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Differential Contributions of Alpha-1 and Alpha-2 Adrenoceptors to Vasoconstriction in Mesenteric Arteries and Veins of Normal and Hypertensive Mice

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Abstract

Mesenteric veins are more sensitive than arteries to the constrictor effects of sympathetic nerve stimulation and α -adrenergic receptor agonists. In the present study, we tested the hypothesis that α_2 -adrenergic receptors (α_2 -ARs) contribute to *in vitro* agonist-induced constriction in veins but not arteries and that α_2 -AR function is down-regulated in mesenteric arteries and veins in deoxycorticosterone acetate-salt (DOCA-salt) hypertension. Norepinephrine (NE) concentration-response curves were similar in SHAM and DOCA-salt arteries and veins indicating that adrenergic reactivity of mesenteric blood vessels is not altered in murine DOCA-salt hypertension *in vitro*. Veins were 30-fold more sensitive to NE than arteries. The α_1 -AR antagonist, prazosin (.003–0.3 μ M), produced concentration-dependent rightward shifts of the NE concentration-response curves in arteries but not veins. The α_2 -AR agonists, clonidine and UK-14,304, did not constrict arteries or veins in the absence or presence of indomethacin (10 μ M) and nitro-L-arginine (NLA; 100 μ M). The α_2 -AR antagonists, yohimbine (.003–0.3 μ M) and rauwolscine (0.1 μ M) did not affect NE responses in SHAM or DOCA-salt arteries but antagonized NE responses in veins. These data indicate that there are different α -AR contractile mechanisms in murine mesenteric arteries and veins. α_1 -ARs, but not α_2 -ARs, mediate direct contractile responses in arteries and veins while α_2 -ARs contribute indirectly to NE-induced constrictions in veins but not arteries *in vitro*. There may be direct protein-protein interactions between α_1 - and α_2 -ARs or between their signaling pathways in veins. This contribution of α_2 -ARs may account for the greater sensitivity of veins compared to arteries to the contractile effects of NE.

Keywords

alpha-1 adrenoceptors; alpha-2 adrenoceptors; mesenteric arteries; mesenteric veins; mouse; DOCA-salt hypertension

1. Introduction

α -Adrenergic receptors (α -ARs) mediate the vascular constrictor effects of endogenous and exogenous catecholamines. α_1 - and α_2 -ARs are expressed by vascular smooth muscle cells and the relative contribution of each receptor type to vasomotor responses is specific to the vascular bed studied. α_1 - and α_2 -ARs mediate contractions of the rabbit (Daly et al., 1988) and dog saphenous vein (Fowler et al., 1984). Itoh et al. (1987) found that phenylephrine (PE), a selective α_1 -AR agonist, was a more potent agonist in canine mesenteric arteries compared to veins while the selective α_2 -AR agonist UK-14,304 was a more potent

constrictor of vein than arteries. These data indicate that arteries and veins express different α -ARs in the same vascular bed. There are also differences in relative expression of α_1 - and α_2 -ARs across vascular beds. For example, in the mesenteric, splenic, renal and femoral vascular beds noradrenergic constrictions are mediated by both α_1 - and α_2 -ARs but the contribution of α_2 -ARs was most prominent in the mesenteric and femoral beds (Polonia et al., 1986).

We have previously shown that murine mesenteric veins are more sensitive than arteries to the constrictor effects of α -AR agonists and veins are resistant to desensitization by adrenergic agonists and to α -AR inactivation by phenoxybenzamine (Pérez-Rivera et al., 2004). In addition, phenoxybenzamine treatment *in vitro* increased the α -AR desensitization rate in veins to that seen in untreated arteries. These data provided functional evidence that murine mesenteric veins have an increased α -AR reserve compared to arteries (Pérez-Rivera et al., 2004). The greater α -AR receptor reserve in veins would contribute to their increased sensitivity to the constrictor effects of NE and to their relative resistance to desensitization compared to arteries.

