PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **189**, 344–352 (1988)

The Influence of Extracellular Calcium on Microvascular Tone in the Rat Cremaster Muscle (42817)

IRVING G. JOSHUA, JOHN T. FLEMING, AND JESSICA P. DOWE

Department of Physiology and Biophysics, University of Louisville, Louisville, Kentucky 40292

Abstract. In vivo responses of arterioles and venules to changes in bath calcium concentrations were observed in the cremaster muscle of male Sprague–Dawley rats. Small arterioles (2A, 3A) initially exposed to a solution containing calcium (2.55 m*M*) significantly dilated in response to a 0-calcium bath. Reexposure to calcium (>0.65 m*M*) caused 2A and 3A arterioles to constrict to diameters similar to the initial control values. In contrast, large arterioles (1A) and all venules (1V, 2V, 3V) were unresponsive to exposure to a 0-calcium solution or to reexposure to calcium (0.65–5.10 m*M*). Treatment with mefenamic acid (10 μg/ml), a prostaglandin synthesis inhibitor, produced marked constriction of arterioles but not of venules, suggesting the involvement of endogenous vasodilator prostaglandins in the regulation of resting diameters of arterioles. In the presence of mefenamic acid, 1A arterioles dilated when exposed to a 0-calcium solution and constricted back to control diameters following reintroduction of calcium into the bath. These data demonstrate heterogeneity in the responsiveness of cremasteric microvessels to changes in extracellular calcium. The small arterioles were most responsive to calcium. The lack of response by the largest arterioles appears to be due to the dilator influences of endogenous prostaglandins.

Several microvascular studies have suggested a heterogeneity in the function and responses of various levels of microvessels of skeletal muscle. Fleming and Joshua (1) and Fleming *et al.* (2) have observed that large cremasteric arterioles (100–50 μm) are relatively nonresponsive to angiotensin II in comparison to small (<50 μm) arterioles. We (3) have also observed a significant increase in arteriolar reactivity to norepinephrine with progressive decreases in arteriolar diameter. Several microvascular studies (4,5) have also noted that small arterioles in the cremaster muscle have a degree of basal tone much higher than that of large arterioles and venules. Since both vascular tone and agonist-induced constriction require increases in intracellular activator calcium, differences in the rate of extracellular calcium entry could explain the observed heterogeneity in the responses of these microvessels.

Several studies have suggested that small precapillary resistance vessels are highly dependent on extracellular calcium as a source of activator calcium. We (6) have also observed a significant potentiation of the responses of large arterioles to norepinephrine following elevations in external calcium via changes in bath calcium concentrations. Hindlimb perfusion studies (7) suggest that small pre- and postcapillary resistance vessels are more dependent than larger vessels on external calcium for norepinephrine-induced constriction and myogenic tone.

The major objective of the present study was to determine whether there is a heterogeneity in the response of arterioles and venules in the rat cremaster muscle to alteration in external calcium. In addition, we assessed the involvement of local endogenous prostaglandins and/or endogenously released norepinephrine in modulating microvascular responses to changes in external calcium, since these agents have been shown to play a role in determining basal levels of tone in arterioles of skeletal muscle.

Methods. *Animals and surgical anesthesia.* Sixty-five male Sprague–Dawley rats, 5 to 6 weeks old and weighing 144 to 200 g, were used in these studies. Each rat was anesthetized with an intraperitoneal administra-

0037-9727/88 $1.50

tion of urethane (800 mg/kg) and α-chloralose (60 mg/kg). Rectal temperature was maintained at 37°C with a heating pad placed under the animal. The trachea was intubated to ensure a patent airway, and the animals were allowed to breathe room air spontaneously. The left femoral artery was cannulated for measurement of arterial pressure.

Cremaster preparation. The cremaster muscle was prepared using the technique described by Miller and Wiegman (8). A small incision in the scrotum exposed the cremaster surrounding the right testicle, and it was gently dissected free from the testicle with care to maintain the vascular and neural connections. Throughout surgery, the cremaster was kept moist with a physiological solution.

