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Interaction between α_1- and α_2-adrenoreceptors contributes to enhanced constrictor effects of norepinephrine in mesenteric veins compared to arteries

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Abstract

Mesenteric veins are more sensitive than arteries to the constrictor effects of sympathetic nerve stimulation and α-adrenoceptor agonists. We tested the hypothesis that α_1- and α_2-adrenoceptors interact to enhance adrenergic reactivity of mesenteric veins. We studied neurogenic and agonist-induced constrictions of mesenteric veins and arteries *in vitro*. Norepinephrine concentration-response curves were left-shifted in veins compared to arteries. UK 14,304 (0.01–1 μM, α_2-adrenoceptor receptor agonist) did not constrict arteries or veins but enhanced constriction and Ca^{2+} signals mediated by α_1-adrenoceptor stimulation in veins. Yohimbine (α_2-adrenoceptor receptor antagonist) and MK912 (α_{2C}-adrenoceptor receptor antagonist), but not α_{2A}- or α_{2B}-adrenoceptor antagonists, produced rightward shifts in norepinephrine concentration-response curves in veins. Pharmacological studies revealed that α_{1D}-adrenoceptors mediate venous constrictions. Norepinephrine responses in veins from α_{2C}-adrenoceptor knock-out (KO) mice were not different from wild type veins. Yohimbine inhibited norepinephrine constrictions in α_{2C}-adrenoceptor KO veins suggesting that there is upregulation of other α_2-adrenoceptors in α_{2C}-KO mice. These data indicate that α_{1D}- and α_{2C}-adrenoceptors interact in veins but not arteries. This interaction enhances venous adrenergic reactivity. Mesenteric vein-specific α_2-adrenoceptor linked Ca^{2+} and perhaps other signaling pathways account for enhanced venous adrenergic reactivity.

Keywords

splanchnic circulation; veins; sympathetic nervous system; α-adrenoceptor; intracellular calcium; vasoconstriction

1. Introduction

Sympathetic nerves regulate blood pressure, in part, by regulating arterial and venous tone (Anderson, et al., 1989, Guyenet, 2006). Small arteries are the main determinants of total peripheral resistance while veins are capacitance vessels (Martin et al. 1998). Sympathetic

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nerves are the major regulator of venous tone and capacitance (Pang, 2001) which directly affect venous return to the heart and cardiac output (Guyton, 1955; Greenway and Lautt 1986). Because blood pressure is a product of total peripheral resistance and cardiac output, regulation of venous tone contributes to regulation of arterial pressure.

Veins have not been studied as extensively as arteries in relation to overall hemodynamics and most studies of veins have used large conduit veins (Gavin et al., 1997). The hemodynamic function of large conduit veins differs from that of splanchnic veins (including mesenteric veins) which are important due to their dense sympathetic nerve supply and high compliance (Pang, 2001). These characteristics impact the pathophysiology of hypertension where there is reduced venous capacitance (Ferrario, et al. 1970; Ricksten et al., 1981; London et al., 1985). When mesenteric capacitance is reduced, blood redistributes to the heart (Greenway and Lautt 1986) increasing cardiac output, which occurs in pre-hypertensive humans (Drukteinis and Roman, 2007). These findings point to the relevance of studies of the factors that regulate venous tone (Fink, 2009).

Mesenteric veins are more sensitive to adrenergic stimulation than mesenteric arteries (Hottenstein and Kreulen, 1987; Perez-Rivera et al., 2004, Luo et al., 2003). This might be due to artery-vein differences in α-adrenoceptor expression. α_2-Adrenoceptors potentiate constrictions mediated by α_1-adrenoceptors in mesenteric veins but not arteries (Perez-Rivera et al., 2007) confirming that α_2-adrenoceptors play a more prominent role constriction of veins than arteries (Flavahan et al., 1984, Ruffolo, 1986, Patel et al., 1981). A direct coupling between α_1- and α_2-adrenoceptors may mediate the functional interaction as occurs in the cauda epididymis (Haynes and Hill, 1996) and glial cells (Wilson and Minneman 1991). Interactions between α_1- and α_2 adrenoceptors are common in heterologous receptor expression systems (Reynen et al., 2000).

α-Adrenoceptors are G-protein coupled receptors and signaling via one receptor linked pathway may be affected by inputs from G-protein coupled receptors coupled to other signaling pathways (Milligan et al., 2006). In addition, α-adrenoceptors can form heterodimers (Hague et al., 2004; 2006 Uberti et al., 2005) that have functional properties different from monomeric receptors (Levac et al., 2002; Milligan et al., 2003). For example, α_{1D}-adrenoceptors, which mediate adrenergic constrictions in mesenteric veins (Daniel et al. 1997), can form heterodimers with other adrenoceptor subtypes (Hague et al., 2004, 2006; Uberti et al., 2005).

In the present study we tested the hypothesis that α_{1D}- and α_2-adrenoceptors interact in murine mesenteric veins but not arteries. We used a pharmacological approach to identify the α_2-adrenoceptor subtype that interacts with α_{1D}-adrenoceptors in mesenteric veins. This interaction contributes to the higher adrenergic reactivity of mesenteric veins compared to arteries.

