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Differential Distribution of β-Adrenergic Receptor Subtypes in Blood Vessels of Knockout Mice Lacking β_1- or β_2-Adrenergic Receptors

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ABSTRACT

β-Adrenergic receptors (β-AR) are essential regulators of cardiovascular homeostasis. In addition to their prominent function in the heart, β-AR are located on vascular smooth muscle cells, where they mediate vasodilating effects of endogenous catecholamines. In this study, we have investigated in an isometric myograph different types of blood vessels from mice lacking β_1- and/or β_2-adrenergic receptor subtypes (β_1-KO, β_2-KO, $\beta_1\beta_2$-KO). In wild-type mice, isoproterenol induced relaxation of segments from thoracic aorta, carotid, femoral and pulmonary arteries, and portal vein. The relaxant effect of β-receptor stimulation was absent in femoral and pulmonary arteries from β_1-KO mice. In aortic and carotid arteries and in portal veins, the vasodilating effect of isoproterenol was reduced in mice lacking β_1- or β_2-receptors. However, in these vessels the vasodilating effect was only abolished in double KO mice lacking both β_1- and β_2-receptors. Vessel relaxation induced by forskolin did not differ between wild-type and KO mice. Similar contributions of β_1- and β_2-receptors to isoproterenol-induced vasorelaxation were found when vessels from KO mice were compared with wild-type arteries in the presence of subtype-selective β-receptor antagonists. These studies demonstrate that β_1-adrenergic receptors play a dominant role in the murine vascular system to mediate vasodilation. Surprisingly, β_2-receptors contribute to adrenergic vasodilation only in a few major blood vessels, suggesting that differential distribution of β-adrenergic receptor subtypes may play an important role in redirection of tissue perfusion.

β-Adrenergic receptors (β-ARs), members of the G-protein-coupled receptor superfamily, mediate the effects of catecholamines in the sympathetic nervous system. Using techniques of molecular cloning, three distinct β-AR subtypes have been identified (β_1-AR, β_2-AR, β_3-AR) (for reviews, see Benovic et al., 1988; Bylund et al., 1994). One of the important functions of β-ARs is the regulation of blood pressure and vascular smooth muscle tone. Activation of β-ARs in the peripheral vasculature leads to vascular smooth muscle relaxation, which is manifested as a hypotensive blood pressure response in humans and in animals (Allwood et al., 1963). During times of stress, β-AR mediated vascular relaxation may help redirect the cardiac output to tissues that have an increased oxygen demand (Goldenberg et al., 1950).

Based on early pharmacological studies, the β_2-AR was shown to be the major vascular β-AR subtype (Lands et al., 1967). Additional pharmacological studies, however, demonstrated a role for the other β-AR subtypes in the vasculature. Pharmacological experiments in dogs have revealed the presence of β_1-ARs in the vasculature (Taira et al., 1977; Vatner et al., 1985; Nakane et al., 1988). In addition, the rat coronary and mesenteric arteries have been shown to possess functional β_1-ARs (Abdelrahman et al., 1990; Zwaveling et al., 1996). Recent reports also demonstrate that β_3-AR activation can lead to hypotensive responses caused by peripheral vasodilation (Enoksson et al., 1995). Most importantly, one report suggests that also in the human vascular system, β_1-adrenergic receptors may play a dominant role over the β_2-mediated effects (Wellstein et al., 1988).

Further insights into the roles of individual β-AR subtypes in cardiovascular homeostasis have resulted from studies on genetically engineered mice (Rohrer et al., 1996, 1999; Chruscinski et al., 1999). In vivo studies on β_1-AR knockout, β_2-AR knockout, and β_1-/β_2-AR double knockout mice have implicated all three β-AR subtypes in mediating hypotensive responses to exogenous catecholamines. In β_2-AR knockout mice the hypotensive blood pressure response to the β-recep-

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ABBREVIATIONS: β-AR, β-adrenergic receptor; KO, knockout; PG, prostaglandin.

tor agonist isoproterenol was significantly blunted, demonstrating a role for β_2-ARs in mediating vascular relaxation (Chruscinski et al., 1999). The fact that a hypotensive response remained in β_2-AR knockout mice, however, suggests that additional β-AR subtypes can mediate vascular relaxation. In $\beta_1\beta_2$-AR double knockout mice, the hypotensive response to isoproterenol was further attenuated, demonstrating a role for the β_1-AR in mediating vascular relaxation (Rohrer et al., 1999). Residual hypotensive responses to isoproterenol in $\beta_1\beta_2$-AR double knockout mice are presumably caused by β_3-AR activation. Interestingly, hypotensive responses to the β_3-receptor agonist CL316243 were exaggerated in $\beta_1\beta_2$-AR double-knockout mice, suggesting up-regulation of β_3-ARs as part of a compensatory process (Rohrer et al., 1999).