The rat was transferred to a board designed so that the rat's hindlegs straddled a 50-ml tissue bath. Silk sutures suspended the cremaster in a flat position over an optical port in the bath filled with a physiological salt solution (in m*M*): 25.5 $NaHCO_3$, 112.9 NaCl, 4.7 KCl, 1.19 KH_2PO_4, 1.19 $MgSO_4 \cdot 7H_2O$, and 11.6 dextrose. The bath calcium concentration was varied between 0.0 and 5.10 m*M* by the addition of $CaCl_2 \cdot 2H_2O$. Bath calcium concentrations were verified using a Technicon Autoanalyzer. The bath temperature was maintained at 34.5°C (the *in situ* crotal sac temperature of conscious rats) with an immersed insulated heating coil. Bath pO_2 (25–40 Torr), pCO_2 (34–45 Torr), and pH (7.40 ± 0.05) were controlled by varying the amounts of carbon dioxide and nitrogen bubbled through the bath. The bubbling of these gases also ensured complete mixing of drugs. The bath pH was continuously monitored using a pH electrode, and bath gases were periodically checked with a blood gas analyzer (IL 213).

The preparation was positioned on the stage of a trinocular microscope for observation of the cremaster microcirculation via closed-circuit television microscopy. The image of the cremaster was recorded on videotape and displayed on a calibrated monitor at approximately 1000 to 1600× magnification. Luminal diameters (with the column of blood) were measured from the television monitor screen at 1-min intervals during control and experimental periods.

Experimental protocol. Cremaster arterioles and venules were selected for observation on the basis of the vascular branching pattern (3). The largest arteriole entering the cremaster was termed the first-order arteriole (1A), and the large venule paired with the 1A was termed the first-order venule (1V). Arterioles branching from the 1A were termed second-order arterioles (2A). Similarly, venules which fed into the 1V were termed second-order venules (2V). Subsequent vessel branches from the 2A and tributaries to the 2V were termed, respectively, third-order arterioles (3A) and third-order venules (3V). A single arteriole or venule at any one level was observed in each rat.

In the first series of experiments, 28 rats were used to determine the effect of varying concentrations of bath calcium on the diameters of arterioles and venules. Control diameters of the vessels were measured during a 10-min control period as the cremaster was bathed in our standard solution (2.55 m*M* calcium). Then the cremaster was exposed to a 0-calcium solution for 20 min. At 10-min intervals the calcium concentration of the bath solution was increased (0.65, 1.28, 2.55, and 5.10 m*M* calcium) and the arteriolar and venular diameters were measured every minute. After completion of the calcium dose–response protocol, the tissue was washed with the standard solution. The cremaster was then exposed to a bath concentration of sodium nitroprusside (10^{-5} *M*), which produces maximal dilation of microvessels in the cremaster (9), and the response was observed for 5 min.

A second group of eight animals was used to determine whether constrictor responses of 3A to changes in bath calcium concentration are due to an α-adrenergic receptor stimulation by endogenous catecholamines. Initially, the diameters of 3A were observed in the standard bath solution (2.55 m*M* calcium) for a 10-min control period. The α-adrenergic blocking agent phentolamine was then added to the bath (bath concentration

10^{-6} M) and arteriolar diameters were observed for 30 min. The bath solution was then exchanged for a solution containing 0-calcium and phentolamine for a period of 30 min. Finally the 0-calcium bath was exchanged for the standard solution (2.55 mM calcium) and phentolamine, and the microvascular response was observed for an additional 20-min period. The concentration of phentolamine used in this protocol has been shown to block the constrictor effect of a high dose of norepinephrine (10^{-7} M) on the 3A vessels.

In the third series of experiments, the influence of endogenous prostaglandins on the responses of arterioles and venules to changes in bath calcium concentration was investigated in 22 rats. After a 5-min control period in the standard, calcium-containing bath solution, the cremaster was exposed to the prostaglandin synthesis inhibitor mefenamic acid (10 μg/ml) for 50 min and then to increasing bath concentrations of calcium (0.0–5.1 mM) in the continued presence of mefenamic acid. In six additional animals we observed the response of 1A to changes in bath calcium concentrations in the presence of both mefenamic acid and phentolamine (10^{-6} M).

Statistical analysis. Data were statistically analyzed by analysis of variance followed by a Newman–Keuls analysis for multiple comparisons between means (10). All values are expressed as means ± SEM; probability levels of less than 0.05 were considered significant.

Preparation of drugs. Mefenamic acid (Warner–Lambert/Parke–Davis) was dissolved in a saline–sodium carbonate (1 mg/ml) solution (pH 9.5) to give a stock solution of 1 mg/ml. The addition of 0.5 ml of the stock to the 50-ml cremaster bath gave a mefenamic acid bath concentration of 10 μg/ml (2.8×10^{-5} M). Phentolamine (Ciba) was dissolved in saline to give a stock solution of 3.17 mg/ml (10^{-4} M), which, when added (0.5 ml) to the cremaster bath, provided a bath concentration of 10^{-6} M. Sodium nitroprusside (Mallinckrodt) was prepared daily in distilled water and stored in a dark cabinet until use. A norepinephrine stock solution (10^{-5} M) was prepared daily by dissolving norepinephrine bitartrate (Sigma) in distilled water with ascorbic acid (1 mg/ml) to prevent inactivation.