Increased venous reactivity and resistance to desensitization by α -ARs would play a role in blood pressure control and overall hemodynamics. Decreases in venous capacitance (resulting from increased venomotor tone) shift blood to the heart and the arterial circulation thereby increasing arterial pressure (Pang, 2001). Therefore, changes in venous capacitance can contribute to the development of hypertension. For example, humans with borderline hypertension exhibit decreased venous distensibility and redistribution of blood volume towards thoracic veins and the heart (Mark, 1984; Takeshita and Mark, 1979). In addition, patients with salt-sensitive hypertension show a greater decrease in venous distensibility during salt-loading than do subjects with salt-resistant hypertension (Draaijer et al., 1993). These changes can also occur in animal models of salt sensitive hypertension. In deoxycorticosterone acetate (DOCA)-salt hypertensive rats, venomotor tone is increased (Fink et al., 2000). The primary determinant of venomotor tone is sympathetic nerve activity and sympathetic input to veins is elevated in DOCA-salt hypertensive rats (Fink et al., 2000). As α_2 -ARs can play an important role in contractile responses to adrenergic agonists, a differential role of α_2 -ARs in arteries and veins could contribute to differences in adrenergic reactivity. Hypertension is also associated with changes in vascular α -AR constrictor mechanisms. For example α_1 -AR reactivity is increased in large mesenteric arteries from DOCA-salt hypertensive rats (Perry and Webb, 1988). In addition, nitro-L-arginine-induced hypertension in rats is associated with an upregulation of α_2 -AR receptors in the aorta and in mesenteric arteries (Kanagy, 1997). Finally, α_2 -AR agonists can relax arteries via an action at endothelial α_2 -ARs which couple to release of nitric oxide and other vasodilators (Vanhoutte, 2001). Therefore, our objective was to determine whether α_2 -AR stimulation contributes to the increased sensitivity of mesenteric veins to α -AR agonists by examining contractile responses of murine mesenteric arteries and veins in the presence or absence of α_1 or α_2 -AR specific agonists and antagonists. As sympathetic nerve activity is elevated in DOCA-salt hypertension (Fink et al., 2000), we also examined whether or not α -AR function is down-regulated in arteries and veins from DOCA-salt hypertensive rats in response to elevated sympathetic input to venous smooth muscle cells.

2. Methods

2.1. Animals

C57/BL male mice (25–30g) were obtained from Charles River Labs (Portage, MI). Upon arrival at the animal care facility, mice were maintained according to the standards approved by the Michigan State University All-University Committee on Animal Care and Use. Mice were individually housed in clear plastic cages with free access to standard pelleted chow

(Harlan/Teklad 8640 Rodent Diet) and tap water. Mice were housed in temperature and humidity-controlled rooms with a 12 hours on/12 hours off light cycle. Animals were allowed a period of 2–3 days of acclimatization prior to entry into any experimental protocol.

2.2. Preparation of DOCA-salt hypertensive animals

Mice were unilaterally nephrectomized under anesthesia using a solution containing ketamine (500 mg/ml) and xylazine (20 mg/ml) in a 9:1 ratio, respectively. Animals within the weight range used (25 – 30g) received about 80 μ L of the anesthetic. Standard surgical procedures were used to prepare DOCA-salt hypertensive mice (Perez-Rivera et al., 2005a; 2005b). SHAM mice were unilaterally nephrectomized, but they received no DOCA pellet implantation and were given tap water. Both groups of mice were placed on standard pelleted rodent chow. After recovery, the mice were housed under standard conditions for 4 weeks after which systolic BP was determined by the tail-cuff method.

2.3. In-vitro preparation of mesenteric vessels

Mice were euthanized with a lethal dose of pentobarbital (50 mg/kg i.p.). The small intestine and associated mesenteric vessels were removed and placed in oxygenated (95% O₂, 5% CO₂) Krebs' solution of the following composition (mmol): NaCl 117, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 25, glucose 11. A segment of the intestine with the 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 (200 – 300 μ m outside 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) and superfused with warm (37°C) Krebs' solution at a flow rate of 7 ml min⁻¹. All preparations were allowed a 20 min equilibration period during which the vessels relaxed to a stable resting diameter.

2.4. Video monitoring of vessel diameter

Video monitoring of vessel diameter—The output of a black-white video camera (DAGE-MTI 100, IN) attached mounted on the inverted microscope (Olympus CK-2) was fed to a frame grabber card (Picolo, Euresys Inc., TX) mounted in a personal computer. Video images were analyzed online using Diamtrak^R edge-tracking software (version 3.5, <http://www.diamtrak.com>) which provided a continuous measure of artery or vein outside diameter during experiments. Drug induced responses were measured in unpressurized blood vessels. Changes in diameter of 0.5 μ m could be resolved.

2.5. Concentration-response studies

All drugs were added in known concentrations to the superfusing Krebs' solution. Control concentration-response curves were obtained in arteries (0.1 – 30 μ M) and veins (0.1 nM – 3 μ M) after application of NE (Sigma-Aldrich, St. Louis, MO). Each agonist concentration was applied for 3 min and there was a 20-minute interval between successive applications. The contribution of α_1 -ARs to NE constrictor responses was studied by comparing curves in the absence and in the presence of the selective α_1 -AR antagonist prazosin (.003 – 0.3 μ M; Sigma-Aldrich). A role for α_2 -ARs in mediating contractile responses to NE was studied by comparing curves in the absence and presence of the selective α_2 -AR antagonists yohimbine (.003 – 0.3 μ M; Sigma-Aldrich) and rauwolscine (0.1 μ M; Sigma Aldrich). We also directly tested for the presence of contractile α_2 -ARs in arteries and veins by challenging blood vessels with the α_2 -AR agonists clonidine (0.1 – 10 μ M; Sigma-Aldrich) and UK-14,304

