

# Increased Reactivity of Murine Mesenteric Veins to Adrenergic Agonists: Functional Evidence Supporting Increased $\alpha_1$ -Adrenoceptor Reserve in Veins Compared with Arteries

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## ABSTRACT

These studies examined adrenergic reactivity of mesenteric arteries and veins from deoxycorticosterone acetate-salt (DOCA-salt) hypertensive and sham control mice. We measured constrictions in unpressurized arteries and veins by monitoring vessel diameter using computer-assisted video microscopy in vitro. Veins were more sensitive than arteries to the constricting effects of norepinephrine (NE) and phenylephrine (PE), but the  $\alpha_2$ -agonists clonidine and UK 14,304 [5-bromo-6-(2-imidazolyl-2-yl-amino)-quinoxaline] did not constrict arteries or veins. Reactivity was not altered in arteries or veins from DOCA-salt mice. We next investigated the mechanism of increased venous reactivity to NE and PE by studying desensitization to maximum concentrations of NE and PE. Sham arteries

desensitized to NE and PE more than DOCA-salt arteries, whereas DOCA-salt and sham veins maintained 80% of the initial NE and PE constriction. To determine whether the increased reactivity and resistance to desensitization in veins was due to a greater  $\alpha$ -adrenoceptor reserve, vessels were incubated with the alkylating agent phenoxybenzamine (PBZ; 0.3, 3, 10, and 30 nM). The NE-elicited initial constriction was reduced by PBZ (3, 10, and 30 nM) in sham but only by PBZ (30 nM) in DOCA-salt veins. All doses of PBZ blocked NE responses in sham and DOCA-salt arteries. These data suggest that mesenteric veins express more  $\alpha_1$ -adrenoceptors than arteries, accounting for greater reactivity and resistance to desensitization compared with arteries.

The sympathetic nervous system (SNS) is an important contributor to hypertension and other cardiovascular diseases (de Champlain, 1990). The main effector of the SNS, which plays an important role in the regulation of vascular tone, is the catecholamine norepinephrine (NE) and to a lesser extent, epinephrine (McCulloch and McGrath, 1998). These vasoactive agents modulate vascular tone by directly acting on specific receptor proteins present on vascular smooth muscle cells.  $\alpha$ -Adrenergic receptors play a fundamental role in regulation of systemic arterial blood pressure and blood flow (Piascik and Pérez, 2001).

Blood pressure regulation is dependent on total peripheral vascular resistance (TPR) and cardiac output (CO) (Beavers et al., 2001). Hypertension can result from an increase in either CO or TPR. In established hypertension, the usual hemodynamic abnormality is increased TPR (Ferrario et al.,

1970; Smith and Hutchins, 1979; Schobel et al., 1993). Since small arteries are the main site of vascular resistance, many studies have compared the ability of NE to contract arteries from normotensive and hypertensive individuals. Data derived from these studies are conflicting. Some studies have shown an enhanced reactivity of arteries from deoxycorticosterone acetate (DOCA)-salt rats to adrenergic agonists compared with control (Ekas and Lokhandwala, 1980; Longhurst et al., 1988; Meggs et al., 1988; Perry and Webb, 1988). Other studies showed that sensitivity to adrenergic agonists is normal in caudal (Hermsmeyer et al., 1982) and mesenteric arteries (Luo et al., 2003) of DOCA-salt rats.

Increased CO also can contribute to increases in blood pressure. The splanchnic bed is a major blood reservoir containing up to 30% of total blood volume (Greenway, 1983). This capacitance function largely resides in systemic veins and venules. A reduction in capacitance of systemic veins will shift blood from peripheral vascular beds toward the thoracic cavity (Ricksten et al., 1981). In this way, augmented venous return leads to higher stroke volume and CO. Data from animal and human studies support a role for decreased ve-

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**ABBREVIATIONS:** SNS, sympathetic nervous system; NE, norepinephrine;  $\alpha_1$ ,  $\alpha$ -1; DOCA, deoxycorticosterone acetate; PE, phenylephrine; PBZ, phenoxybenzamine; CO, cardiac output; MCFP, mean circulatory filling pressure; TPR, total peripheral vascular resistance; UK 14,304, 5-bromo-6-(2-imidazolyl-2-yl-amino)-quinoxaline.

nous capacitance in the development of hypertension, as increased CO often occurs in the initial stages of experimental hypertension (Ferrario et al., 1970; Smith and Hutchins, 1979) as well as in the early stages of human hypertension (Schobel et al., 1993).

Mean circulatory filling pressure (MCFP) is the effective driving force for venous return to the heart. MCFP is elevated in renal hypertensive dogs; two-kidney, one-clip hypertensive rats; spontaneously hypertensive rats; and in the DOCA-salt hypertensive rats (Ferrario et al., 1970; Edmunds et al., 1989; Martin et al., 1998; Fink et al., 2000). MCFP is dependent on venoconstrictor tone and blood volume. Most studies performed with animals and hypertensive humans have revealed that blood volume does not increase in hypertension (Ferrario et al., 1970; Schobel et al., 1993). Therefore, increased MCFP in hypertension development is primarily due to venoconstriction. Multiple factors determine venomotor tone, but sympathetic-mediated vasoconstriction is the most important (Pang, 2001). However, little is known about venous reactivity to NE in hypertension.

We sought to test the hypothesis that increased venoconstriction in hypertension is due to enhanced reactivity to NE. We studied vascular reactivity in a murine model of DOCA-salt hypertension. In this salt-sensitive, low-renin experimental model, SNS activity has been found to play an important role (de Champlain, 1990), and venous capacitance has been shown to be decreased by the SNS as determined by changes in MCFP (Fink et al., 2000), making this hypertension model relevant for the studies performed here. Furthermore,  $\alpha_1$ -adrenoceptor antagonists are effective antihypertensive agents in DOCA-salt hypertension (Nabata et al., 1985).

