Increased vascular sensitivity to catecholamines characterizes mineralocorticoid hypertension. The present study investigated three possible sites that may account for this abnormality: agonist affinity, Ca\(^{2+}\) release from intracellular stores, and Ca\(^{2+}\) sensitivity of the contractile proteins. Adult male Sprague-Dawley rats underwent uninephrectomy and were implanted subcutaneously with deoxycorticosterone acetate (DOCA; 200 mg/kg, 1% NaCl: 0.2% KCl drinking water, 4–6 weeks). Control rats were sham treated. Helical strips of mesenteric arteries were placed in muscle baths for measurement of isometric force development. Although the ED\(_{50}\) for norepinephrine was significantly lower in arteries from DOCA rats (pD\(_{2}\), 8.21 ± 0.15) than in those from sham controls (pD\(_{2}\), 7.24 ± 0.11), agonist affinity, determined by partial blockade with phenoxybenzamine, did not differ between the two groups. In contrast, norepinephrine-stimulated \(^{45}\)Ca\(^{2+}\) efflux in the absence of extracellular Ca\(^{2+}\) was significantly greater in arteries from DOCA rats than in those from sham rats. In the presence of ryanodine to deplete intracellular Ca\(^{2+}\) stores, force development to Ca\(^{2+}\) was not different in saponin-permeabilized vessels from DOCA rats, indicating that the Ca\(^{2+}\) sensitivity of the contractile proteins was not altered in DOCA hypertension. We conclude that increased vascular sensitivity to norepinephrine in mineralocorticoid hypertension is related to increased release of Ca\(^{2+}\) from a subcellular store and not to changes in agonist affinity or to the contractile protein interaction. Based on previous reports, it is likely that this abnormality reflects a postreceptor change in signal transduction, but there is also evidence to suggest that an increase in the number of \(\alpha\)-adrenergic receptors may be involved. (Hypertension 1992;19:734–738)

KEY WORDS • adrenergic receptors • norepinephrine • calcium • vascular smooth muscle • deoxycorticosterone • mineralocorticoid hypertension • sarcoplasmic reticulum

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Increased vascular sensitivity to catecholamines has been well documented in various forms of hypertension and has been implicated in the development of increased peripheral vascular resistance. From a mechanistic standpoint, changes at a number of levels may contribute to observed differences in functional responses of vascular smooth muscle between hypertensive and normotensive control animals: alterations in receptor number or binding properties, changes in postreceptor signal transduction, and cellular changes that may directly affect a functional response, such as differences in the contractile proteins. The present study investigated three possible sites that may account for augmented contractile responsiveness to norepinephrine in mesenteric arteries from mineralocorticoid hypertensive rats. To evaluate agonist affinity, we determined the dissociation constant for norepinephrine using partial, irreversible \(\alpha\)-adrenergic blockade with phenoxybenzamine. We assessed agonist-induced mobilization of intracellular calcium stores by norepinephrine-stimulated \(^{45}\)Ca\(^{2+}\) efflux. Finally, we examined the function of contractile proteins using contractile responses to calcium in saponin-permeabilized arteries. Based on previous work in arteries from aldosterone and deoxycorticosterone acetate (DOCA) hypertensive rats, we hypothesized that the increase in vascular sensitivity to norepinephrine in mineralocorticoid hypertension is not due to a change in receptor affinity for the agonist but can be attributed to a change or changes occurring in the sequence of events after receptor activation.