(0.1 – 10 μ M; Sigma-Aldrich, St. Louis, MO) in the absence or the presence of the cyclooxygenase inhibitor, indomethacin (10 μ M), or the nitric oxide synthase inhibitor *N*-nitro-L-arginine (NLA; 100 μ M). All antagonists were applied for 20 minutes prior to agonist application. A single concentration-response curve for each agonist was obtained in each preparation.

2.6. Data analysis

Agonist-induced constrictions are expressed as a percent reduction of the resting diameter). Half maximal effective agonist concentration (EC_{50}) and maximum response (E_{max}) were calculated from a least-squares fit of individual agonist concentration response curves using the following logistic function from Origin 7.0 (Microcal Software, Inc, Northampton, MA):

$$Y = \{ (E_{min} - E_{max}) / [1 + (x/EC_{50})^n] \} + E_{max}$$

where E_{min} is the minimum response (set at 0), n is the slope factor. Data are expressed as mean $\pm$ SEM.

Agonist concentration-response curves in the presence or absence of antagonists were analyzed by plotting the negative logarithm of the ratio of effective agonist concentrations (50% maximal response) in the presence and absence of the antagonist minus 1 [$\log$ dose ratio (DR) – 1] vs. the negative logarithm of the antagonist concentration (Arunlakshana and Schild, 1959). The X-intercept yields the pA_2 value).

Statistical differences between groups were assessed by Student's two-tailed unpaired t-test. When more than two groups were compared, analysis of variance (ANOVA) was used with Student-Newman-Keuls multiple comparison as a post test. $P < 0.05$ was considered statistically significant. All statistical analyses and 95% confidence interval (CI) calculations were performed using GraphPad InStat (GraphPad Software, San Diego, CA).

3. Results

3.1. General

Four weeks after the start of DOCA-salt treatment, systolic blood pressure in DOCA-salt ($n=93$) mice was significantly higher than in SHAM ($n=93$) mice (122 ± 1 mmHg vs 92 ± 1 mmHg, respectively; $P < 0.05$). The initial resting diameter of mesenteric arteries from SHAM and DOCA-salt mice was 138 ± 4 μ m and 135 ± 4 μ m, respectively ($P > 0.05$). The initial diameter of mesenteric veins from SHAM and DOCA-salt mice was 185 ± 5 μ m and 190 ± 7 μ m, respectively ($P > 0.05$).

3.2. Prazosin blocks α_1 -ARs in mesenteric arteries and veins

In the first set of experiments, we assessed the relative contribution of α_1 -AR activation to NE-induced constrictions of mesenteric arteries and veins from SHAM and DOCA-salt hypertensive rats. We examined NE-induced constrictions arteries and veins in the absence and presence of prazosin, an α_1 -AR antagonist. Prazosin alone did not change blood vessel diameter. NE produced a concentration-dependent constriction of arteries but there were no differences in the NE concentration-response curves obtained in SHAM and DOCA-salt arteries (Fig. 1A,B and Table 1). In SHAM arteries, prazosin produced parallel rightward shifts of the NE concentration-response curve without changing the E_{max} (Fig. 1A, Table 1). Schild analysis of these rightward shifts (Fig. 1C) produced a Schild plot with a slope of 0.9 ± 0.1 (95% CI: 0.6 – 1.1) that was not different from unity. Similar data were obtained in

arteries from DOCA-salt rats (Fig. 1B, Table 1) where the Schild plot (Fig. 1D) yielded a line with a slope of 0.8 ± 0.08 (95% CI: 0.6 – 1.0) that was not different from unity.

NE constricted veins in a concentration-dependent manner (Fig. 2A, Fig. 2B, and there were no differences between concentration response curves obtained in veins from SHAM and DOCA-salt rats (Table 1). However, veins were up to 30-fold more sensitive (determined by comparisons of EC_{50} values) than arteries to the constricting effects of NE (Table 1). Prazosin inhibited NE-induced constrictions in SHAM (Fig. 2A, Table 1) and DOCA-salt (Fig. 2B, Table 1) veins. However, prazosin did not produce concentration-dependent rightward shifts in the NE concentration-response curves obtained in veins (Fig. 2A, 2B). Schild plots for SHAM veins (Fig. 2C) had a slope of 0.3 ± 0.2 significantly less than 1 (95% CI: 0.2 – 0.8). Similarly, the Schild plot from data obtained in DOCA-salt veins also had a slope (0.5 ± 0.2) that was significantly less than 1 (95% CI: 0.1 – 0.9).