We compared acute reactivity and time-dependent desensitization to  $\alpha$ -adrenergic agonists in small arteries and veins of DOCA-salt and sham control mice. We did these studies because if altered vascular responses to NE contribute to hypertension (a chronic condition), those responses should be either larger or more sustained in vessels from hypertensive versus normotensive mice. We also examined potential mechanisms behind differences in vascular reactivity of arteries and veins.

## Materials and Methods

**Animals.** C57/BL male mice (25–30 g) used in these experiments were obtained from Charles River Breeding Laboratories (Portage, MI). On arrival at the animal care facility, mice were maintained according to the standards approved by the Michigan State University All-University Committee on Animal Use and Care. Mice were individually housed in clear plastic cages with free access to standard pelleted chow (8640 Rodent Diet; Harlan Teklad, Madison, WI) and tap water. Mice were housed in temperature- and humidity-controlled rooms with a 12/12-h on/off light cycle. Animals were allowed a period of 2 to 3 days of acclimatization prior to entry into any experimental protocol.

**DOCA-Salt Surgery.** Mice were unilaterally nephrectomized under anesthesia provided by intraperitoneal injection of approximately 70 to 80  $\mu$ l of a solution containing ketamine (100 mg/ml) and xylazine (20 mg/ml) in a 9:1 ratio, respectively. The skin over the left flank was shaved, and a 1.5-cm incision was made through the skin and underlying muscle caudal to the rib cage. The left kidney was exteriorized and removed after ligation of the renal artery and vein with 4-0 silk sutures (Ethicon, Inc., Somerville, NJ). The muscle and skin layers were then closed separately with 4-0 silk sutures. A small

area between the shoulder blades of the back was shaved, and a 1-cm incision was made through which DOCA-salt pellets were implanted subcutaneously, resulting in a dose of 150 mg/kg DOCA. All DOCA mice were given salt water containing 1% NaCl and 0.2% KCl. Normotensive sham mice were also unilaterally nephrectomized, received no DOCA pellet implantation, and were given tap water. Both groups were placed on standard pelleted rodent chow. After recovery, the mice were housed under standard conditions for 4 weeks after which systolic blood pressure was determined by the tail-cuff method.

**In Vitro Preparation of Mesenteric Vessels.** Mice were anesthetized, and the small intestine with its associated mesenteric vessels was removed and placed in oxygenated (95% oxygen, 5% carbon dioxide) Krebs' physiological saline solution of the following composition: 117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, and 11 mM glucose. A segment of the intestine with associated vessels was removed and pinned flat in a silicone elastomer-lined (Sylgard; Dow Corning, Midland, MI) petri dish. A section of mesentery containing vessels close to the mesenteric border was cut out using fine scissors and forceps. The preparation was transferred to a smaller silicone elastomer-lined recording bath and pinned flat. Second- or third-order mesenteric veins or arteries (100–200- $\mu$ m diameter) were isolated for study by carefully clearing away the surrounding fat tissue. The recording bath containing the preparation was mounted on the stage of an inverted microscope (Olympus CK-2; Tokyo, Japan) and superfused with warm (37°C) Krebs' solution at a flow rate of 7 ml/min. All preparations were allowed a 20-min equilibration period during which the vessels relaxed to a stable resting diameter.

**Kidney and Cardiac Ventricle Weight.** Kidneys and cardiac ventricles from sham control and DOCA-salt mice were excised, blotted dry, and weighed. Tissue weight was normalized to body weight.

**Video Monitoring of Vessel Diameter.** The output of a black-and-white video camera (Hitachi model KP-111; Yokohama, Japan) attached to the microscope was fed to a PC Vision Plus frame-grabber board (Imaging Technology Inc., Woburn, MA) mounted in a personal computer. The video images were analyzed using computer software (Diamtrak, Adelaide, Australia). The digitized signal was converted to an analog output (DAC-02 board; Keithley Megabyte, Tauton, MA) and fed to a chart recorder (EZ Graph; Gould, Inc., Cleveland, OH) for a record of vessel diameter. Changes in vessel diameter as small as 1.8  $\mu$ m could be resolved.

**Concentration-Response Studies.** All drugs were added in known concentrations to the superfusing Krebs' solution. Concentration-response curves were obtained after application of the adrenergic agonists NE and PE (Sigma-Aldrich, St. Louis, MO). Each agonist concentration was applied for 3 min, and there was a 20-min interval between successive applications. A single concentration-response curve was obtained from each preparation.

**Desensitization Studies.** Mesenteric vessels were taken from sham and DOCA-salt mice, isolated, and prepared as described in the sections above. In this series of experiments, vasoconstriction of arteries and veins was examined using NE (veins,  $10^{-6}$  M; arteries,  $10^{-5}$  M) and PE (veins/arteries,  $10^{-5}$  M), concentrations that elicited maximal constrictions in these vessels. The adrenergic agonist was continuously applied to the superfusing Krebs' solution, and blood vessels were exposed to the adrenergic agonist for 30 min. The vasoconstrictor state of arteries and veins at different time points was examined.