2. Materials and methods

2.1 Animals

C57/Bl6 male mice (25–30g) were purchased from Charles River Breeding Laboratories (Portage, MI). In the animal care facility, mice were maintained according to the standards approved by the Institutional Animal Care and Use Committee at Michigan State University. Mice were housed individually in clear plastic cages with free access to standard chow (Harlan/Teklad 8640 Rodent Diet) and tap water.

Mice heterozygous for the neomycin-disrupted locus coding for the α_{2C}-adrenoceptor (http://jaxmice.jax.org/strain/002512.html) were purchased from Jackson Laboratories (Bar

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Harbor, ME). These mice have been described previously (Link et al., 1995). As the colony expanded, heterozygotes were bred and their offspring (9–12 weeks of age) were used. An established PCR protocol (http://jaxmice.jax.org/strain/003557.html) was followed to identify the genotype of individual pups.

2.2 In vitro preparation of mesenteric arteries and veins

Mice were euthanized with a lethal dose of pentobarbital (50 mg/kg, i.p.), and the small intestine with its associated mesentery was removed and placed in oxygenated (95% O_2, 5% CO_2) Krebs' solution of the following composition (mM): 117, NaCl; 4.7, KCl; 2.5, $CaCl_2$; 1.2, $MgCl_2$; 1.2, NaH_2PO_4; 25 $NaHCO_3$ and 11, glucose. A piece 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 blood vessels were isolated for study by carefully clearing away the surrounding fat. The recording bath containing the preparation was mounted on the stage of an inverted microscope (Olympus CK-2, Optical Analysis Corp. Nashua, NH) 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. The output of a black and white video camera (Hitachi model KP-111; Yokohama, Japan) attached to the microscope was fed to a frame grabber card (Picolo, Euresys Inc., TX, USA) mounted in a personal computer. The video images were analyzed real-time using Diamtrak Edge-tracking software (version 3.5, Diamtrak, Adelaide, Australia), which tracks the distance between the outer edges of blood vessel in the observation field. Changes in vessel diameter as small as 1 μm could be resolved.

2.3 Adrenoceptortor Antagonist studies

After tissue equilibration, adrenoceptor antagonists were applied for 20 minutes before norepinephrine concentration-response curves were generated. Norepinephrine, phenylephrine and UK 14,304 (5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline) were used as adrenoceptor agonists. Propranolol was used to block β-ardenoceptors. BRL44408 (2-[(4,5-Dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole), imiloxan and MK 912 ((2S, 12bS)1′,3′-dimethylspiro(1,3,4,5′,6,6′,7,12b-octahydro-2H-benzo[b]furo[2,3-a]quinolizine)-2,4′-pyrimidin-2′-one) were used as α_{2A}- α_{2B}- and α_{2C}-adrenoceptor antagonists respectively. 5-methylurapadil (5-MU), L-765-314 (benzyl (2S)-4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-2-(tert-butylcarbamoyl)piperazine-1-carboxylate) and BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) were used as α_{1A}-α_{1B}- and α_{1D}-adrenoceptor antagonists respectively. pK_i and pK_B values of these antagonists for α-adrenoceptor subtypes are summarized in Table 1. Previous work has shown that there is good correlation between affinity estimates for these adrenoceptor antagonists obtained in radioligand binding (pK_i) and functional assays (pK_B) (Docherty, 1999). All drugs were purchased from Sigma-Aldrich (St. Louis, MO) and were added in known concentrations to the Krebs' solution. A single adrenoceptor agonist concentration-response curve was obtained from each preparation. To avoid possible desensitization to norepinephrine, separate preparations were used to construct concentration response curves in the absence and presence of α-adrenoceptor antagonists. However, a control curve and a paired curve in the presence of an α-adrenoceptor antagonist were always obtained on the same day using tissues from the same mouse.

2.4 Simultaneous measurement of diameter and intracellular Ca^{2+} in mesenteric veins

Venous smooth muscle cell intracellular Ca^{2+} was measured *in vitro*. Mesenteric veins were cannulated with glass micropipettes and pressurized to 6 mm Hg with no luminal flow, as

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described previously (Burns et al., 2004). Maximal outside diameter was obtained in 0 mM Ca^{2+} solution.

Emitted fluorescence of the ratiometric Ca^{2+}-sensitive dye Fura 2 (Molecular Probes, Invitrogen, Carlsbad, CA) was used as an index of $[Ca^{2+}]_{in}$. Fura 2-AM was added to the bath for 60 min at room temperature to load venous smooth muscle cells in already cannulated blood vessels. After Fura 2-AM was washed out of the vessel chamber, 30 min were allowed for dye de-esterification at 37°C. Fluorescence was measured using a microscope-based photometry system (Photon Technologies International, Birmingham, NJ, USA) as previously described (Burns et al. 2004). Fura-2 was excited with alternating 340 and 380nm wavelength light with a DeltaRam X high-speed multi-wavelength illuminator, and Fura 2 emission was measured at 510nm with a D-104 photomultiplier at 0.8Hz. The illuminator and photometer were mounted on a Nikon TE 300 inverted microscope (Nikon Instruments Inc., Melville, NY, USA) equipped with x20 (N.A. 0.5) and x40 (N.A. 0.75) Plan Fluor long working-distance objectives. FeliX software (Photon Technologies International) was used to control the illuminator and photometer and for data acquisition. After subtracting the background and normalizing the 340/380 ratio representing concentration of Ca^{2+}, changes in global Ca^{2+} upon drug application are presented as % change from baseline. Veins were washed between drug applications until baseline diameter was restored.