Results. The mean arterial blood pressure for all rats (N = 65) in these experiments was 95 ± 1 mm Hg. Blood pressure was not significantly affected by additions of calcium chloride, mefenamic acid, norepinephrine, nitroprusside, or phentolamine to the cremaster muscle bath.

The responses of the three levels of arterioles and venules to alterations in bath calcium concentration are illustrated in Fig. 1. Exposure to a 0-calcium solution caused significant dilation of 2A and 3A arterioles, but no significant changes in the diameters of larger arterioles (1A) or any of the venules (1V, 2V, 3V). Reintroduction of calcium (0.65 mM calcium and higher) caused 2A and 3A vessels to constrict to diameters that were not significantly different from control diameters. Again, 1A and all venules showed no significant response to manipulation of the bath calcium concentration.

A comparison of arteriolar diameters following exposure to the 0-calcium solution and to nitroprusside indicates that diameters of 1A (107 ± 6 vs 111 ± 9 μm, respectively) and 2A (71 ± 8 vs 69 ± 9 μm, respectively) were not significantly different during these two conditions. However, 3A vessels exposed to nitroprusside showed a significant dilation above values recorded with 0-calcium (26 ± 2 vs 35 ± 5 μm). In the case of all venules, the diameters observed following exposure to nitroprusside were not significantly different from diameters observed during exposure to the standard (2.55 mM calcium) or the 0-calcium solutions.

Arterioles responded to NE (10^{-7} M) alone with constriction to 35 ± 6% of control diameter. After a 30-min exposure to 10^{-6} M phentolamine, which did not alter resting arteriolar diameters, application of NE failed to produce a significant constriction. Exposure to 0 mM calcium in the presence of phentolamine (Fig. 2) produced a significant arteriolar dilation (46 ± 6%). Reintroduction of calcium (2.55 mM) to the bath in the presence of phentolamine caused the arterioles to