3.3. Yohimbine and rauwolscine inhibit α_2 -ARs in veins but not arteries

We studied α_2 -AR mediated contractile responses in mesenteric vessels by measuring NE-induced constrictions in the presence or absence of selective α_2 -AR antagonists. Yohimbine did not alter the resting diameter or NE-induced constrictions of SHAM or DOCA-salt arteries (Fig. 3A, 3B, Table 2). However, yohimbine antagonized NE-induced constrictions of SHAM and DOCA-salt veins (Fig. 4A, 4B, Table 2) rats. Nevertheless, yohimbine did not cause concentration-dependent and parallel rightward shifts in the NE concentration response curve. As a consequence, this resulted in nonlinear Schild plots in SHAM (Fig. 4C) and DOCA-salt veins (Fig. 4D). Control experiments that looked at concentration-response curves to PE, a selective α_1 -AR agonist, in the absence and presence of yohimbine were performed in veins to rule out the possibility that yohimbine was acting at α_1 -ARs. Yohimbine (30 nM) did not antagonize contractile responses to PE in SHAM (Fig. 5A, Table 3) or DOCA-salt (Fig. 5B, Table 3) veins.

To determine whether the inhibition of NE-induced contraction in veins but not arteries was specific for yohimbine, we examined NE-induced contractile responses in arteries and veins in the absence and presence of rauwolscine. Rauwolscine (100 nM) did not antagonize NE-induced constrictions in SHAM (Fig. 6A, Table 3) or DOCA-salt (Fig. 6B, Table 3) arteries. However, rauwolscine did cause a rightward shift of NE concentration-response curves in veins from SHAM (Fig. 7A, Table 3) and DOCA-salt (Fig. 7B, Table 3) rats.

3.4. α_2 -AR agonists do not constrict arteries or veins: lack of effect of inhibitors of nitric oxide synthase or cyclooxygenase

Our previous work showed that neither clonidine or UK 14,304 (α_2 -AR agonists) constriction mesenteric arteries or veins from rats (Luo et al., 2003) or mice (Perez-Rivera et al., 2004). Similar results were obtained in the present study where it was found that neither clonidine (0.1–10 μ M) or UK-14,304 (0.1–10 μ M) caused more than 10% constriction of arteries or veins (data not shown). As activation of endothelial α_2 -ARs results in endothelium-dependent vasorelaxation (Bockman et al., 1996; Figueroa et al., 2001), it is possible that α_2 -AR mediated release of vasodilators could antagonize any contractile effects of α_2 -AR agonists on vascular smooth muscle. For this reason, clonidine and UK-14,304 were studied in the presence of the cyclooxygenase inhibitor, indomethacin (10 μ M), or the nitric oxide synthase inhibitor *N*-nitro-L-arginine (NLA; 100 μ M) which inhibit cyclooxygenase and nitric oxide synthase, respectively. Even in the presence of these inhibitors, clonidine and UK-14,304 caused < 10% constriction of arteries or veins (data not shown).

3.5 Interaction between α_1 - and α_2 -AR agonists in veins

The results obtained using α -AR receptor antagonists indicate that both α_1 and α_2 -AR contribute to NE-induced constrictions of mesenteric veins. However, while PE, caused direct venoconstriction, α_2 -AR agonists alone did not constrict veins. It is possible that α_2 -ARs do not mediate direct venoconstriction but instead they may sensitize the α_1 -AR contractile mechanism. To test this hypothesis, PE concentration-response curves were obtained in the absence and presence of UK 14,304 (0.1 μ M). It was found that UK 14,304 shifted the PE curve to the left without changing the maximum response (Figure 8). The PE EC₅₀ in the absence of UK 14,304 was 127 ± 26 nM and in the presence of UK 14,304 this value was 42 ± 20 nM ($n = 5$, $P < 0.05$). UK 14,304 also changed the steepness of the PE concentration-response curve. The slope factor for the PE alone concentration-response curve was 0.95 ± 0.1 while in the presence of UK 14,304 the slope factor for the PE concentration-response curve was reduced to 0.5 ± 0.1 ($P < 0.05$).

4. Discussion

4.1. Contribution of α_2 -ARs to constriction in veins but not arteries

In vitro studies have consistently shown that α_1 -ARs are the predominant receptor mediating NE-induced contraction of mesenteric arteries in the rat (Hussain and Marshall, 2000) and mouse (Yamamoto and Koike, 2001). In the present study, Schild analysis revealed that prazosin acted as a simple competitive antagonist vs. NE in arteries confirming previous data that α_1 -ARs mediate NE-induced constrictions of mesenteric arteries in the mouse. This conclusion is also supported by our data showing that yohimbine and rauwolscine (α_2 -AR antagonists) do not affect NE-induced arterial constrictions. Furthermore, clonidine and UK 14,304 (α_2 -AR agonists) did not constrict mesenteric arteries. Similar data using UK 14,304 have also been obtained in pressurized mesenteric arteries from the mouse (Flavahan, 2005). We conclude that α_2 -ARs are not involved in NE-induced constrictions of murine mesenteric arteries.