**Effect of Phenoxybenzamine (PBZ) on NE- or PE-Elicited Initial Constriction and the Vasoconstrictor Reactivity of Mesenteric Arteries and Veins on a 30-Min Incubation Period with Adrenergic Agonists.** The PBZ-pretreatment protocol was performed according to a previously published protocol (Watts et al., 1996). After the initial 20-min equilibration period, tissues were incubated for 10 min with one concentration of PBZ (0.3, 3, 10, and 30 nM) followed by a 30-min incubation in 100  $\mu$ M sodium thiosul-

fate ( $\text{Na}_2\text{S}_2\text{O}_3$ ). Preparations were then washed for an additional 30 min with Krebs' physiological saline solution after which they were challenged with maximal concentrations of the adrenergic agonists NE (veins,  $10^{-6}$  M; arteries,  $10^{-5}$  M) and PE (veins/arteries,  $10^{-5}$  M). The initial constriction and vasoconstrictor reactivity of arteries and veins throughout a 30-min period were examined.

**Data Analysis.** Constrictions of blood vessels to the different treatments are expressed as percentage constriction (percentage reduction from the resting diameter). Half-maximal effective agonist concentration ( $\text{EC}_{50}$ ) and maximum response ( $E_{\text{max}}$ ) were calculated from a least-squares fit of individual agonist concentration-response curves using the following logistic function from Origin 5.0 (Origin-Lab Corp., Northampton, MA):  $Y = (E_{\text{min}} - E_{\text{max}}) / [1 + (x/\text{EC}_{50})^n] + E_{\text{max}}$ , where  $E_{\text{min}}$  is the minimum response and was constrained to zero and  $n$  is the slope factor. All data are expressed as mean  $\pm$  S.E.M. Statistical differences between groups were assessed by Student's two-tailed unpaired  $t$  test. When more than two groups were compared, an analysis of variance was used with Student-Newman-Keuls multiple comparison as a post test.  $P < 0.05$  was considered statistically significant. All statistical analyses were performed using GraphPad InStat for Windows 95 (GraphPad Software Inc., San Diego, CA).

## Results

**General.** Four weeks after the start of DOCA-salt treatment, systolic blood pressure in the DOCA-salt treated mice ( $n = 56$ ) was significantly higher than systolic blood pressure in the sham ( $n = 47$ ) control mice ( $123 \pm 1$  mm Hg versus  $101 \pm 1$  mm Hg,  $P < 0.05$ ). In agreement with other studies documenting hypertrophy of kidneys and cardiac ventricles of DOCA-salt rats (Young et al., 1994) and mice (Peng et al., 2001), kidney and ventricular weight, when normalized for body weight, were higher in the DOCA-salt treated group ( $10.0 \pm 0.2$  mg/g body weight versus  $8.1 \pm 0.1$  mg/g body weight and  $4.7 \pm 0.1$  mg/g body weight versus  $4.0 \pm 0.09$  mg/g body weight, respectively;  $P < 0.05$ ). The inner diameter of mesenteric arteries from sham and DOCA-salt mice was  $154 \pm 8.1$   $\mu\text{m}$  and  $166.2 \pm 6.9$   $\mu\text{m}$ , respectively ( $P > 0.05$ ). The diameter of mesenteric veins from sham and

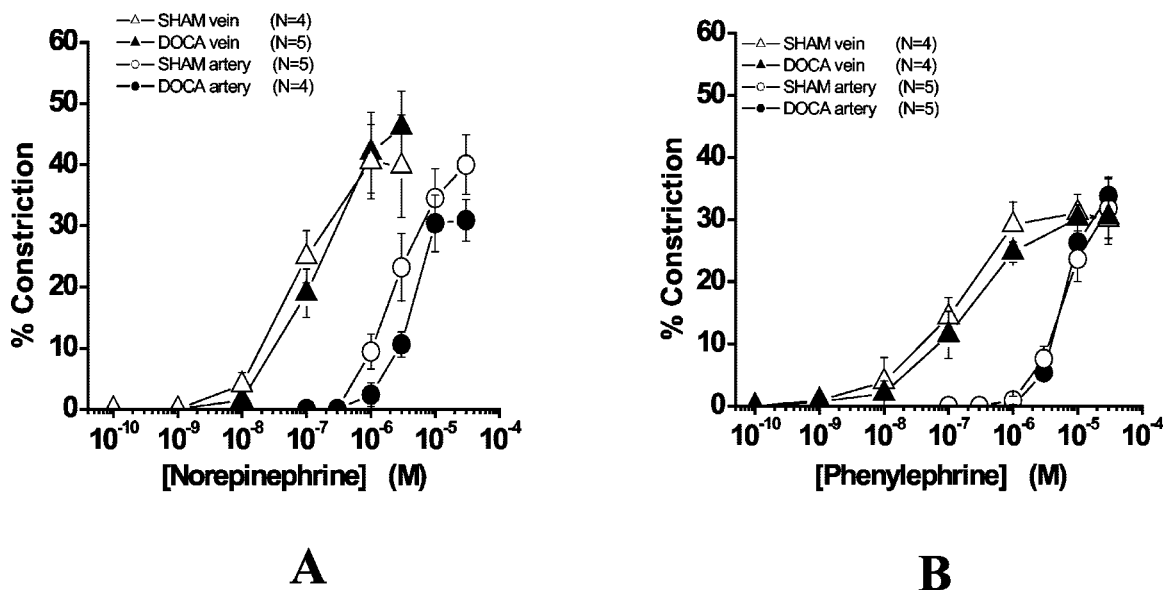
DOCA-salt mice was  $193.7 \pm 3.2$   $\mu\text{m}$  and  $169.8 \pm 5.1$   $\mu\text{m}$ , respectively ( $P < 0.05$ ).