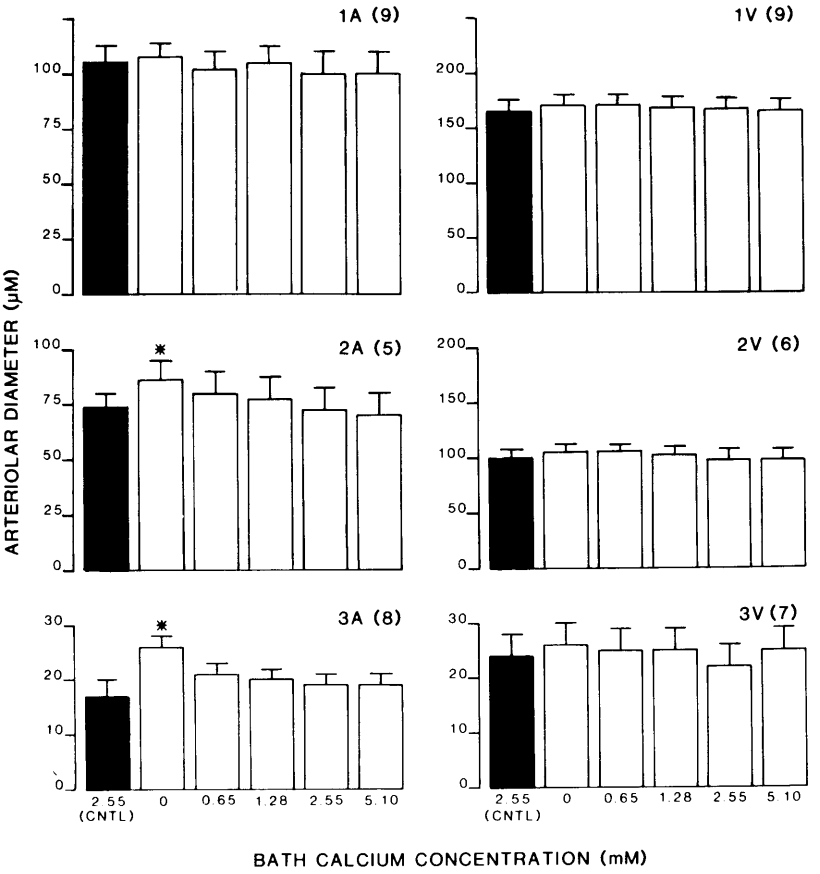

FIG. 1. Diameters of first- (1A, 1V), second- (2A, 2V), and third- (3A, 3V) order arterioles and venules in response to increasing bath calcium concentrations. Data points represent diameters in micrometers (means ± SEM) at each bath calcium level. Numbers in parantheses indicate the number of vessels in each group. Solid bars represent control diameters in the standard bath solution (2.55 m*M* calcium). An asterisk indicates a significant difference ($P < 0.05$) from control diameters.

constrict to diameters comparable to control diameters (13 ± 1 vs 13 ± 2 μm).

Exposure to mefenamic acid produced a significant reduction in 1A diameters after 30 min. Significant reductions in 2A and 3A diameters were observed at 15 and 5 min after exposure, respectively. At the end of the 50-min exposure period, the diameters of 1A, 2A, and 3A were reduced by 24, 36, and 42%, respectively (Fig. 3). Exposure to mefenamic acid had no significant effect on resting diameters of venules. Exposure to the 0-calcium solution in the presence of mefenamic acid caused significant dilation of all arterioles (Fig. 3). Reintroduction of calcium (0.65 m*M* or higher) to the bath caused a constriction of 1A to values similar to control values, while 2A and 3A vessels constricted to initial control diameters in the presence of mefenamic acid at calcium concentrations of 1.28 and 2.55 m*M* respectively. In contrast, the venules (1V, 2V, 3V) did not significantly constrict in mefenamic acid alone. Exposure to 0 m*M* calcium in the presence of mefenamic acid did not dilate the venules nor did reintroduction of calcium to the bath significantly affect venule diameter.

Figure 4 illustrates the responses of 1A to changes in bath calcium following exposure

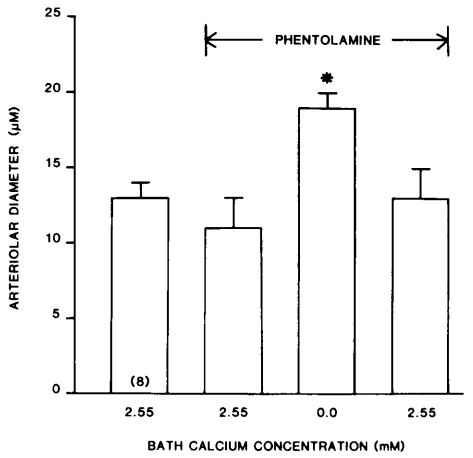

FIG. 2. Effect of phentolamine on arteriolar (3A) responses to changes in bath calcium concentration. Bars represent means ± SEM. The number in the parentheses indicates the number of vessels observed. The asterisk indicates a significant difference ($P < 0.05$) from diameters at 2.55 m*M* calcium in the presence of phentolamine.

to both phentolamine and mefenamic acid. Mefenamic acid alone caused a significant arteriolar constriction. Phentolamine in the continued presence of mefenamic acid had no significant effect on vessel diameters. However, exposure to a 0 m*M* calcium solution in the presence of these two agents produced a significant dilation which was reversed by reintroduction of calcium (2.55 m*M*) into the bath.

Discussion. In our current study, we observed the effects of a bath medium containing no calcium on diameters of arterioles and venules in the rat cremaster. The cremaster, a relatively thin tissue (approximately 150–250 μm thick) weighing approximately 200 mg, was continually exposed to 50 ml of bath medium. We feel that the relatively large volume of the bath would make it the primary determinant of tissue extracellular calcium concentration. The possibility exists, however, that in the smaller arterioles low concentrations of extracellular calcium, derived from perfusing blood or from calcium bound in the extracullelar matrix (11), may be present at the level of the vascular smooth muscle during exposure to 0-calcium. This seems to be the most likely explanation for the additional dilation (above diameters observed in the 0-calcium bath) produced by exposure to nitroprusside for third order arterioles. Sutter *et al.* (7) have suggested that the smaller precapillary vessels are the most dependent on extracellular calcium for activator calcium. Similarly, preliminary studies with the calcium entry blocker diltiazem (unpublished observation) also appear to produce dilation above that observed with 0-calcium bath alone. Clearly, however, current studies demonstrate a marked effect of changes in "extracellular" calcium on microvascular diameters and the interaction of prostaglandins.