Schild analysis revealed that, in veins, prazosin did not act as a simple competitive antagonist. This result suggests that NE may act at more than one receptor site. It is possible that α_2 -ARs contribute to the prazosin-resistant response in mesenteric veins. However, we found that neither clonidine nor UK 14,304 constricted arteries or veins suggesting that α_2 -ARs do not mediate direct NE-induced constriction of mesenteric blood vessels. We further explored the contribution of α_2 -ARs to NE-induced constrictions of mesenteric blood vessels by examining NE concentration-response curves in the presence of yohimbine or rauwolscine. Both of these α_2 -AR antagonists competitively antagonized NE-induced constrictions in veins suggesting that α_2 -ARs contribute via an indirect mechanism to these responses.

The suggestion that α_2 -ARs indirectly contribute to NE-induced constrictions of mesenteric veins is also supported by our data showing that UK 14,304 potentiates PE-induced constrictions of veins. UK 14,304 did not cause venous constriction but it did cause a leftward shift in the PE concentration-response curve. Similar data have been obtained in rat tail artery where it was shown that UK 14,304 caused a leftward shift in the concentration constriction curve for methoxamine, an α_1 -AR agonist. In this same study it was shown that UK 14,304 alone did not cause arterial constriction (Chen et al., 1999). Therefore, the proposed interaction between α_1 -AR and α_2 -ARs is not unique to murine mesenteric veins. Taken together data suggest that activation of α_2 -ARs sensitizes veins to the constrictor effects of α_1 -AR activation. In addition, the slope factor for the PE concentration-curve was reduced in the presence of UK 14304. The decline in slope factor indicates that UK 14,304 alters the interaction of PE with the α_1 -AR or with the associated contractile mechanism.

4.3. Veins are more sensitive than arteries to NE: contribution of α_2 -ARs

Veins are more sensitive than arteries to the constrictor effects of sympathetic nerve stimulation (Hottenstein and Kreulen, 1987; Luo et al., 2003). In the present studies we confirmed that are more sensitive than arteries to the contractile effects of NE (Luo et al., 2003). Data from the present study suggest that a contribution of α_2 -ARs to NE-induced constriction of veins but not arteries contributes to greater noradrenergic reactivity of veins compared to arteries. It should also be pointed out that there is also likely to be differential expression of α_1 -AR receptor subtypes or post-receptor signaling in arteries and veins as veins were also more sensitive to the contractile effects of PE, a selective α_1 -AR agonist. This latter result agrees with previous studies in rats (Luo et al., 2003) and mice (Pérez-Rivera et al., 2004) that showed an increased reactivity of mesenteric veins over arteries to stimulation by PE.

Although we showed that α -ARs contribute to NE induced venous constriction, α_2 -AR agonists did not cause direct venoconstriction. This contrasts with previously published work showing that both α_1 -AR and α_2 -AR agonists constrict veins (Fowler et al., 1984; Itoh et al., 1987; Daly et al., 1988; Daniel et al., 1997). While α_2 -AR agonists can constrict some veins, these responses are generally smaller than those caused by α_1 -AR agonists (Fowler et al., 1984; Daniel et al., 1997). The smaller constriction caused by α_2 -AR compared to α_1 -AR agonists in veins might be due to lower concentrations of α_2 -AR compared to α_1 -ARs. However, binding studies revealed that in canine mesenteric vein, α_2 -AR concentrations are higher than those for α_1 -ARs (Daniel et al., 1997). An alternative explanation is that α_2 -AR receptors may couple very inefficiently to the signaling mechanisms producing constriction in veins. This suggestion is consistent with the observation that canine mesenteric veins require pre-depolarization with elevated extracellular potassium concentration in order to potentiate responses caused by α_2 -AR agonists (Daniel et al., 1987). Murine mesenteric veins may express fewer α_2 -ARs than mesenteric veins in other species and these receptors may couple very poorly to the intracellular signaling pathways leading to venoconstriction. However, even modest activation of these signaling mechanisms by α_2 -AR may be sufficient to sensitize venous smooth muscle to the constrictor effects of α_1 -AR activation.