Methods

Under sodium pentobarbital anesthesia (50 mg/kg i.p.), adult male Sprague-Dawley rats (250–300 g; Charles River Laboratories, Inc., Portage, Mich.) underwent left nephrectomy and were given subcutaneous, Silastic implants impregnated with DOCA (200 mg/kg). Control rats received a sham treatment; a left nephrectomy was performed, and Silastic without DOCA was implanted. All animals were maintained on standard rat chow, and drinking water was supplemented with 1% NaCl and 0.2% KCl. Experiments were performed after 4–6 weeks of DOCA treatment. At the time of experimentation, animals were anesthetized,
FIGURE 1. Left panel: Line plot shows contractile responses to norepinephrine in mesenteric arteries from deoxycorticosterone acetate (DOCA) hypertensive (○, n=6) and normotensive sham (●, n=6) rats before and after partial inactivation of α-adrenergic receptors with phenoxybenzamine. Contraction is expressed as percentage of maximal contractile response to norepinephrine in the absence of the antagonist. Each point represents mean±SEM. Right panel: Plot shows relation between sensitivity to norepinephrine (pD^2) and the dissociation constant for the agonist (pK^) in mesenteric arteries from DOCA hypertensive (○) and normotensive (●) rats. Each point represents mean±SEM. Dashed line and associated 95% confidence interval represent the theoretical relation between pD^2 and pK^ values when sensitivity is entirely determined by agonist affinity (slope=1). Mean values for DOCA hypertensive rats fall outside the derived confidence interval for the theoretical regression line.

killed by pneumothorax, and the superior mesenteric artery was removed and placed in physiological salt solution (PSS). Arteries were cleaned of adherent fat and connective tissue and were cut into helical strips. The endothelium was then removed by gentle rubbing of the luminal surface with a cotton swab. For contractile experiments, strips were mounted vertically in a tissue bath for measurement of isometric force, as previously described.5 Unless otherwise noted, the composition of the PSS was (mM): NaCl 130, KCl 4.7, KH2PO4 1.18, MgSO4·7H2O 1.17, CaCl2·2H2O 1.6, NaHCO3 14.9, dextrose 5.5, and CaNa2 EDTA 0.03.

α-Adrenergic Receptor Affinity for Norepinephrine

A passive tension of 600 mg was applied to each strip (0.8x10 mm), and strips were allowed to equilibrate for 90 minutes in warmed (37°C), aerated (95% O2-5% CO2) PSS. Norepinephrine was then cumulatively added to the tissue bath in half-log increments at concentrations ranging from 10^-10 to 10^-4 M in the presence of 10^-6 M cocaine, to block neuronal uptake. Two concentration–response curves to norepinephrine were performed in each strip, with the second curve being performed after a 15-minute incubation with phenoxybenzamine (10^-7 M), an irreversible α-adrenergic receptor antagonist. Phenoxybenzamine was rinsed from the tissue bath for 15 minutes before the addition of norepinephrine. The dissociation constant (K,) for norepinephrine was calculated according to methods described by Furchgott.3 Equieffective concentrations of norepinephrine before (A) and after (A’) phenoxybenzamine were determined for responses equivalent to 20–80% of the maximal norepinephrine contraction in the absence of antagonist. The K, was then determined using values derived from the relation between the reciprocals of these concentrations (1/A against 1/A’): K,=slope−1/intercept.

Radioactive Calcium Efflux

45Ca^2+ efflux from intact vascular segments was measured using methods described by Leitjen and van Breemen.6 Briefly, vascular strips (0.8x10 mm) were incubated in PSS (37°C; 95% O2-5% CO2) containing 45Ca^2+ (2 μCi/ml) for 1 hour. After loading, the tissue was rinsed twice in nonlabeled PSS (1.6 mM CaCl2) and then passed at 1-minute intervals through a series of vials containing calcium-free PSS (1 mM EGTA). Norepinephrine (10^-6 M) was added to the vials 5 minutes after the efflux curve was initiated for a 3-minute period. Radioactivity in tissue and effluent samples was counted by liquid scintillation. Fractional tissue 45Ca^2+ content, normalized to wet weight of tissue, was then determined.