To further define the roles of individual β-AR subtypes in the peripheral vasculature, we have studied β-AR mediated relaxation in isolated blood vessels from the various β-AR knockout models. Using a small vessel myograph, we studied the function of adrenergic receptor subtypes in isolated segments of mouse large conduit arteries, smaller muscular arteries and veins. The results demonstrate that the β_1-adrenergic receptor subtype dominates over the β_2-subtype in mediating vasorelaxation in the murine vasculature.

Materials and Methods

Generation of Knockout Mice. Mice lacking functional β_1- and/or β_2-adrenergic receptors have been generated previously (Rohrer et al., 1996, 1999; Chruscinski et al., 1999). All mice were maintained under specified pathogen-free conditions and animal studies were in accordance with the University and government authorities guidelines. Mice were genotyped by Southern blot analysis as described previously (Rohrer et al., 1996; Chruscinski et al., 1999). β_1-Receptor KO chimeric mice were originally crossed with C57BL/6J $\times$ DBA/2 F_1 hybrid mice (Rohrer et al., 1996), whereas the β_2-receptor deletion was crossed onto an FVB/N background (Chruscinski et al., 1999). Wild-type mice for the present studies were from the C57BL/6J $\times$ DBA/2 background as well as from the inbred C57BL/6J strain. Initial experiments had demonstrated that isoproterenol-induced vasorelaxation did not differ between these and the FVB/N strain.

Myograph Studies. Adult mice (3–6 months old) were sacrificed via cervical dislocation and various vessels were dissected from the animal. Vessels were placed in a physiological salt solution consisting of 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM $MgSO_4$, 1.18 mM KH_2PO_4, 24.9 mM $NaHCO_3$, 10 mM glucose, and 0.03 mM EDTA. Vessels were stored at 4°C before being placed in the myograph. Before mounting, excess connective tissue was dissected away from the vessels. A single tungsten wire (40 μm diameter) was passed through the lumen of the vessel with care taken not to damage the endothelium. This single wire was attached to one of the supports on a computer-controlled, automated myograph (Myo500A; J.P. Trading, Aarhus, Denmark). A second tungsten wire was then passed through the lumen of the vessel and attached to the second support. One of the supports was attached to a drive motor and micrometer, allowing control of movement and measurement of distances. The second support was connected to a force transducer to measure the wall tension developed by the vessel. During the time that the vessel was being mounted, the physiological salt solution described above was present in the myograph bath. The temperature of the bath was maintained at 37°C and 5% CO_2/95% O_2 was bubbled into the salt solution. A computer-assisted normalization protocol was then performed to set the pretension on the vessel. This normalization protocol has been described previously (Mulvany and Halpern, 1976, 1977). Briefly, to determine the length-tension relationship for each vessel, the computer adjusted the support connected to the micrometer. Based on this relationship, it was possible to estimate the diameter (L_{100}) that the vessel would have if it were experiencing a transmural pressure of 100 mm Hg. For vessels that were part of the arterial vascular system, the diameter of the vessel was set to 0.9 $\times$ L_{100}. Because venous and pulmonary pressures are much lower than the pressure of the systemic circulation, vessels studied from these vascular beds were normalized to a transmural pressure of 30 mm Hg.

After the normalization procedure was complete, vessels were challenged with a high-potassium solution (same as physiological salt solution described above but with 42.7 mM NaCl and 80 mM KCl) to determine whether they were viable. Vessels that demonstrated a contraction in response to the high potassium solution were used for further studies. During the last 15 min of the equilibration period, prazosin (final concentration, 0.3 μM) was added to the bath to block the activation of α_1 adrenergic receptors. After the equilibration period, prostaglandin $F_{2\alpha}$ (final concentration, 3 μM) or phenylephrine (final concentration, 10 μM) was then added to the bath to precontract the vessel. Vessels precontracted with phenylephrine were not incubated with prazosin. Increasing concentrations of isoproterenol were then added to the bath to stimulate β-ARs and relax the vessel. In cases in which no relaxation was observed with isoproterenol, forskolin (final concentration 1 μM) was added to the bath to directly stimulate adenylyl cyclase and relax the vessel. For some vessels, β-receptor subtype-selective antagonists were added to

TABLE 1

β-Adrenergic receptor subtypes mediating relaxation of isolated mouse blood vessels. Internal diameter was determined after normalization of wall tension corresponding to an intraluminal pressure of 100 mm Hg (means ± S.E.M., n = 6–9 vessels). Relaxation of precontracted vessels to stimulation of β-ARs by isoproterenol is indicated: Y, yes; N, no.

