larger veins.

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