2.5 Data analysis

Artery and vein constrictor responses are expressed as percent constriction from the resting diameter. Half-maximal effective α-adrenoceptor agonist concentration (EC_{50}) and maximum response (E_{max}) were calculated from a least-squares fit ofindividual α-adrenoceptor agonist concentration-response curves using the following logistic function (Origin 7.0 (Origin-Lab Corp., Northampton, MA):

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

where E_{min} is the minimum response and was constrained to zero and n is the slope factor. All data are expressed as mean ± S.E.M. Statistical differences between group means for E_{max} and EC_{50} values were assessed by Student's two-tailed unpaired t test. A P value < 0.05 was considered statistically significant.

3. Results

3.1 Norepinephrine is a more potent constrictor of veins compared to arteries

We found that mesenteric veins were more sensitive the constrictor effects of norepinephrine compared to arteries (Fig. 1). The EC_{50} for norepinephrine-induced constriction of veins was 0.02 ± 0.004 μM and for arteries this value was 4.0 ± 0.6 μM ($P < 0.05$, n=6).

As norepinephrine can act at α_1- and α_2- and β-adrenoceptors in blood vessels, we next tested if any of these receptors were responsible for increased venous reactivity to norepinerphrine. The β-adrenoceptor antagonist propranolol did not affect norepinephrine concentration response curve in veins (Fig. 2A). However, yohimbine (0.3 μM), an α_2-adrenoceptor antagonist, caused a 8-fold rightward shift in the norepinephrine concentration response curve in mesenteric veins (Fig. 2B); the control EC_{50} was 0.01 ± 0.002 μM while in the presence of yohimbine this value was 0.08 ± 0.01 μM ($P < 0.05$, n=6). Yohimbine did not affect norepinephrine responses in arteries (Fig. 2C).

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3.2 An α_2-adrenoceptor agonist, UK 14,304 increases Ca^{2+} in mesenteric veins

The data presented above suggest that α_2-adrenoceptors contribute indirectly to norepinephrine-induced constriction of mesenteric veins. In order to begin to probe the mechanism responsible for this facilitation, we simultaneously measured constriction and intracellular Ca^{2+} in veins when low concentrations of the α_1-adrenoceptor agonist, phenylephrine (0.1 μM) and UK 14,304 (0.1 μM) were applied individually and together. When applied individually, phenylephrine and UK 14,304 produced little or no constriction of veins (Fig. 3A). However, combined drug application caused a constriction that was significantly greater than the sum of the individual responses ($P < 0.05$, Fig. 3A). While UK14,304 did not constrict veins, it produced a 13 ± 4% increase in intracellular Ca^{2+} over baseline (Fig. 3B). Phenylephrine increased intracellular Ca^{2+} by 18 ± 1% over baseline. Combined application of phenylephrine and UK 14,304 produced an additive increase in intracellular Ca^{2+} (Fig. 3B).

3.3 α-adrenoceptor subtypes in murine mesenteric veins

We next used a pharmacological approach to identify functional α_1- and α_2-adrenoceptor subtypes in venous smooth muscle. Phenylephrine concentration-response curves were obtained in the absence and presence of α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor antagonists (Fig. 4; Table 1). Only the α_{1D}-adrenoceptor antagonist, BMY 7378, caused a rightward shift in the phenylephrine concentration response curve (Fig. 4C, Table 2).

We next attempted to identify the α_2-adrenoceptor subtype that contributes to norepinephrine-induced constriction of veins using α-adrenoceptor subtype selective antagonists. BRL44408, an α_{2A}-adrenoceptor antagonist (Fig. 5A), and imiloxan, an α_{2B}-adrenoceptor antagonist (Fig. 5B), did not change norepinephrine concentration response curves. Only the α_{2C}-adrenoceptor antagonist, MK 912, caused a significant rightward shift in the norepinephrine concentration response curve (Fig. 5C). The control norepinephrine EC_{50} was 0.007 ± 0.001 μM while in the presence of MK 912 (0.01 μM) this value was 0.04 ± 0.009 μM ($P < 0.05$, n=6).

3.4 Studies in mesenteric veins from α_{2C}-adrenoceptor knockout (KO) mice

To test the hypothesis that α_{2C}-adrenoceptors contribute to norepinephrine-induced constrictions of mesenteric veins, we used tissues from α_{2C}-adrenoceptor KO mice. There were no differences in norepinephrine concentration-response curves in veins from wild type and α_{2C}-adrenoceptor KO mice (Fig. 6A). The E_{max} and EC_{50} values in wild type mesenteric veins were 53 ± 3% and 21 ± 6 nM (n=10) respectively. The E_{max} and EC_{50} values in α_{2C}-adrenoceptor KO mesenteric veins were 52 ± 2% and 29 ± 2 nM (n=14); these values were not different from those in wild type veins ($P > 0.05$). In addition, yohimbine (0.3 μM) produced a 6-fold rightward shift in the norepinephrine concentration-response curve in veins from α_{2C}-adrenoceptor KO mice (Fig. 6B); the control and yohimbine treated EC_{50} values were 44 ± 13 (n=6) and 264 ± 95 (n=5) nM, respectively ($P < 0.05$). This was similar to the rightward shift caused by yohimbine in veins from wild type mice. Yohimbine did not change the E_{max} value (Fig. 6B). To test the possibility that other α_2-adrenoceptor subtypes compensated for the loss of a functional α_{2C}-adrenoceptor, we used veins from the KO mice and tested the effects α_{2A}- and α_{2B}-adrenoceptor antagonists on norepinephrine constrictions. The α_{2B}-adrenoceptor antagonist, imiloxan (1 μM) did not change the norepinephrine concentration response curve, while the α_{2A}-adrenoceptor antagonist, BRL44408 (0.3 μM), reduced the maximum norepinephrine-induced constrictions of veins from α_{2C}-adrenoceptor KO mice (Fig. 6C). The control E_{max} value was 47 ± 3% (n=5) while in the presence of BRL44408 this value was 34 ± 3% (n=8; $P < 0.05$). There were no differences in the norepinephrine EC_{50} value in the absence (44 ± 11 nM) or presence (100 ± 41 nM; $P > 0.05$) of BRL44408.