We observed that intermediate (2A) and small (3A) arterioles in the cremaster microcirculation show a dependency on extracellular calcium for maintenance of intrinsic tone. The removal of calcium from the bathing solution causes dilation, whereas the addition of calcium causes constriction. The increase in arteriolar tone in these small arterioles after reexposure to calcium suggests that the major source of activator calcium for intrinsic tone in vascular smooth muscle of small resistance vessels is derived from extracellular sources. Maximal intrinsic tone (minimum vessel diameter) occurred at bath calcium concentrations of 1.28–2.55 m*M* calcium.

Smaller arterioles (3A) of the cremaster muscle are more sensitive to norepinephrine-induced constriction than larger vessels (3); thus the intrinsic tone observed in these smaller vessels may be due to endogenous norepinephrine. We therefore conducted experiments to determine if the intrinsic tone of these small vessels and the constrictor response to reintroduction of calcium to the bath were due to constrictor effects of endogenous norepinephrine. We found that the intrinsic tone of these vessels remained even after exposure of the vessels to a concentration of phentolamine which produced almost total blockade of the response to a high dose of exogenous norepinephrine (Fig. 2). Thus, the intrinsic tone of these vessels is not due to stimulation by endogenous norepinephrine. We also observed that phentol-

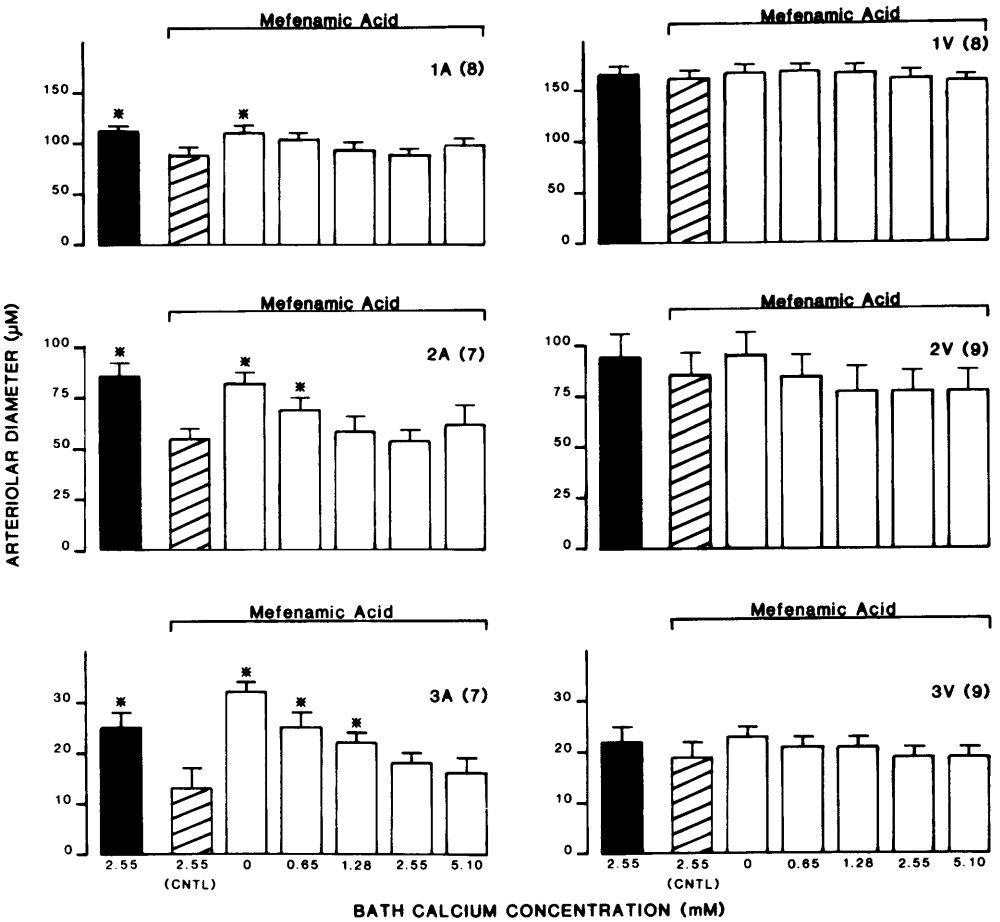

FIG. 3. Effect of mefenamic acid on the responses of arterioles (1A, 2A, 3A) and venules (1V, 2V, 3V) to changes in bath calcium concentrations. Bars represent means ± SEM; numbers in parentheses indicate the number of vessels in each group. Solid bars represent diameters in the standard bath solution (2.55 m*M* calcium), while striped bars represent control vessel diameters in the standard bath solution following a 50-min exposure to mefenamic acid (10 μg/ml). Asterisks indicate significant differences ($P < 0.05$) from control diameters in the presence of mefenamic acid (striped bars).