**$\alpha_1$ -Adrenergic Receptors Mediate Constrictions of Arteries and Veins.** The adrenergic agonist NE produced a concentration-dependent constriction of mesenteric veins ( $10^{-10} - 3 \times 10^{-6}$  M) and arteries ( $10^{-7} - 3 \times 10^{-5}$  M) from DOCA-salt and sham control mice (Fig. 1A). Similarly, the selective  $\alpha_1$ -adrenergic receptor agonist, PE, produced a concentration-dependent constriction of mesenteric veins ( $10^{-10} - 3 \times 10^{-5}$  M) and arteries ( $10^{-7} - 3 \times 10^{-5}$  M) in both treatment groups (Fig. 1B). NE and PE were both more potent in constricting mesenteric veins from sham and DOCA-salt mice as there was a leftward shift in the concentration-response curve obtained in veins when compared with arteries (Fig. 1, A and B; Table 1). However, the magnitude of responses of veins and arteries to various doses of NE and PE were similar between DOCA-salt and sham groups (Fig. 1, A and B; Table 1).

The role of  $\alpha_2$ -adrenergic receptors in mediating vasoconstriction of arteries and veins from DOCA-salt and sham control mice was assessed. The  $\alpha_2$ -adrenoceptor agonists clonidine ( $10^{-7}$  M -  $10^{-5}$  M) and UK 14,304 ( $10^{-7}$  M -  $10^{-5}$  M) did not elicit constrictions in mesenteric arteries or veins from sham and DOCA-salt mice.

### Differential Desensitization in Arteries and Veins.

Our concentration-response studies showed that murine mesenteric veins were more sensitive than arteries to the constrictor effects of NE and PE. Mesenteric arteries exhibited similar maximal responses, but higher concentrations were needed to achieve them. Given those differences, we decided to further examine the potential mechanisms behind the marked differences in reactivity between mesenteric arteries and veins to adrenergic stimulation. This next series of experiments explored whether arteries and veins desensitize in a similar way when exposed to maximum concentrations of NE and PE. The concentrations used in this series of experiments were those responsible for inducing a maximal re-



**Fig. 1.** Concentration-response curves for the adrenergic agonists norepinephrine (A) and phenylephrine (B) obtained in mesenteric arteries and veins from sham control and DOCA-salt mice. Veins were more sensitive to the contractile effects of the agonists. Vascular reactivity was not altered in DOCA-salt vessels compared with their sham controls. Data are mean  $\pm$  S.E.M.  $N$  indicates the number of animals from which preparations were obtained.

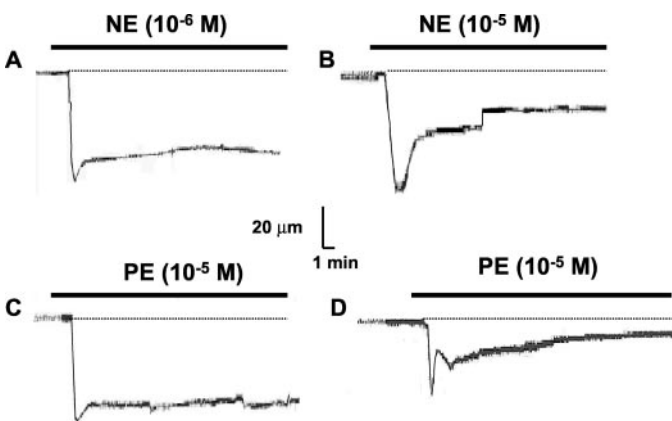
TABLE 1

Response of mesenteric arteries and veins from sham control and DOCA-salt mice to the adrenergic agonists PE and NE. Data are expressed as mean  $\pm$  S.E.M. Numbers in parentheses refer to the number of animals from which the data were obtained.  $E_{\max}$  is the maximum constriction based on data fitted to a logistic equation.  $EC_{50}$  is the negative logarithm of the molar concentration of agonist producing half-maximal constriction.

	$E_{\max}$		$EC_{50}$	
	Vein	Artery	Vein	Artery
	%		-log M	
NE				
SHAM	40.6 $\pm$ 7.5 (4)	39.3 $\pm$ 11.7 (5)	7.2 $\pm$ 0.2 <sup>a</sup> (4)	5.7 $\pm$ 0.1 (5)
DOCA-salt	48.8 $\pm$ 6.0 (5)	31.9 $\pm$ 3.7 (4)	6.8 $\pm$ 0.2 <sup>a</sup> (5)	5.5 $\pm$ 0.01 (4)
PE				
SHAM	30.5 $\pm$ 2.8 (4)	32.9 $\pm$ 5.0 (5)	7.1 $\pm$ 0.2 <sup>a</sup> (4)	5.3 $\pm$ 0.1 (5)
DOCA-salt	31.0 $\pm$ 3.7 (4)	34.2 $\pm$ 3.7 (5)	6.8 $\pm$ 0.3 <sup>a</sup> (4)	5.2 $\pm$ 0.05 (5)

<sup>a</sup> Significantly different compared with respective artery  $EC_{50}$ .

sponse in arteries and veins according to our concentration-response studies (Fig. 1). NE produced an initial peak constriction in both arteries ( $10^{-5}$  M) and veins ( $10^{-6}$  M) from DOCA-salt and sham control mice (Fig. 2, A and B). However, arteries exhibited a time-dependent desensitization as their diameter returned to the initial resting diameter during the 30-min agonist application (Fig. 2B). This effect was more prominent in sham arteries compared with DOCA-salt arteries. After 30 min of continuous agonist exposure, the diameter of sham arteries was about 10% of the initial peak constriction caused by NE, whereas in DOCA-salt arteries, the response declined to about 50% of the initial peak constriction (Fig. 3A). However, mesenteric veins maintained a tonic constriction despite continuous exposure to NE (Fig. 2A). After 30-min exposure, DOCA-salt and sham vein diameter was about 80% of the initial peak constriction elicited by NE (Fig. 3A). Continuous exposure of arteries and veins to the selective  $\alpha_1$ -adrenergic receptor agonist PE ( $10^{-5}$  M) revealed a marked difference between mesenteric arteries and veins. Incubation with PE elicited a constriction in arteries and veins (Fig. 2, C and D). PE responses in DOCA-salt and sham arteries (Fig. 3B) completely desensitized upon continuous exposure to PE. However, after 30-min exposure to PE, the diameter of DOCA-salt and sham veins was between 80 to 90% of the initial peak constriction (Fig. 3B).