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4. Discussion

4.1 Indirect contribution of α_2-adrenoceptors to norepinephrine-induced constrictions of mesenteric veins

Mesenteric veins are more sensitive to the constrictor effects of sympathetic nerve stimulation and to norepinephrine than mesenteric arteries (Hottenstein and Kreulen, 1987; Smyth et al., 2000; Luo et al., 2003; Perez Rivera et al., 2004; Park et al., 2007). Therefore, we studied postjunctional mechanisms that could contribute to the enhanced adrenergic reactivity of mesenteric veins *in vitro*. Previous work suggested that α_2-adrenoceptors contribute to norepinephrine-induced constrictions of murine mesenteric veins but not arteries (Perez-Rivera et al., 2007). This was confirmed in the present study where we showed that yohimbine inhibits norepinephrine-mediated constriction of veins but not arteries. However, the α_2-adrenoceptor agonist, UK 14,304, did not constrict mesenteric veins. Although UK 14,304 did not directly constrict veins, it produced a more than additive constriction when co-applied with phenylephrine. This suggests that there is a synergistic interaction between constrictor pathways activated by α_1- and α_2-adrenoceptors in mesenteric veins. Facilitation of α_1-adrenoceptor coupled Ca^{2+} signaling by α_2-adrenoceptors is one mechanism by which this interaction could occur. However, combined application of UK 14,304 and phenylephrine produced only an additive increase in intracellular Ca^{2+} so other mechanisms must account for the synergistic interaction between α_1- and α_2-adrenoceptors in mesenteric veins. It is possible that there is a functional interaction between α_1- and α_2-adrenoceptors in MV as occurs in the cauda epididymis (Haynes and Hill, 1996) and primary glial cell cultures (Wilson and Minneman 1991). Functional interactions between α_1-adrenoceptors and α_2-adrenoceptors occur in heterologous receptor expression systems (Reynen et al., 2000). There may also be artery vs. vein differences in signaling mechanisms downstream from increases in intracellular Ca^{2+}

4.2 α_1- and α_2-adrenoceptor subtypes in mesenteric veins

We used a pharmacological approach to identify the α_1-adrenoceptor subtype mediating α-adrenoceptor agonist induced constriction of mesenteric veins. We found that the α_{1D}-adrenoceptor antagonist, BMY 7378, inhibited constrictions caused by phenylephrine while α_{1A}- and α_{1B}-adrenoceptor antagonist did not affect these responses. We conclude that α_{1D}-adrenoceptors mediate constriction of murine mesenteric veins as shown previously for the canine mesenteric vein (Daniel et al. 1997).

We next identified the α_2-adrenoceptor subtype expressed by mesenteric veins. Previous work showed that the α_{2C}-adrenoceptor contributes to constrictions of the porcine pulmonary vein (Görnemann et al., 2007). Our pharmacological studies using α_2-adrenoceptor subtype selective antagonists indicated that α_{2C}-adrenoceptors contribute to norepinephrine-induced venoconstriction. BRL44408 has 25 and 100-fold higher affinity for the α_{2A}- over the α_{2B}- and α_{2C}-adrenoceptors respectively making this drug useful for identifying responses mediated by α_{2A}-adrenoceptors. However, antagonists for the α_{2B}-adrenoceptor (imiloxan) and the α_{2C}-adrenoceptor (MK912) are only 10–20-fold more selective over other subtypes. This modest selectivity limits their usefulness for receptor identification. Nonetheless, the rightward shift of the norepinephrine concentration-response curve caused by MK912 closely resembles the shift produced by yohimbine. Furthermore, BRL44408 and imiloxan did not change norepinephrine concentration response curves. Taken together, our data and those published previously (Gavin et al., 1997) indicate that α_{2C}-adrenoceptors contribute to norepinephrine-induced constriction of mesenteric veins.