amine did not abolish the constrictor response of 3A to the readdition of calcium to the bath in the presence of phentolamine (Fig. 2), indicating that an adrenergic receptor mechanism probably does not mediate the constrictor response of the small vessels to calcium. The absence of an effect of phentolamine on the microvascular response to calcium is of added importance in light of the fact that extracellular concentration has been shown to be of importance in regulating neurotransmitter release (12).

Similarly, Lombard *et al.* (13) demonstrated that tetrodotoxin or phentolamine has no effect on the resting diameters of small arterioles (43–10 μm) in the hamster cheek pouch. These authors speculated that tone in these arterioles was due to intrinsic activity of vascular smooth muscle (myogenic) or to nonadrenergic constrictor influences such as angiotensin II, vasopressin, or other hormonal systems.

Previously, we (6) investigated the influence of bath calcium concentrations on the

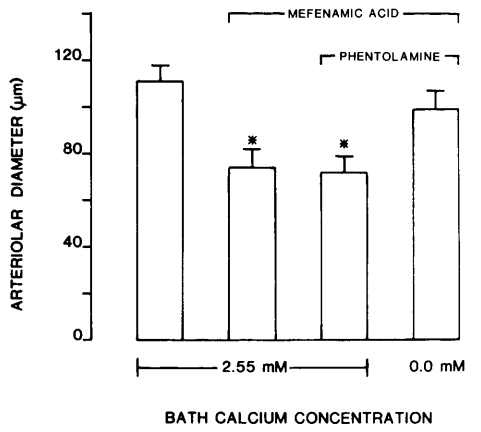

FIG. 4. Effect of mefenamic acid (10 μm/ml) and phentolamine (10^{-6} *M*) on the responses of large arterioles (1A) to changes in bath calcium concentrations. Bars represent means ± SEM for six arterioles. Asterisks indicate significant differences ($P < 0.05$) from diameters during exposure to the control bath solution (2.55 m*M* calcium) in the presence of mefenamic acid.

response of large arterioles (1A) in the cremaster to norepinephrine. Arteriolar constriction due to norepinephrine was significantly attenuated by reducing the bath calcium concentration from 2.55 to 1.28 m*M*, suggesting that in skeletal muscle, extracellular calcium plays an important role as a source of activator calcium for agonist (norepinephrine)-induced constriction at the level of large arterioles. Sutter *et al.* (7) have shown that lowering the calcium concentration in the perfusate to the rat hindquarter reduces the response of microvessels in the periphery to norepinephrine to a much greater extent than that in the larger upstream precapillary vessels. Collectively, these data suggest that arterioles in skeletal muscle are highly dependent on extracellular calcium for both intrinsic tone and agonist-induced constriction.

Another interesting finding was the heterogeneity in the responses of the various arteriolar levels to changes in bath calcium concentration. Essentially, the large arterioles (1A), unlike the smaller arterioles, showed no significant response to increases in bath calcium level from 0 to 5.1 m*M*. Vanhoutte (14) suggested that the heterogeneous behavior of vascular smooth muscle to constrictor stimuli can be attributed to factors related to anatomical location and the local tissue environmental conditions (e.g., temperature, partial pressure of oxygen, local production of autacoids, and autonomic innervation). In previous studies we (3) and others (4) have found that large arterioles in the microcirculation of the rat cremaster lack significant tone, whereas small arterioles have substantial intrinsic tone (5, 15, 16). Therefore, the heterogeneity in the responses to calcium is possibly due to the presence or absence of initial intrinsic tone.

In recent studies, we (1, 2) observed a heterogeneity in the response of arterioles in the cremaster muscle to angiotensin-induced constriction. Large arterioles were unresponsive to topically applied angiotensin II, while small arterioles showed a marked initial constriction. However, in the presence of a prostaglandin synthesis blocker (mefenamic acid or indomethacin), the larger arterioles constricted during exposure to angiotensin II. Thus, Fleming *et al.* (2) showed that the heterogeneity in the arteriolar response to angiotensin II was due to the selective dilator effect of endogenous prostaglandins on the larger arterioles. On the basis of these results, we hypothesized that the large arterioles (1A) of the cremaster muscle are unresponsive to changes in bath calcium concentrations because of the dilator influence of endogenous prostaglandins. We tested this hypothesis by pretreating the cremaster with the prostaglandin synthesis inhibitor mefenamic acid, and then observing the responses of larger arterioles and venules to changes in bath calcium concentration.