Calcium Sensitivity of Contractile Elements

After equilibration and control contractile responses to norepinephrine in PSS, mesenteric artery strips (0.3x5 mm) were skinned using the technique of Saida and van Breemen.7 Strips were exposed to a skinning solution with 80 μM saponin for 15 minutes. They were then placed in a relaxing solution of the following composition (mM): potassium propionate 130, Tris maleate 20, MgCl2 4, and EGTA 2. In the presence of ryanodine (10^-5 M) to deplete intracellular calcium stores, concentration–response curves to calcium (10^-8 to 10^-4 M) were generated by exposure of the strips to a series of EGTA-buffered calcium solutions containing 2.4×10^-4 M calmodulin.

Drugs

Norepinephrine (Levophed bitartrate, Sterling Drug, Inc., New York) and cocaine were obtained from the University of Michigan Hospital Pharmacy. All other chemicals used in this study were purchased from Sigma Chemical Co., St. Louis, Mo.
Statistical Analysis

Data are expressed as mean±SEM. For calculation of effective dose concentrations, contraction was expressed as a percentage of the maximal response, and concentrations producing a half-maximal response (ED₅₀) were then determined visually by a plot of the percentage of response against the log concentration of the agonist. These values are expressed as negative logarithms (pD₂ values). Statistical analyses were performed by unpaired and paired Student’s t tests. The criterion for statistical significance was a value of p<0.05.

Results

Mesenteric arteries from DOCA hypertensive rats were more sensitive to norepinephrine than those from sham rats, as evidenced by a leftward shift in the concentration–response curve and a significantly greater pD₂ value (Figure 1). The pD₂ value for norepinephrine was 8.21±0.15 (antilog, 6.10×10⁻⁶ M) in hypertensive rats as compared with 7.24±0.11 (antilog, 5.72×10⁻⁶ M) in sham rats, indicating a 10-fold increase in the sensitivity of arteries from hypertensive rats. Maximal force development to norepinephrine did not differ between the two groups (DOCA, 625±26 mg; sham, 619±34 mg). After vessels were treated with phenoxybenzamine, the slope of the concentration–response curve and the maximal response to norepinephrine were reduced in all arteries tested (Figure 1, left panel). In phenoxybenzamine-treated vessels, the maximal contractile response to norepinephrine, expressed as a percentage of the maximal control response to the agonist, was not significantly different between DOCA hypertensive (78±2%) and sham (75±2%) rats.

Unlike pD₂ values, the pKᵣ for norepinephrine did not differ significantly in mesenteric arteries from DOCA hypertensive rats (pKᵣ, 6.89±0.08; antilog, 1.29×10⁻⁷ M) as compared with those from sham rats (pKᵣ, 6.54±0.21; antilog, 2.92×10⁻⁷ M). The relation between these variables is illustrated in the right panel of Figure 1. When sensitivity is determined only by agonist affinity, the theoretical relation between pD₂ and pKᵣ values has a slope of unity, depicted by the dashed line in the figure, indicating a 1:1 correspondence between sensitivity and receptor affinity for the agonist. In the present experiments, mean pD₂ and pKᵣ values for arteries from sham rats were within the 95% confidence band for the theoretically expected relation, suggesting that the mean pD₂ value approximates the value for pKᵣ. In contrast, pD₂ and pKᵣ values in arteries from DOCA hypertensive rats fell outside the derived 95% confidence band, indicating that factors other than receptor affinity are involved in the determination of sensitivity in these vessels. However, this result is also consistent with an increase in the number of receptors.

To evaluate norepinephrine-induced release of calcium from intracellular stores, ⁴⁵Ca²⁺ efflux from mesenteric artery strips was measured in calcium-free PSS. Norepinephrine (10⁻⁶ M) stimulated ⁴⁵Ca²⁺ efflux in all tissues. As shown in Figure 2, the magnitude of stimulated efflux was significantly greater, by approximately 30%, in arteries from DOCA hypertensive rats than in those from sham rats.