4.4. Functional interaction between α_1 - and α_2 -ARs

The data present here indicate that in order to see a contribution of α_2 -ARs to contractile responses in veins, co-activation of both α_1 - and α_2 -ARs is necessary. Similar receptor interactions occur in chinese hamster lung fibroblasts expressing α_1 -ARs and α_{2A} -ARs (Reynen et al., 2000). In those studies, NE did not increase $[Ca^{2+}]_i$ in cells expressing only α_1 -ARs but NE did cause an increase in $[Ca^{2+}]_i$ in cells co-expressing α_1 -AR and α_{2A} -AR. The effects of NE were blocked by subtype selective concentrations of α_1 - and α_2 -AR antagonists. Selective agonists of α_1 -AR and of α_2 -ARs agonists applied alone did not have any effect on $[Ca^{2+}]_i$ release but when added together induced a robust stimulation of $[Ca^{2+}]_i$. These data are similar to our own where we showed that concentrations of PE that normally do not constrict veins do elicit a constriction when the same concentrations are applied in the presence of UK 14,304. As Reynen et al. (2000) stated, this phenomenon could be of physiological importance in vascular smooth muscle cells (in particular venous smooth muscle cells) that express functional α_1 -ARs and α_2 -ARs. Our data provide evidence that this functional interaction occurs in venous smooth muscle cells that normally co-express α_1 -ARs and α_2 -ARs at physiologically relevant levels. This is not always the case in heterologous expression systems. Previous studies in veins have also suggested that a functional interaction occurs between α_1 -ARs and α_2 -ARs (Daly et al., 1987) and this interaction may be important to the hemodynamic function of veins (see below)

An important question is how this receptor interaction might occur. Is it due to a direct physical interaction between α_1 - and α_2 -ARs or an interaction involving the signaling cascades activated by both receptors? It is known that α -ARs could interact with other receptor systems in ways that are receptor-specific. For example, in mouse atria, angiotensin and bradykinin receptors interact with α_2 -AR (Cox et al., 2000; Trendelenburg et al., 2003) and protein kinase C activation plays a role in this functional interaction (Mota and Guimaraes, 2003). More detailed experiments are necessary to determine whether or not there is direct coupling of α_1 - and α_2 -ARs or if post receptor events are responsible for the functional interaction in veins.

4.5 DOCA-salt hypertension does not alter adrenergic reactivity in arteries or veins

We did not find any differences in adrenergic reactivity between DOCA-salt arteries and veins compared to their SHAM counterparts. This is in contrast to the studies by Luo et al. (2003) who showed a decreased reactivity of DOCA-salt veins but no difference in reactivity between SHAM and DOCA-salt arteries. Other studies performed in DOCA-salt rats have found that mesenteric arterial adrenergic reactivity is enhanced (Longhurst et al., 1988; Perry and Webb, 1988). Potential reasons for the discrepancies seen are differences in size of the vessels studied or the different methods used to assess vascular reactivity. It should also be noted that despite significant increases in blood pressure in DOCA-salt mice, the degree of hypertension in mice is much less than that reported for rats (Johns et al., 1996). Therefore, increases in blood pressure seen in DOCA-salt mice may have been sufficient to stimulate the signaling mechanisms leading to changes in adrenergic reactivity in the mesenteric vasculature.

4.6. Conclusions

There are different α -AR contractile mechanisms in murine mesenteric arteries and veins: α_1 -ARs mediate constriction in arteries and veins whereas α_2 -ARs contribute to responses in veins but not arteries. This difference in adrenoceptor function and pharmacology contributes to artery vs. vein differences in adrenergic reactivity: veins are more sensitive than arteries to the constrictor effects of NE. Our data also indicate that there is a functional interaction between α_1 - and α_2 -ARs or their signaling mechanisms in veins but not arteries. Finally, adrenergic reactivity in murine mesenteric arteries and veins is not altered in DOCA-salt hypertension.

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Figure 1. Effect of prazosin on NE-induced constrictions of SHAM (A) and DOCA-salt (B) mesenteric arteries. Prazosin produced concentration-dependent and parallel rightward shifts in the NE-concentration-response curves with no changes in maximal response. (C) Schild plot for prazosin antagonism of NE-induced contractile responses in SHAM mesenteric arteries. (D) Schild plot for prazosin antagonism of NE-induced contractile responses in DOCA-salt mesenteric arteries. In both cases, the slope of the plot was not different from 1.0 (see text). Data are mean $\pm$ SEM. N indicates the number of animals from which preparations were obtained.

Figure 2.

Effect of prazosin on NE- induced constriction of SHAM (A) and DOCA-salt (B) mesenteric veins. Prazosin (3 nM) produced a significant rightward shift in NE concentration-response curves in SHAM and DOCA-salt veins with no change in maximal response among treatment groups. Higher concentrations of prazosin did not produce further rightward shifts in NE concentration response curves. (C) Schild plot for prazosin antagonism of NE-induced contractile responses in SHAM mesenteric arteries. (D) Schild plot for prazosin antagonism of NE-induced contractile responses in DOCA-salt mesenteric arteries. In both cases, the slope of the plot was significantly less than 1.0 (see text). Data are mean $\pm$ SEM. N indicates the number of animals from which preparations were obtained.