Exposure to mefenamic acid caused constriction of all levels of arterioles, while venules were unaffected. Our data corroborate the previous finding of Faber *et al.* (17, 18) and Fleming and Joshua (1). In the current study, the degree of 1A constriction in response to mefenamic acid was not lessened by phentolamine (Fig. 4). Therefore, we believe that the tone induced in these vessels following prostaglandin synthesis inhibition is most likely due to an attenuation of the

dilator influence of endogenous prostaglandins rather than to an increase in the neuronal release of norepinephrine and/or an increase in arteriolar reactivity to endogenous norepinephrine. The possibility also exists, however, that exposure to mefenamic acid causes a constriction which is mediated by both a decrease in vasodilator prostaglandins and an increase in vasoactive leukotrienes produced by a shift from the cyclooxygenase pathway to the lipoxygenase pathway (19).

Following exposure to mefenamic acid, all arterioles responded similarly to manipulations of bath calcium concentrations, i.e., dilating when exposed to 0-calcium and constricting when calcium was reintroduced into the bath (Fig. 2). These results suggest that all arteriolar levels depend on extracellular calcium as a source of activator calcium but that under normal conditions the dilator influence of endogenous prostaglandins masks the constrictor response of large arterioles (1A) to increases in extracellular calcium.

The reason for the absence of a venular response to manipulations in bath calcium (Fig. 1) or to inhibition of endogenous prostaglandins (Fig. 3) in our current study is not known. These vessels may lack the mechanisms necessary for generating nonadrenergic intrinsic tone, and the lack of intrinsic tone may be the factor responsible for the absence of a response to changes in bath calcium concentration. The lack of a venular response to inhibition of local prostaglandin synthesis corroborates previous studies (2). Since histological studies by Rhodin (20) suggest that venules above 50 μm in diameter possess vascular smooth muscle, we do not think that the lack of effect especially in the large (1V and 2V) venules is due to absence of smooth muscle. Previous studies with venules in the rat cremaster (7, 21) indicate that these venules are capable of constricting in response to an adrenergic agonist, norepinephrine, and therefore the results would also suggest that these vessels possess a significant vascular smooth muscle component. The possibility exists, however, that the small reduction in diameters of venules observed following topical exposures to vasoconstrictor agents is passive and due to a fall in venular intraluminal pressures produced by upstream precapillary vessel constriction.

We thank Dr. Frederick N. Miller for his helpful suggestions and Mary Englebert for her technical assistance. This research was supported by a grant from the American Heart Association, National Research Service Award HL-06790, and a Fellowship Award from the Kentucky Heart Association.

1. Fleming JT, Joshua IG. Mechanism of the biphasic arteriolar response to angiotensin II. Amer J Physiol **247**:H88–H94, 1984.
2. Fleming JT, Harris PD, Joshua IG. Endogenous prostaglandins selectively mask large arteriole constriction to angiotensin II. Amer J Physiol **253**:H1573–H1580, 1987.
3. Joshua IG, Wiegman DL, Harris PD, Miller FN. Progressive microvascular alteration with hypertension development. Hypertension **6**:61–67, 1984.
4. Hutchins PM, Darnell AE. Observation of a decreased number of small arterioles in spontaneously hypertensive rats. Circ Res (Suppl I), **34** and **35**:I-161–I-165, 1974.
5. Prewitt RL, Chen IH, Dowell RF. Development of microvascular rarefaction in the spontaneously hypertensive rat. Amer J Physiol **241**:H306–H310, 1981.
6. Joshua IG, Harris PD, Wiegman DL, Miller FN, Jauchem JR. Calcium modulation of microvascular sensitivity during renovascular hypertension. Proc Soc Exp Biol Med **167**:442–447, 1981.
7. Sutter MC, Hallback M, Jones JV, Folkow B. Contractile responses to noradrenaline: Varying dependence on external calcium of consecutive vascular segments of perfused rat hindquarters. Acta Physiol Scand **99**:166–172, 1977.
8. Miller FN, Wiegman DL. Anesthesia induced alteration of small vessel responses to norepinephrine. Eur J Pharmacol **44**:331–337, 1977.
9. Miller FN, Joshua IG, Anderson GL. Quantitation of vasodilation-induced macromolecular leakage by *in vivo* fluorescent microscopy. Microvasc Res **24**:56–67, 1982.
10. Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa State Univ. Press, pp258–298, 1967.
11. Lodge NJ, Van Breemen C. Mobilization of extracellular bound calcium during high potassium and norepinephrine stimulation of the rabbit aorta. Blood Vessels **22**(5):234–243, 1985.
12. Katz B, Miledi R. The timing of calcium action during neuromuscular transmission. J Physiol **189**:535–544, 1967.