Concentration–response curves to calcium were performed in saponin-permeabilized strips of mesenteric artery to assess the calcium sensitivity of contractile elements. The pD₂ value for calcium was not significantly different in arteries from DOCA hypertensive rats (pD₂, 6.42±0.08; antilog, 3.83×10⁻⁷ M) as compared with those from sham rats (pD₂, 6.43±0.05; antilog, 3.74×10⁻⁷ M). There was no significant difference in the maximal force development to calcium in saponin-permeabilized arteries from hypertensive (78±13 mg) and sham (71±13 mg) rats.

Discussion

The present study confirms previous reports that arteries from rats with mineralocorticoid hypertension exhibit an increase in sensitivity to norepinephrine and examines possible mechanisms that may underlie altered sensitivity in this model of the disorder. The data indicate that in mesenteric arteries from DOCA hypertensive rats there is no change in receptor affinity for the agonist. However, norepinephrine-stimulated ⁴⁵Ca²⁺ efflux is increased, an observation that implicates a role for enhanced mobilization of intracellular calcium in augmented responsiveness to the agonist. The finding that the calcium sensitivity of contractile elements is not changed in DOCA hypertension argues against the presence of a general alteration in the contractile proteins of the vascular smooth muscle cell. Together, the results of this study suggest that augmented responsiveness to norepinephrine in arteries from DOCA hypertensive rats is related to an enhanced mobilization of intracellular calcium. Based on previous reports, it is likely that this abnormality reflects a postreceptor change in signal transduction, but there is also evidence that an increase in the number of α-adrenergic receptors may be involved.

Most studies of the affinity of α-adrenergic receptors in hypertension have used measures of affinity for competitive antagonists such as phentolamine or prazosin. Recently, however, Nyborg and Bevan have shown that agonist affinity for norepinephrine is...
increased in mesenteric arterioles from spontaneously hypertensive rats (SHR) using the noncompetitive α-adrenergic antagonist benextramine. Other studies using dissociation constants and radioligand binding of competitive antagonists have not demonstrated a change in α-adrenergic receptor affinity in the SHR vasculature.\textsuperscript{11,12} Interestingly, in rabbit arteries, variations in agonist affinity for norepinephrine among different vessels are not accompanied by similar variations in affinity for the antagonist prazosin.\textsuperscript{13} Observed discrepancies between evaluations of the binding properties based on estimates of antagonist versus agonist affinity seem to imply, as Nyborg and Bevan\textsuperscript{10} suggest, a lack of identity in receptor recognition sites for antagonists as compared with agonists.

Using functional studies of contraction and 45Ca\textsuperscript{2+} efflux as well as radioligand binding techniques, Smith et al\textsuperscript{4} have shown that affinity for several α-adrenergic antagonists is not altered in thoracic aortas from aldosterone hypertensive rats. Moreover, the dissociation constant for norepinephrine in hypertensive aortas, determined by measures of 45Ca\textsuperscript{2+} efflux before and after partial inactivation of receptors with dibenamine, did not differ from control. The finding that agonist affinity of α-adrenergic receptors is not altered in mesenteric arteries from DOCA hypertensive rats based on contractile experiments is in accord with this result. In the present study, the dissociation constant for norepinephrine in DOCA hypertensive rats (1.29×10\textsuperscript{-7} M) is similar to that determined by Smith et al\textsuperscript{4} in aldosterone hypertensive rats (4.8×10\textsuperscript{-7} M) but is 10-fold less than the dissociation constant for the agonist in mesenteric arterioles from SHR (1.29×10\textsuperscript{-6} M).\textsuperscript{10} Although we found no evidence of a change in agonist affinity, a decrease in receptor affinity for the α₁-antagonist [(±)-BE, the radiolabeled derivative of 2-[(4-hydroxyphenyl)-ethyl aminomethyl] tetracaine or HEAT, has been reported in mesenteric arteries from DOCA hypertensive rats.\textsuperscript{1} This disparity may be related to methodological differences but also suggests that affinity may differ between agonists and antagonists, supporting postulated differences in receptor recognition sites for these two classes of compounds.