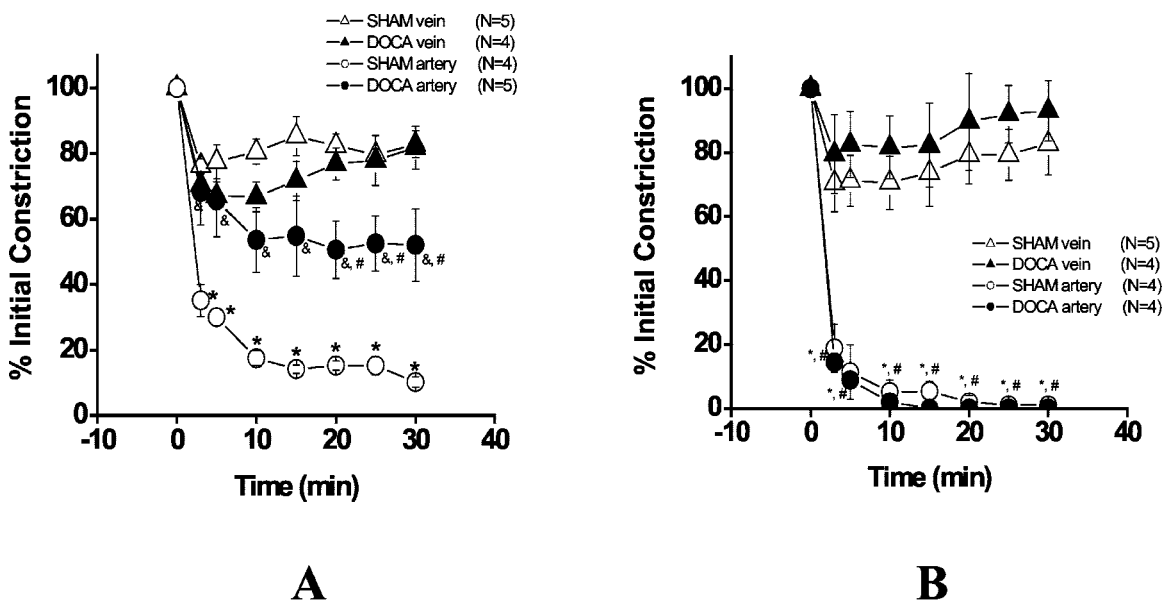
**Fig. 2.** Representative traces showing maintained constrictions in a vein (A and C) but not an artery (B and D) when exposed to maximum concentrations of NE or PE. Agonists were applied at the indicated concentration during the period indicated by the bar above each trace. The first 15 min of incubation are shown.

**$\alpha$ -Adrenoceptor Alkylation Studies with PBZ: Effects on Agonist-Induced Initial Constriction.** To further determine whether the increased reactivity to adrenergic agonists seen in veins was due to differences in adrenergic receptor concentrations, we incubated the vessels with the  $\alpha$ -adrenoceptor alkylating agent PBZ (0.3, 3, 10, and 30 nM) and compared the effects on the NE- or PE-elicited initial peak constriction. Incubation of sham veins with PBZ (0.3 nM) did not affect their initial constriction in response to NE (Fig. 4A). However, higher PBZ concentrations (3, 10, and 30 nM) produced a significant concentration-dependent reduction in the NE-elicited peak constriction (Fig. 4A). DOCA-salt mesenteric veins were more resistant to PBZ-alkylating effects as the peak contractile response to NE was significantly inhibited only at the highest (30 nM) PBZ concentration (Fig. 4A).

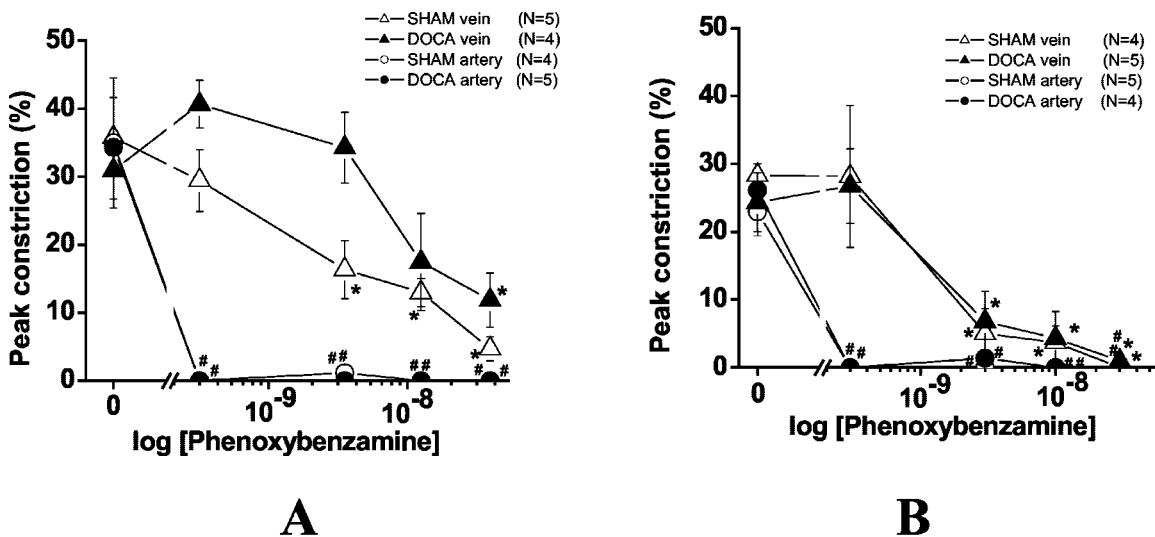
Preincubation of sham veins with PBZ (0.3 nM) did not affect the peak constriction elicited by PE compared with control responses (Fig. 4B). However, incubation with higher concentrations (3, 10, and 30 nM) of PBZ significantly inhibited peak constriction (Fig. 4B). A similar inhibition was seen in DOCA-salt veins (Fig. 4B) as PBZ (0.3 nM) did not affect the peak contractile response seen after PE application compared with veins not exposed to PBZ. However, incubation at the higher doses (3, 10, and 30 nM) significantly inhibited the peak contractile response (Fig. 4B).