Because of the limited selectivity of imiloxan and MK912, we used α_{2C}-adrenoceptor KO mice to verify the contribution of this receptor to norepinephrine-induced constriction of

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mesenteric veins. We predicted that veins from α_{2C}-adrenoceptor KO mice would be less sensitive to norepinephrine and that yohimbine would not change the norepinephrine concentration response curve. However, veins from the α_{2C}-adrenoceptor KO animals did not exhibit attenuated sensitivity to norepinephrine and yohimbine produced a rightward shift in the concentration response curve in veins from these animals. This finding could be attributed to physiological compensation in the KO mice, where other α_2-adrenoceptor subtypes assume the function of the deleted α_{2C}-adrenoceptor. In our experiments, the α_{2A}-adrenoceptor compensated for the loss of functional α_{2C}-adrenoceptors because in α_{2C}-adrenoceptor KO veins, but not in wild type veins, BRL44408 inhibited norepinephrine responses. There are many examples where gene KO leads to upregulation of other proteins. For example, chloroethylclonidine, an antagonist of the α_{1B}-adrenoceptor, was more effective in inhibiting norepinephrine-mediated constrictions of femoral arteries from α_{1D}-adrenoceptor KO mice compared to arteries from wild type mice. These authors concluded that there was an increased functional role for the α_{1B}-adrenoceptor in the α_{1D}-adrenoceptor KO mice (Zacharia et al., 2005). α_{1D}-Adrenoceptors are the predominant receptor mediating α-adrenoceptor agonist-induced constriction of the carotid artery in mice (Deighan et al., 2005). However, in α_{1D}-adrenoceptor KO mice, α_{1A}-adrenoceptors mediate agonist induced constrictions of the carotid artery, a result that is consistent with compensatory upregulation of α_{1A}-adrenoceptors (Deighan et al., 2005). Another example of physiological compensation in the adrenoceptor family occurs in β_1/β_2-adrenoceptor KO mice that exhibit reduced muscarinic receptor density in the heart (Rohrer et al., 1999). In addition, these mice exhibit an exaggerated hypotensive response to a β_3-adrenoceptor agonist suggesting upregulation of β_3 receptor function in these mice. These studies demonstrate that genetic deletion of one adrenoceptor subtype can lead to upregulation of other adrenoceptors.

4.3 Implications for understanding differential adrenergic reactivity in mesenteric arteries and veins

Our data indicate that differential α_{1D}- and α_2-adrenoceptor expression in mesenteric arteries and veins contributes to increased sensitivity of mesenteric veins to the constrictor effects of norepinephrine. In mesenteric veins, α_2-adrenoceptors link to a Ca^{2+}-dependent signaling pathway which sensitizes mesenteric veins to the constrictor effects caused by α_1-adrenoceptor activation (Crowley et al., 2002).

Previous studies using tissues and cell lines have demonstrated a functional interaction between α_1- and α_2-adrenoceptors (Fukui et al., 2005). For example, direct coupling between endogenously expressed α_1-adrenoceptors and recombinant α_{2A}-adrenoceptors occurs in Chinese hamster lung cells (Reynen et al., 2000). In these studies, norepinephrine did not raise Ca^{2+} in cells only expressing native α_1-adrenoceptors but after transfection with α_{2A}-adrenoceptors, norepinephrine caused an increase in intracellular Ca^{2+} that was blocked by α_1- and α_2-adrenoceptor antagonists. This type of response also occurs in the cauda epidymis of guinea pig where α_2-adrenoceptor activation potentiates Ca^{2+} influx through L-type Ca^{2+} channels when α_1-adrenoceptors are activated simultaneously (Haynes and Hill 1996). α_2-Adrenoceptor activation alone did not cause Ca^{2+} entry. This mechanism is plausible as adrenergic responses of veins require Ca^{2+} influx (Aburto et al., 1993). Therefore, further functional studies in veins and venous smooth muscle cells are needed to identify pathways underlying the functional interaction between α_{1D}- and α_2-adrenoceptors.

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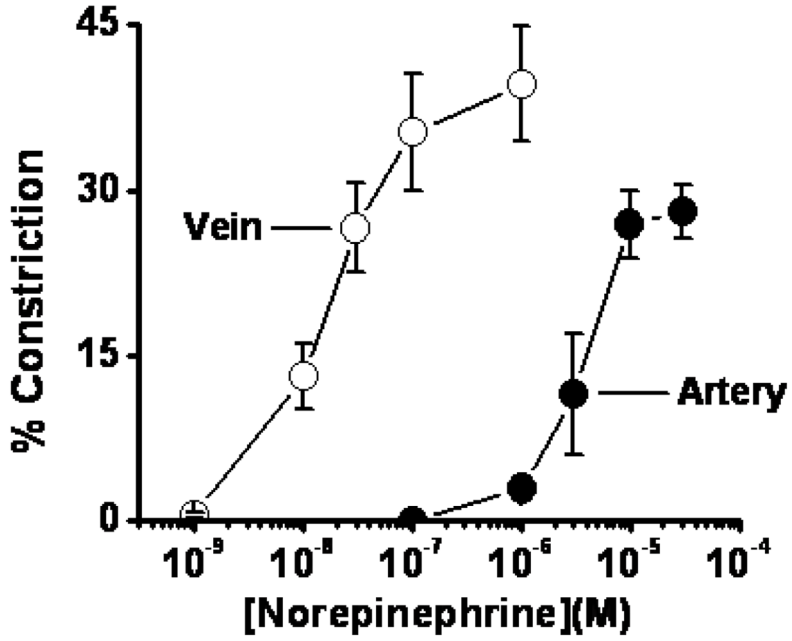

Fig. 1.

Mesenteric veins are more sensitive than mesenteric arteries to the constrictor effects of norepinephrine. The norepinephrine concentration response curve is shifted to the left in mesenteric veins (n=5) relative to arteries (n=6). Data are mean ± S.E.M.