Meggs et al\textsuperscript{1} observed that, although affinity for the antagonist [(±)-BE] was decreased in mesenteric arteries from DOCA hypertensive rats, binding capacity was approximately twice that found in control arteries. This receptor upregulation stands in contrast to the finding of Smith et al\textsuperscript{4} that the binding capacity for [(±)-HEAT or [H]prazosin is not altered in aortas from aldosterone hypertensive rats. Studies in other tissues from DOCA hypertensive rats have shown that the binding capacity for α-adrenergic antagonists in the heart and kidney is either decreased or unchanged, whereas several areas of the brain show increases in the binding of adrenergic antagonists.\textsuperscript{40} A recent autoradiographic characterization indicates that there is a downregulation of α₁ and α₂ receptors in peripheral tissues of rats with genetic, mineralocorticoid, and renal hypertension.\textsuperscript{44} Although the present study does not exclude the possibility that an increase in the number of α-adrenergic receptors may contribute to augmented sensitivity to norepinephrine, it seems unlikely that this explanation would account for the 10-fold difference observed between DOCA hypertensive and sham arteries.

Postreceptor mechanisms postulated to contribute to enhanced vascular sensitivity to norepinephrine in hypertension involve agonist-induced hydrolysis of membrane phospholipids by phospholipase C and subsequent production of the second messengers inositol 1,4,5-trisphosphate and diacylglycerol.\textsuperscript{7} It has been suggested that inositol 1,4,5-trisphosphate-mediated mobilization of intracellular calcium is an important step in the activation of contraction in vascular smooth muscle.\textsuperscript{12} Based on measures of 45Ca\textsuperscript{2+} efflux, this study demonstrates that norepinephrine-induced release of intracellular calcium is increased in mesenteric arteries from DOCA hypertensive rats. Previous work from our laboratory has shown that phasic contractile responses to norepinephrine in calcium-free solution are also augmented in these vessels.\textsuperscript{16} This augmented responsiveness in calcium-free solution occurred over the entire concentration range of the agonist, indicating that altered mobilization of calcium from the subcellular store contributes to the increased sensitivity to norepinephrine in arteries from DOCA hypertensive rats. The observed increase in sensitivity was not seen with phasic contractile responses to caffeine, indicating that this abnormality is not due to a change in the size of the intracellular calcium store. Importantly, in aortas from aldosterone hypertensive rats, findings of increased norepinephrine-stimulated production of phosphoinositide metabolites\textsuperscript{2-4} are consistent with the interpretation that changes in postreceptor signal transduction play a role in the enhanced mobilization of intracellular calcium in DOCA hypertension. Increases in phasic contractile responses to norepinephrine under calcium-free conditions have also been reported in SHR.\textsuperscript{13,18} However, in this model of hypertension, increases in agonist-induced 45Ca\textsuperscript{2+} efflux have not been demonstrated.\textsuperscript{18}

The present study provides no evidence to indicate that the calcium sensitivity of the contractile elements is altered in DOCA hypertension; there was no difference in pD\textsubscript{2} values for calcium between saponin-permeabilized mesenteric arteries from hypertensive and sham rats. In fact, the EC\textsubscript{50} value for calcium in these vessels, 0.38 and 0.37 μM, respectively, was virtually identical to the value of 0.36 μM noted in a previous study of sensitivity to calcium in chemically skinned aortas from aldosterone hypertensive and control rats.\textsuperscript{19} Similar results have been obtained in studies of skinned vascular preparations from SHR.\textsuperscript{20,21}

In conclusion, the results of this study indicate that augmented sensitivity to norepinephrine in mesenteric arteries from DOCA hypertensive rats is not accompanied by a change in agonist affinity or a change in the contractile protein interaction. However, the findings suggest that the increased sensitivity is associated with an augmented mobilization of intracellular calcium.

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