Figure 3. Yohimbine did not affect NE concentration response curves in SHAM (A) or DOCA-salt (B) mesenteric arteries. Data are expressed as mean $\pm$ SEM. N indicates the number of animals from which preparations were obtained.

Figure 4.

Effect of yohimbine on NE-induced constriction of SHAM (A) and DOCA-salt (B) mesenteric veins. Yohimbine produced a significant rightward shift in the concentration-response curve of SHAM and DOCA-salt veins. Agonist responses are expressed as percent change from resting diameter. (C) Schild plot for yohimbine antagonism of NE-induced contractile responses in SHAM mesenteric veins (D) Schild plot for yohimbine antagonism of NE-induced contractile responses in DOCA-salt mesenteric veins. In both cases, the slope of the plot was significantly different from 1.0 (see text). Data are mean $\pm$ SEM. N indicates the number of animals from which preparations were obtained.

Figure 5. Yohimbine did not affect constrictions induced by phenylephrine (PE) in SHAM (A) or DOCA-salt (B) mesenteric veins. PE responses are expressed as percent change from resting diameter. Data are mean $\pm$ SEM. N indicates the number of animals from which preparations were obtained.

Figure 6. Rauwolscine did not affect NE-induced constriction of SHAM (A) and DOCA-salt (B) mesenteric arteries. NE-induced responses are expressed as percent change from resting diameter. Data are mean $\pm$ SEM. N indicates the number of animals from which preparations were obtained.

Figure 7. Effect of rauwolscine on NE-induced constriction of SHAM (A) and DOCA-salt (B) mesenteric veins. Rauwolscine produced a significant rightward shift in the concentration-response curve in veins from both groups. Data are mean $\pm$ SEM. N indicates the number of animals from which preparations were obtained.

Figure 8.

UK 14,304 potentiates PE-induced constrictions of mesenteric veins. Concentration response curves for PE-induced constriction of mesenteric veins were obtained in the absence and presence of UK 14,304 at a concentration (0.1 μ M) that did not cause direct venous constriction. UK 14,304 caused a leftward shift in the PE concentration response curve without changing the maximum response. UK 14,304 also caused a decrease in the slope factor of the PE concentration-response curve (see text). Data are mean $\pm$ SEM (n = 5).

Table 1
Properties of NE concentration response curves in arteries and veins from SHAM and DOCA-salt mice in the absence and presence of prazosin

Data are expressed as mean $\pm$ SEM. Numbers in parentheses are the number of animals from which the data were obtained. $E_{\max}$ is the maximum constriction. EC_{50} is the negative logarithm of the agonist concentration producing half maximal constriction.

	$E_{\max}$ (%)		EC_{50} (- log M)	
	ARTERY	VEIN	ARTERY	VEIN
SHAM				
NE (control)	25.7 $\pm$ 3.1 (5)	38.8 $\pm$ 4.7 (8)	5.7 $\pm$ 0.08 (5)	7.2 $\pm$ 0.2 (8)
NE/Prazosin (3 nM)	23.9 $\pm$ 2.9 (4)	32.6 $\pm$ 2.4 (6)	5.1 $\pm$ 0.2* (4)	6.3 $\pm$ 0.3* (6)
NE/Prazosin (30 nM)	24.0 $\pm$ 7.0 (4)	32.0 $\pm$ 3.2 (7)	4.5 $\pm$ 0.1* (4)	6.1 $\pm$ 0.3* (7)
NE/Prazosin (300 nM)	23.4 $\pm$ 2.3 (4)	28.8 $\pm$ 3.0 (7)	3.5 $\pm$ 0.2* (4)	5.7 $\pm$ 0.3* (7)
DOCA-salt				
NE (control)	28.1 $\pm$ 2.9 (5)	33.3 $\pm$ 2.8 (12)	5.8 $\pm$ 0.06 (5)	7.4 $\pm$ 0.2 (12)
NE/Prazosin (3 nM)	19.4 $\pm$ 2.5 (4)	26.2 $\pm$ 3.8 (8)	5.1 $\pm$ 0.09* (4)	6.3 $\pm$ 0.2* (8)
NE/Prazosin (30 nM)	18.6 $\pm$ 3.8 (4)	28.5 $\pm$ 3.0 (7)	4.5 $\pm$ 0.06* (4)	5.8 $\pm$ 0.3* (7)
NE/Prazosin (300 nM)	23.8 $\pm$ 1.3 (4)	29.8 $\pm$ 8.5 (5)	3.7 $\pm$ 0.1* (4)	5.4 $\pm$ 0.2* (5)

* indicates $P < 0.05$ -vs- control.