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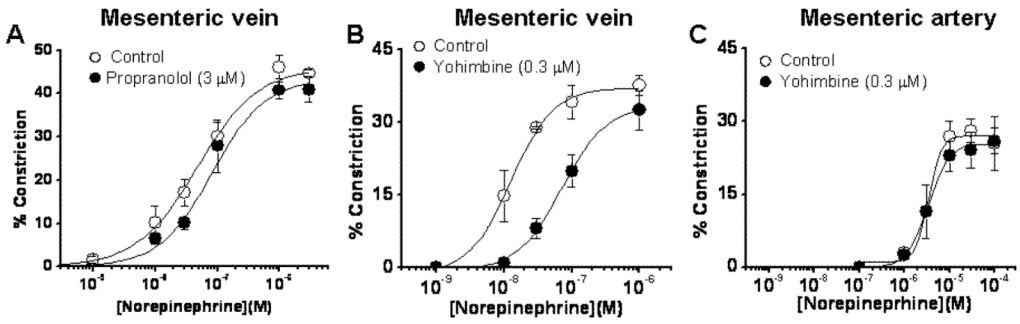

Fig. 2.

α_2-adrenoceptors contribute to norepinephrine induced norepinephrine induced constrictions of mesenteric arteries but not veins. (A) Propranolol, a β-adrenoceptor antagonist, did not alter the norepinephrine concentration response curve in mesenteric veins (n=4). Yohimbine causes a rightward shift in the norepinephrine concentration response curve in mesenteric veins (n=6) (**A**) but not arteries (n=6) (**B**). Data are mean ± S.E.M.

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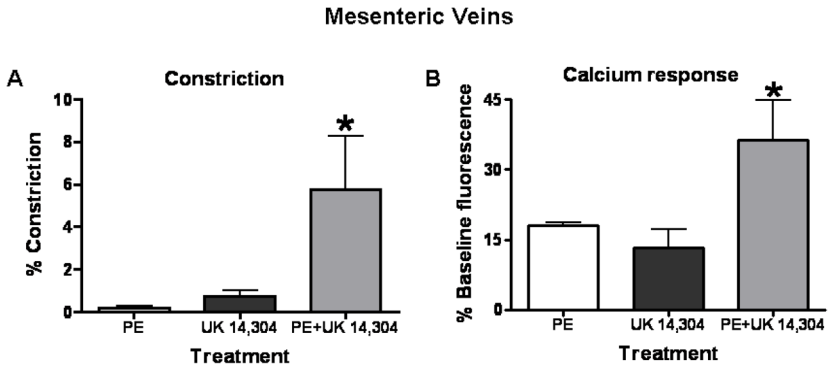

Fig. 3.

UK 14,304 potentiated constrictions of mesenteric veins caused by phenylephrine. (**A**) Low concentrations of UK 14,304 and phenylephrine applied individually caused little venoconstriction. However when the same concentrations were applied simultaneously they caused a more than additive constriction. (**B**) Fura-2 based Ca^{2+} imaging in mesenteric veins revealed, that phenylephrine or UK14,304 applied alone produced modest increases in intracellular Ca^{2+}; agonist co-application caused an additive response. *indicates significantly different from responses caused by individual agonist application ($P < 0.05$, $n = 5$). Data are mean ± S.E.M.

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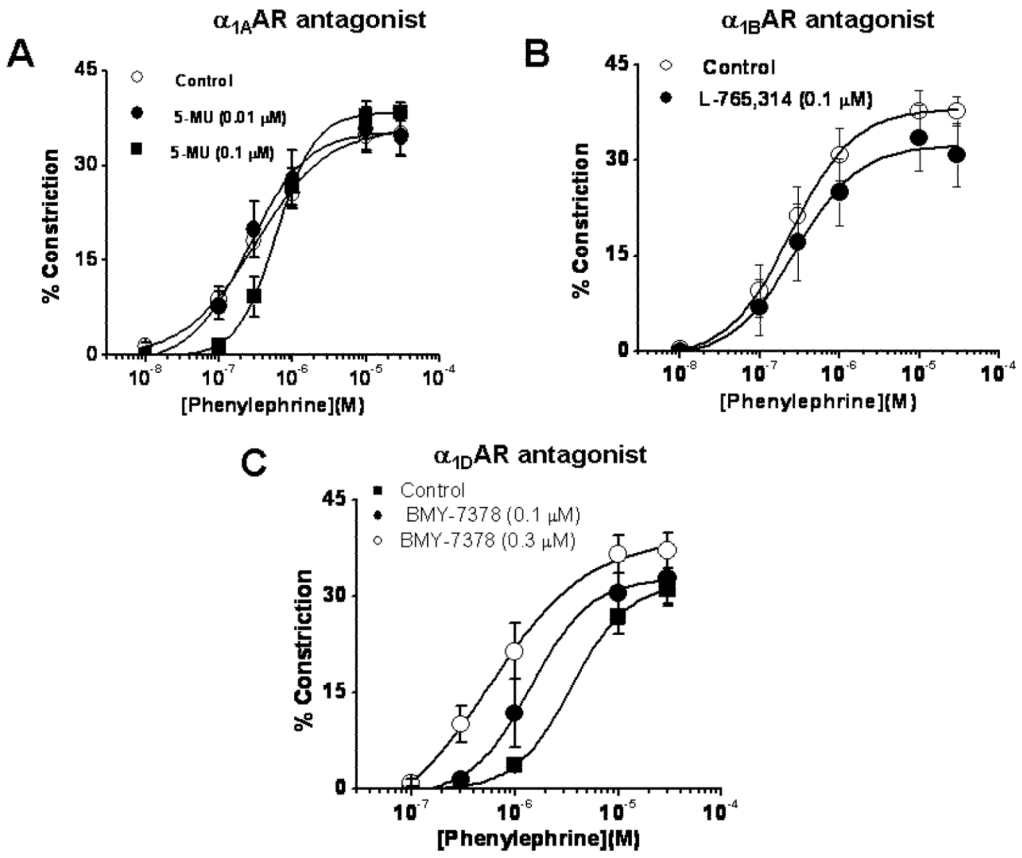