13. Lombard JH, Chenoweth JL, Stekiel WJ. Non-neural vascular smooth muscle tone in arterioles of hamster cheek pouch. Microvasc Res **29**:81–88, 1985.
14. Vanhoutte PM. Heterogeneity or homogeneity? In: Bevan, Ed. Vascular Neuroeffector Mechanisms. New York, Raven Press, pp331–332, 1980.
15. Bohlen HG. Arteriolar closure mediated by hyperresponsiveness to norepinephrine in hypertensive rats. Amer J Physiol **5**:H157–H164, 1979.
16. Miller FN, Wiegman DL, Joshua IG, Nolph KD, Rubin J. Effects of vasodilators and peritoneal dialysis solution on the microcirculation of the rat cecum. Proc Soc Exp Biol Med **161**:605–608, 1979.
17. Faber JE, Harris PD, Joshua IG. Microvascular response to blockade of prostaglandin synthesis in rat skeletal muscle. Amer J Physiol **243**:H51–H60, 1982.
18. Faber JE, Harris PD, Miller FN. Microvascular sensitivity to PGE_2 and PGI_2 in skeletal muscle of decerebrate rat. Amer J Physiol **243**:H844–H851, 1982.
19. Lefer AM. Eicosanoids as mediators of ischemia and shock. Fed Proc **44**:275–280, 1985.
20. Rhodin JAG. Ultrastructure of mammalian venous capillaries, venules and small collecting veins. J Ultrastruct Res **25**:452–500, 1968.
21. Wiegman DL, Joshua IG, Morff RJ, Harris PD, Miller FN. Microvascular responses to norepinephrine in renovascular and spontaneously hypertensive rats. Amer J Physiol **236**:H545–H548, 1979.

Received December 8, 1987. P.S.E.B.M. 1988, Vol. 189.
Accepted September 2, 1988.

Statement of ownership, management, and circulation required by the Act of October 23, 1962, Section 4369, Title 39, United States Code: of

PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

Published monthly (except August) by Academic Press, Inc., 1 East First Street, Duluth, MN 55802. Number of issues published annually: 11. Editor: Dr. M. R. Nocenti, Society for Experimental Biology and Medicine, 630 West 168th Street, New York, NY 10032.
Owned by Society for Experimental Biology and Medicine, 630 West 168th Street, New York, NY 10032. Known bondholders, mortgagees, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages, and other securities: None.
Paragraphs 2 and 3 include, in cases where the stockholder or security holder appears upon the books of the company as trustee or in any other fiduciary relation, the name of the person or corporation for whom such trustee is acting, also the statements in the two paragraphs show the affiant's full knowledge and belief as to the circumstances and conditions under which stockholders and security holders who do not appear upon the books of the company as trustees, hold stock and securities in a capacity other than that of a bona fide owner. Names and addresses of individuals who are stockholders of a corporation which itself is a stockholder or holder of bonds, mortgages, or other securities of the publishing corporation have been included in paragraphs 2 and 3 when the interests of such individuals are equivalent to 1 percent or more of the total amount of the stock or securities of the publishing corporation.
Total no. copies printed: average no. copies each issue during preceding 12 months: 5158; single issue nearest to filing date: 4992. Paid circulation (a) to term subscribers by mail, carrier delivery, or by other means: average no. copies each issue during preceding 12 months: 4391; single issue nearest to filing date: 4291. (b) Sales through agents, news dealers, or otherwise: average no. copies each issue during preceding 12 months: 0; single issue nearest to filing date: 0. Free distribution by mail, carrier delivery, or by other means: average no. copies each issue during preceding 12 months: 117; single issue nearest to filing date: 117. Total no. of copies distributed: average no. copies each issue during preceding 12 months: 4508; single issue nearest to filing date: 4408.

(Signed) M. R. Nocenti, Editor