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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 constrictions 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 in 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.

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 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 et al., 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 in the 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

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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]Porcine ciliary artery (Wikberg-Matsson and Simonsen, 2001); [b]Human saphenous vein (Gavin et al., 1997) ; [c]Guinea pig and rabbit cerebral 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); and [i]Rat tail artery (α_{1B}) (Jähnichen et al., 2004).

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 and Low, 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 (25–30 g) 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.

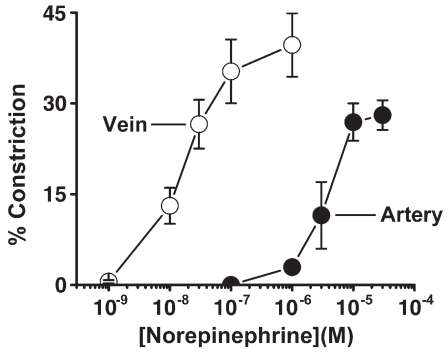

Fig. 1. Mesenteric veins are more sensitive than mesenteric arteries to the constrictor effects of norepinephrine. The norepinephrine concentration–response curve is shifted to the left in mesenteric veins ($n=5$) relative to arteries ($n=6$). Data are mean ± S.E.M.

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 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 (1000 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

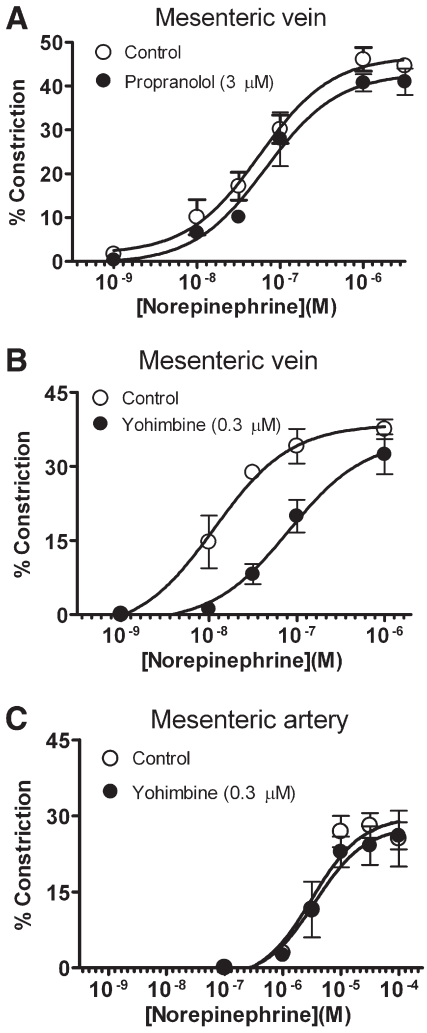

Fig. 2. α_2-Adrenoceptors contribute to norepinephrine-induced constrictions of mesenteric veins but not arteries. (A) Propranolol, a β-adrenoceptor antagonist, did not alter the norepinephrine concentration–response curve in mesenteric veins ($n=4$). Yohimbine causes a rightward shift in the norepinephrine concentration–response curve in mesenteric veins ($n=6$) (A) but not arteries ($n=6$) (B). Data are mean ± S.E.M.