Fig. 4.

α_{1D}-Adrenoceptors mediate phenylephrine-induced constrictions of mesenteric veins. Phenylephrine concentration response curves were unaffected by the α_{1A}-adrenoceptor antagonist 5-methylurapadil (5-MU)(**A**) or the α_{1B}-adrenoceptor antagonist, L-765-314 (**B**). The α_{1D}-adrenoceptor antagonist, BMY-7378 produced rightward shift in the phenylephrine concentration-response curve (**C**). Data are mean ± S.E.M.

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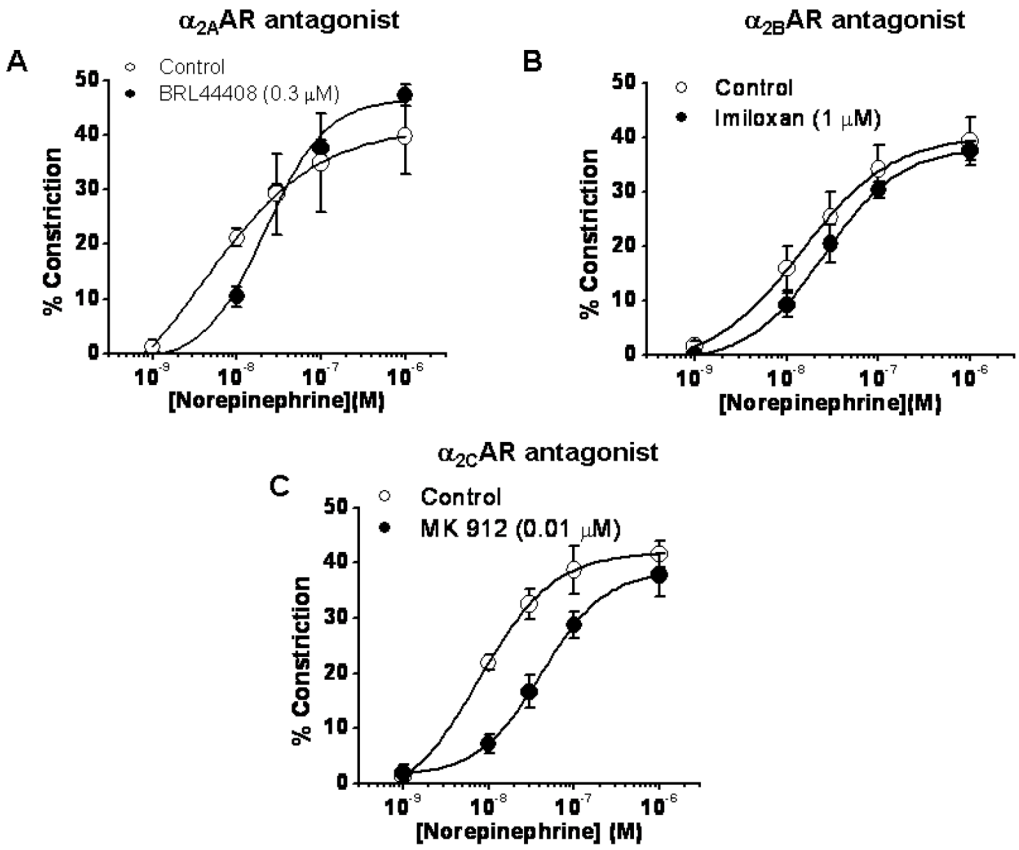

Fig. 5.

α_{2C}-Adrenoceptors contribute to norepinephrine induced constrictions of mesenteric veins. The α_{2A}-adrenoceptor antagonist BRL44408 (**A**) and the α_{2B}-adrenoceptor antagonist imiloxan (**B**) did not change the norepinephrine concentration response curves in mesenteric veins. (**C**) MK912 (α_{2C}-adrenoceptor antagonist) caused a significant rightward shift in the norepinephrine concentration response curve (n=6). Data are mean ± S.E.M.