Incubation of sham control and DOCA-salt mesenteric arteries with all PBZ concentrations completely inhibited their contractile response to NE (Fig. 4A). All PBZ concentrations blocked PE-induced constrictions of sham and DOCA-salt arteries (Fig. 4B).

**Effects of  $\alpha$ -Adrenoceptor Alkylation with PBZ on Desensitization.** The ability of 30-min exposure to NE to desensitize mesenteric veins preincubated with different concentrations of the alkylating agent PBZ was assessed. As preincubation with any PBZ concentration completely inhibited contractile responses in arteries, these studies were not performed in arteries. PBZ (0.3 nM) pretreatment did not change reactivity of sham veins to NE applied for 30 min, as NE caused a sustained constriction (Fig. 5A). In contrast, veins preincubated with PBZ (3 nM) were not able to maintain a contractile response throughout the 30-min period when compared with non-PBZ-treated veins (Fig. 5A). As 30 nM PBZ markedly reduced the peak NE-induced constriction in sham veins (Fig. 4A), we could not assess desensitization in these tissues. DOCA-salt veins were more resistant to the PBZ-inhibitory effect, since only veins incubated with the



**Fig. 3.** Mesenteric arteries but not veins desensitize during a 30-min incubation period with the adrenergic agonists NE (A) and PE (B). Blood vessels were exposed for 30 min to near-maximum agonist concentration. Veins maintained a tonic constriction upon challenge with NE and PE. This tonic constriction was not different between sham control and DOCA-salt veins. Arteries showed a time-dependent desensitization to NE that was more prominent in the sham arteries. PE completely desensitized sham and DOCA-salt arteries. Data are mean  $\pm$  S.E.M. *N* indicates the number of animals from which the preparations were obtained. \*, *P* < 0.05 sham artery versus sham vein; #, *P* < 0.05 DOCA artery versus DOCA vein; &, *P* < 0.05 DOCA artery versus sham artery.



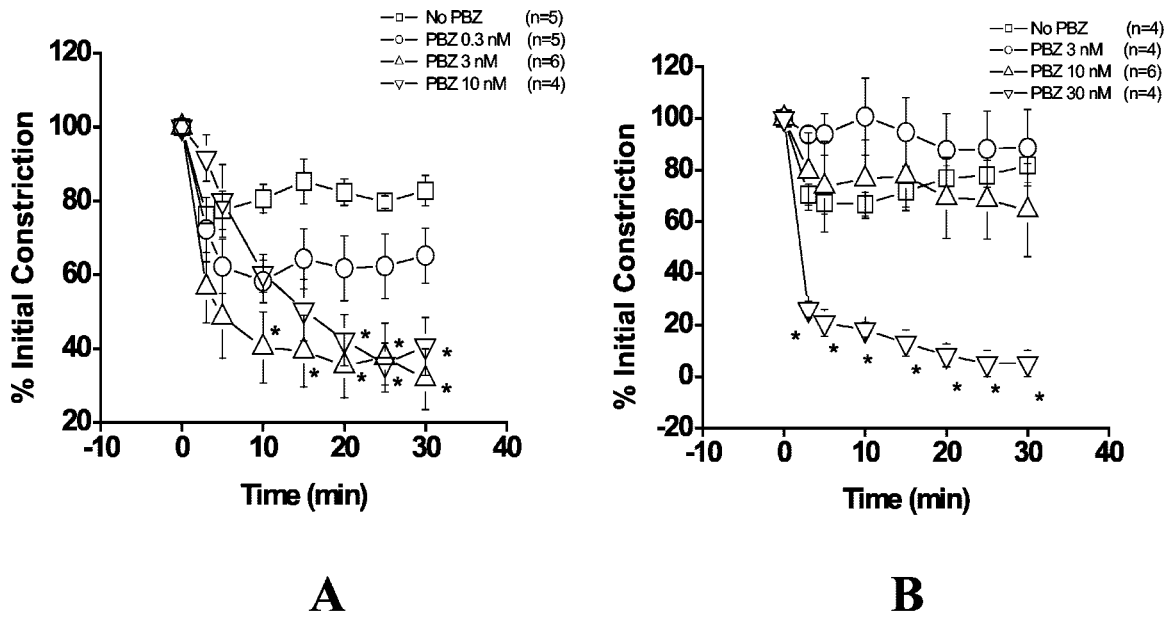
**Fig. 4.** Effect of PBZ on NE- (A) and PE-induced (B) initial constriction in sham control and DOCA-salt arteries and veins. Blood vessels were incubated for 10 min with PBZ (0.3–30 nM) prior to challenge with NE or PE. PBZ (0.3–30 nM) pretreatment completely abolished NE- and PE-elicited constrictions of mesenteric arteries from sham as well as DOCA-salt mice. PBZ (3–30 nM) significantly reduced NE-induced constrictions of sham veins, whereas only PBZ (30 nM) significantly reduced the NE-elicited initial response in DOCA-salt veins. PBZ (3–30 nM) pretreatment significantly inhibited PE-induced constrictions of sham and DOCA-salt veins. Data are mean  $\pm$  S.E.M. from *N* mice. \* and #, *P* < 0.05 versus no PBZ.