Table 2
Response of mesenteric arteries and veins from SHAM and DOCA-salt mice to NE in the absence or presence of yohimbine

Data are mean $\pm$ SEM. Numbers in parentheses are the number of animals from which the data were obtained. $E_{\max}$ is the maximum constriction. EC_{50} is the negative logarithm of the agonist concentration producing half maximal constriction.

	$E_{\max}$ (%)		EC_{50} (-log M)	
	ARTERY	VEIN	ARTERY	VEIN
SHAM				
NE (control)	25.7 $\pm$ 3.1 (5)	38.8 $\pm$ 4.7 (8)	5.7 $\pm$ 0.08 (5)	7.2 $\pm$ 0.2 (8)
NE/Yohimbine (3 nM)	26.8 $\pm$ 2.6 (5)	33.9 $\pm$ 2.6 (4)	5.8 $\pm$ 0.05 (5)	6.4 $\pm$ 0.2 * (4)
NE/Yohimbine (30 nM)	20.5 $\pm$ 2.8 (4)	30.4 $\pm$ 4.0 (5)	5.6 $\pm$ 0.05 (4)	5.3 $\pm$ 0.2 * (5)
NE/Yohimbine (300 nM)	28.8 $\pm$ 1.1 (4)	31.1 $\pm$ 3.1 (6)	5.6 $\pm$ 0.06 (4)	5.1 $\pm$ 0.3 * (6)
DOCA-salt				
NE (control)	28.1 $\pm$ 2.9 (5)	33.3 $\pm$ 2.8 (12)	5.8 $\pm$ 0.06 (5)	7.4 $\pm$ 0.2 (12)
NE/Yohimbine (3 nM)	29.5 $\pm$ 5.3 (4)	32.8 $\pm$ 4.5 (4)	5.7 $\pm$ 0.1 (4)	6.4 $\pm$ 0.2 (4)
NE/Yohimbine (30 nM)	33.6 $\pm$ 6.6 (4)	30.4 $\pm$ 2.7 (6)	5.8 $\pm$ 0.1 (4)	6.4 $\pm$ 0.5 (6)
NE/Yohimbine (300 nM)	35.6 $\pm$ 5.1 (4)	26.4 $\pm$ 6.1 (5)	5.7 $\pm$ 0.09 (4)	5.4 $\pm$ 0.7 * (5)

* indicates $P < 0.05$ -vs- control.

Table 3
Response of mesenteric arteries and veins from SHAM and DOCA-salt mice to NE in the absence or presence of rauwolscine and to PE in the absence and presence of yohimbine

Data are mean $\pm$ SEM. Numbers in parentheses are the number of animals from which the data were obtained. $E_{\max}$ is the maximum constriction. EC_{50} is the negative logarithm of the agonist concentration producing half maximal constriction.

	$E_{\max}$ (%)		EC_{50} (-log M)	
	ARTERY	VEIN	ARTERY	VEIN
SHAM				
NE (control)	25.7 $\pm$ 3.1 (5)	40.0 $\pm$ 3.0 (5)	5.7 $\pm$ 0.08 (5)	7.5 $\pm$ 0.2 (5)
NE/Rauwolscine (100 nM)	26.1 $\pm$ 2.5 (6)	28.7 $\pm$ 2.6 * (6)	5.7 $\pm$ 0.08 (6)	7.0 $\pm$ 0.05 * (6)
PE (control)	n.d.	36.5 $\pm$ 2.8 (9)	n.d.	6.5 $\pm$ 0.1 (9)
PE/Yohimbine (30 nM)	n.d.	39.7 $\pm$ 3.1 (9)	n.d.	6.6 $\pm$ 0.1 (9)
DOCA-salt				
NE (control)	28.1 $\pm$ 2.9 (5)	38.9 $\pm$ 1.2 (8)	5.7 $\pm$ 0.07 (5)	7.2 $\pm$ 0.2 (8)
NE/Rauwolscine (100 nM)	25.3 $\pm$ 1.4 (5)	24.7 $\pm$ 4.0 * (7)	5.8 $\pm$ 0.04 (5)	6.7 $\pm$ 0.1 * (7)
PE(control)	n.d.	34.9 $\pm$ 5.4 (6)	n.d.	6.6 $\pm$ 0.1 (6)
PE/Yohimbine (30 nM)	n.d.	42.2 $\pm$ 6.4 (5)	n.d.	6.4 $\pm$ 0.03 (5)

* indicates $P < 0.05$ -vs- control.