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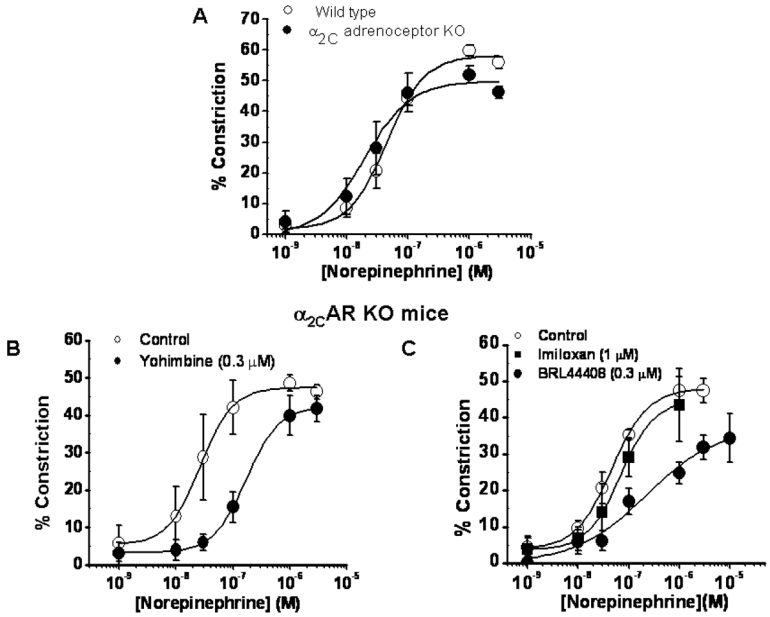

Fig. 6.

Norepinephrine concentration response curves in mesenteric veins from α_{2C}-adrenoceptor KO mice did not differ from curves obtained in veins from wild type mice (**A**). Yohimbine caused a rightward shift in norepinephrine concentration response curve in vein from KO mice (**B**). In mesenteric veins from α_{2C}-adrenoceptor KO mice, the α_{2A}-adrenoceptor antagonist, BRL44408, but not the α_{2B}-adrenoceptor antagonist imiloxan inhibited constrictions caused by norepinephrine (**C**). Data are mean ± S.E.M.

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Table 1

pK_i values determined in radioligand binding assays and pK_B values obtained in functional assays for adrenoceptor (AR) antagonists used to characterize norepinephrine-induced constrictions of mesenteric veins.

Antagonist	pK_i			pK_B		
	α_{2A}-AR	**α_{2B}-AR**	**α_{2C}-AR**	**α_{2A}-AR**	**α_{2B}-AR**	**α_{2C}-AR**
BRL44408	8.2	6.2	6.8	7.8[a]	---	5.7[b]
Imiloxan	5.8	6.9	6.0	---	---	---
MK912	8.9	8.9	10.2	8.9[c]	8.9	10[c] 10.1[d]
	α_{1A}-AR	**α_{1B}-AR**	**α_{1D}-AR**	**α_{1A}-AR**	**α_{1B}-AR**	**α_{1D}-AR**
5-MU	8.8	6.8	7.3	9.2[e], 8.7[g]	7.1[g]	7.9[h]
L 765-314	6.3	8.3	7.3	-----	7.3[i]	-----
BMY 7378	6.6	7.2	9.4	6.7[f], 6.5[g]	7.7[f]	9.0[i]

pKi values obtained from Hussain and Marshall, 1997; Patane et al., 1998. pKB values were obtained where available as follows:

[a] Procine ciliary artery (Wikberg-Matsson and Simonsen, 2001);

[b] Human saphenous vein (Gavin et al., 1997);

[c] guinea pig and rabbit cerbral cortex (Trendelenburg et al., 1996);

[d] Porcine pulmonary vein (Görnemann et al., 2007);

[e] Rat perfused kidney (Blue et al., 1995);

[f] Rat spleen (α_{1B}) and rat vas deferens (α_{1A})(Burt et al., 1995);

[g] Rat portal vein (α_{1A})(Marshall et al., 1996);

[h] Rat aorta (α_{1D})(Hussain and Marshall, 1997);

[i] Rat tail artery (α_{1B})(Jahnichen et al., 2004);

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Table 2

Phenylephrine constriction of mesenteric veins in the absence or presence of antagonists for the α_{1A}-, α_{1B}- and the α_{1D}-adrenoceptors.

	E_{max} (%)	EC_{50} (− log M)
5-methylurapidil (α_{1A}-adrenoceptor antagonist)		
PE (control)	36.5 ± 2.8 (9)	6.5 ± 0.1 (9)
PE/5-MU (0.01 μM)	37.2 ± 3.4 (6)	6.5 ± 0.1 (6)
PE/5-MU (0.1 μM)	40.2 ± 1.4 (8)	6.2 ± 0.1 (8)
L-765,314 (α_{1B}-adrenoceptor antagonist)		
PE (control)	41.6 ± 3.0 (7)	6.5 ± 0.1 (7)
PE/L-765,314 (0.1 μM)	34.3 ± 4.6 (5)	6.4 ± 0.2 (5)
BMY-7378 (α_{1D}-adrenoceptor antagonist)		
PE (control)	37.4 ± 2.7 (11)	6.1 ± 0.1 (11)
PE/BMY-7378 (0.1 μM)	32.7 ± 3.7 (4)	5.8 ± 0.2[a] (4)
PE/BMY-7378 (0.3 μM)	35.9 ± 1.4 (8)	5.3 ± 0.1[a] (8)

Data are mean ± S.E.M. Numbers in parentheses are the number of animals from which data were obtained. E_{max} is the maximum constriction. EC_{50} is the negative logarithm of the molar concentration of agonist producing half maximal constriction.

[a] $P < 0.05$ *vs* control.