highest PBZ concentration (30 nM) failed to maintain contractility to NE applied for 30 min (Fig. 5B). As PE was much less efficacious than NE in stimulating constriction in the blood vessels studied, an analysis examining the effects of a 30-min PE incubation time period on vasoconstriction could not be performed.

### Discussion

**$\alpha_1$ -Adrenergic Receptors Mediate Direct Vasoconstriction of Mesenteric Arteries and Veins.**  $\alpha_1$ -Adrenoceptors mediate vasoconstriction as PE mimicked the con-

stricting effects of NE. Furthermore, the  $\alpha_2$ -adrenoceptor agonists clonidine and UK 14,304 did not constrict any artery or vein. However, others have proposed a vasoconstrictive role for  $\alpha_2$ -adrenoceptors in blood vessels (reviewed by Civantos Calzada and Aleixandre de Artinano, 2001). McCafferty et al. (1999) showed that in the pithed mouse,  $\alpha_{2B}$ -adrenoceptors mediate pressor responses to  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor agonists. It is possible that pressor responses caused by  $\alpha_{2B}$ -adrenoceptor activation are not mediated by vasoconstriction in murine mesenteric vasculature. Alternatively,  $\alpha_2$ -adrenoceptor constriction mechanisms may be active in vivo but not in vitro.



**Fig. 5.** Effect of the alkylating agent PBZ on the time course of NE-induced desensitization of sham control (A) and DOCA-salt (B) veins upon a 30-min exposure period. Blood vessels were incubated for 10 min with PBZ (0.3–30 nM) prior to challenge with NE ( $10^{-6}$  M). Sham veins significantly desensitized when exposed for 30 min to NE when pretreated with PBZ. DOCA-salt veins desensitized significantly only when pretreated (3–10 nM) with the highest PBZ (30 nM) concentration. Data are mean  $\pm$  S.E.M. from *N* number of mice. \*,  $P < 0.05$  versus no PBZ.

**Adrenergic Vascular Reactivity Is Not Altered in DOCA-Salt Mice.** Vascular reactivity of arteries and veins to  $\alpha_1$ -adrenoceptor stimulation is not altered in DOCA-salt compared with sham. Despite the difference in resting venous diameter between sham control and DOCA-salt veins, vascular reactivity was not altered. In agreement with our data, NE responses of subcutaneous veins taken from hypertensive patients were unchanged compared with control subjects (Lind et al., 1997). However, studies performed with DOCA-salt rats showed that mesenteric arterial adrenergic reactivity is enhanced compared with sham rats (Ekas and Lokhandwala, 1980; Longhurst et al., 1988; Perry and Webb, 1988). This discrepancy could be due to the differences in size of the vessels studied or the different methods used to assess vascular reactivity. Suzuki et al. (1994), Longhurst et al. (1988), and Ekas and Lokhandwala (1980) measured perfusion pressure changes of the main branches of the superior mesenteric artery. Perry and Webb (1988) measured isometric force development of large mesenteric arterial strips. We assessed vascular reactivity by measuring diameter changes in unpressurized small mesenteric arteries (<200- $\mu$ m diameter). In addition, there may be different physiological processes regulating adrenergic constriction in mice and rats, as vascular mechanisms can differ between the two species (Douglas et al., 2000). Our studies agree with those in caudal arteries (Hermsmeyer et al., 1982) and mesenteric arteries and veins (Luo et al., 2003), which show that the reactivity to adrenergic agonists does not change in DOCA-salt rats.

**Veins Are More Sensitive to the Vasoconstrictive Effects of NE and PE.** We showed that veins are more sensitive than arteries to adrenergic stimulation. It could be argued that increased venous reactivity is due to the fact that these experiments were carried out in unpressurized vessels, and arteries and veins have different flow-pressure characteristics. However, previous studies have demonstrated that the increased sensitivity of mesenteric veins compared with

arteries to either adrenergic agonists (Naito et al., 1998) or to sympathetic nerve stimulation (Hottenstein and Kreulen, 1987) is maintained when arteries and veins were pressurized to physiological levels. Therefore, increased venous adrenergic reactivity compared with arteries is not a function of vessel pressure.

Given this increased sensitivity of veins to adrenergic agonists, we tested the hypothesis that the increased adrenergic reactivity of veins is due to a larger  $\alpha_1$ -adrenoceptor concentration. The  $\alpha$ -adrenoceptor alkylating agent PBZ was used to assess receptor reserve in arteries and veins. The initial NE-elicited constriction was reduced by low concentrations of PBZ in sham veins but only by the highest PBZ concentration in DOCA-salt veins. All PBZ doses completely inhibited NE responses in arteries. These data suggest that there is a larger  $\alpha_1$ -adrenoceptor reserve in DOCA-salt compared with sham veins. These data also suggest that murine mesenteric veins express more  $\alpha_1$ -adrenoceptors than arteries.

**Veins Are Resistant to Desensitization.** An increased  $\alpha$ -adrenoceptor population in veins led us to predict that veins would be more resistant to desensitization than arteries. Arteries exhibited a time-dependent desensitization by NE that was more prominent in vessels taken from sham mice. In response to continuous exposure to PE, arteries from sham and DOCA-salt mice desensitized completely. Desensitization in arteries was more prominent when the vessels were exposed to PE than NE, suggesting that  $\alpha_2$ -mediated constriction elicited by NE could offset desensitization of  $\alpha_1$ -adrenoceptors. However,  $\alpha_2$ -adrenoceptors do not play a direct vasoconstrictive role in the small arteries and veins studied here (see above). Up-regulation of  $\alpha_1$ -adrenoceptors in DOCA-salt mesenteric arteries could explain why there was not a complete desensitization of these vessels in response to continuous exposure to NE. Up-regulation of  $\alpha_1$ -adrenoceptors occurs in mesenteric arteries of DOCA-salt rats (Meggs et al., 1988). Given this, DOCA-salt arteries

should be more resistant to  $\alpha_1$ -adrenoceptor desensitization than sham arteries upon exposure to PE. That was not found, as both groups of arteries completely desensitized.

Increased postreceptor activation events in DOCA-salt arteries could account for the relative resistance to desensitization seen in those vessels. Phosphatidylinositol activity was found to be greater in mesenteric (Takata et al., 1989) and femoral arteries of DOCA-salt rats, with no apparent change in receptor number or binding affinity (Eid and de Champlain, 1988). On the other hand, mesenteric veins maintained a tonic constriction upon continuous exposure to both NE and PE, suggesting that mesenteric veins have an increased  $\alpha_1$ -adrenoceptor reserve compared with arteries.

$\alpha_1$ -Adrenoceptor subtypes have different susceptibilities to desensitization induced by sustained NE stimulation. In human embryonic kidney 293 cells stimulated continuously with NE, Zhang et al. (1997) showed that the  $\alpha_{1A}$ -subtype easily desensitized. Desensitization of the  $\alpha_{1D}$ -subtype was delayed, with  $\alpha_{1B}$  desensitization being intermediate. Other studies (Chalotorn et al., 2002) have shown that continuous exposure to PE in transiently transfected human embryonic kidney 293 cells increased internalization of  $\alpha_{1A}$ - and  $\alpha_{1B}$ - but not  $\alpha_{1D}$ -adrenoceptors. Internalization was faster for the  $\alpha_{1B}$ -subtype. As there are differences in desensitization and internalization properties of  $\alpha_1$ -adrenoceptors, it will be important to identify the subtype expression in murine mesenteric vessels and to determine whether expression changes in DOCA-salt hypertension.

**PBZ-Pretreated Veins Are Susceptible to Desensitization.** Our studies suggest that there is an increased  $\alpha_1$ -adrenoceptor concentration in veins compared with arteries. The increased receptor concentration could account for the relative resistance of veins to desensitization. We hypothesized that decreasing the  $\alpha_1$ -adrenoceptor reserve in veins would render them more susceptible to desensitization by adrenergic agonists. PBZ-treated veins showed a partial desensitization to NE exposure, similar to that seen in arteries. PBZ-treated sham veins were more susceptible to desensitization compared with DOCA-salt veins, which only desensitized after treatment with the highest PBZ concentration. These results suggest that veins have an increased  $\alpha$ -adrenoceptor population compared with arteries and that there is an up-regulation in DOCA-salt veins compared with sham veins.

Responses to PE in PBZ-treated vessels were also inhibited in sham and DOCA-salt veins. However, the inhibition seen in these vessels was greater than that seen in PBZ-treated veins subsequently challenged with NE. Inhibition of PE responses between sham and DOCA-salt veins upon PBZ pretreatment did not differ. However, responses to PE were completely abolished in mesenteric arteries previously treated with any PBZ concentration. PE may be less efficacious than NE in stimulating constrictions in mesenteric vessels, and this could explain the greater sensitivity to PBZ in veins challenged with PE.

$\alpha_1$ -Adrenoceptors activate a variety of second messenger pathways (Pérez et al., 1993). There could be a larger  $\alpha_1$ -adrenoceptor reserve for one signaling pathway over the other, and there could be preferential activation of one of these pathways in veins as opposed to arteries. This concept of a larger receptor reserve in one signaling pathway over the

other has been shown for the 5-hydroxytryptamine  $2A$  receptor (Kurrasch-Orbaugh et al., 2003).

It could also be that different  $\alpha_1$ -adrenoceptor subtypes are involved in mediating constriction in arteries and veins and that they could differ in their sensitivity to PBZ. Studies performed with  $\alpha_{1B}$ -adrenoceptor knockout mice concluded that  $\alpha_{1A}$ - as well as  $\alpha_{1D}$ -adrenoceptors are involved in vasoconstriction, with a minor role for  $\alpha_{1B}$ -adrenoceptors (Daly et al., 2002). Yamamoto and Koike (2001) also concluded that  $\alpha_{1D}$ -like receptors are present in the mouse mesenteric artery. Whether these receptors play a predominant role in constrictions of murine mesenteric veins is not yet known.

## Conclusion

Murine mesenteric veins are more sensitive than arteries to the constricting effects of NE and PE, and reactivity is not altered in DOCA-salt hypertension. Studies with PBZ indicate that murine mesenteric veins express more  $\alpha_1$ -adrenoceptors than arteries. This would account for the greater venous reactivity to NE and resistance to desensitization compared with mesenteric arteries. Our data also indicate that there is an up-regulation of  $\alpha_1$ -adrenoceptors in DOCA-salt veins. These results support the importance of adrenergic regulation of venomotor tone in the long-term control of arterial blood pressure.

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