Blood Vessel	Internal Diameter	Precontracting Agent	Relaxation to Isoproterenol	β-Adrenergic Receptor Subtype
	μm			
Large arteries				
Thoracic aorta	1102 ± 24	$PGF_{2\alpha}$	Y	$\beta_1 > \beta_2$
Pulmonary artery	829 ± 48	Phenylephrine	Y	β_1
Carotid artery	492 ± 22	$PGF_{2\alpha}$	Y	$\beta_1 > \beta_2$
Femoral artery	412 ± 29	Phenylephrine	Y	β_1
Renal artery	411 ± 10	Phenylephrine	N	
Small arteries				
Epigastric artery	162 ± 06	Phenylephrine	N	
Mesenteric artery	143 ± 14	$PGF_{2\alpha}$	Y	β_1
Distal femoral artery	105 ± 13	Phenylephrine	N	
Veins				
Portal vein	1500 ± 34	$PGF_{2\alpha}$	Y	$\beta_1 = \beta_2$
Femoral vein	375 ± 27	$PGF_{2\alpha}$	Y	β_1
Jugular vein	278 ± 34	$PGF_{2\alpha}$	Y	β_1

the organ bath to determine the contribution of β_1- and β_2-receptors to isoproterenol-induced relaxations. For these experiments, 40 nM CGP-20712A (β_1-receptor antagonist) or 14 nM ICI-118,551 (β_2-receptor antagonist) were used to inhibit β_1- or β_2-receptor mediated responses, respectively (Deighton et al., 1992).

Histological Analysis. For histological analysis of the arterial vessels, mice were anesthetized with tribromoethanol (Engelhardt et al., 1999; Hein et al., 1999) and perfused with 4% glutaraldehyde in phosphate-buffered saline (200 ml per mouse) at a pressure of 100 mm Hg through the apex of the left ventricle. For histological investigation, the heart, aorta, kidney, femoral, and mesenteric arteries were embedded in paraffin or in Epon. Cross-sections and longitudinal sections were digitized using a Zeiss IM35 microscope and morphometric analyses were performed with National Institutes of Health Image and Adobe Photoshop software (Adobe Systems, Mountain View, CA).

Statistical Analysis. Data displayed show means ± S.E.M. For all experiments, one- or two-way analysis of variance tests followed by appropriate post hoc tests or *t* tests were used to determine statistical significance ($p < 0.05$) using Prism 2.0 software (GraphPad Software, San Diego, CA).

Results

As part of a survey of β-AR mediated relaxation in the vasculature, three types of vessels were studied with the myograph: large conduit vessels, smaller resistance vessels, and veins. Studies on large conduit vessels included the thoracic aorta, carotid artery, femoral artery, and renal artery as parts of the systemic circulation. The pulmonary artery was included as a large conduit artery from the pulmonary circulation (Table 1). Vascular morphology was unaltered in β-receptor KO mice (Fig. 1) compared with wild-type mice. Morphometric analysis of femoral arteries did not reveal any differences in femoral artery wall diameter or medial smooth muscle cell area between vessels from wild-type mice and β-receptor-deficient animals (not shown), indicating that the deletion of β_1- or β_2-adrenergic receptor subtypes did not affect vascular structure.

As illustrated in Fig. 2, isolated femoral arteries from wild-type and $\beta_1\beta_2$-KO mice showed similar increases in wall tension upon stimulation with 80 mM K^+ or the α_1-receptor agonist phenylephrine. Similarly, maximal vasoconstriction of segments from the thoracic aorta, carotid artery, renal artery, and pulmonary artery did not differ between wild-type and β_1-KO, β_2-KO, or $\beta_1\beta_2$-KO mice (data not shown), indicating that contractile function was not affected by deletion of the β-adrenergic receptor genes. However, deletion of both β_1- and β_2-receptors ($\beta_1\beta_2$-KO) completely abolished the vasodilatory effect of isoproterenol in isolated femoral artery segments (Fig. 2b). The isoproterenol-induced relaxation was independent of the endothelium, because inhibition of NO-release or mechanical disruption of the endothelium did not affect the β-receptor–mediated vasorelaxation (data not shown). Direct activation of adenylyl cyclase by forskolin led to a similar decrease in wall tension in wild-type and in $\beta_1\beta_2$-KO femoral arteries (Fig. 2).

Surprisingly, vascular relaxation of the murine femoral artery was dependent solely on the β_1-receptor subtype (Fig. 3a). Maximal vasorelaxation and the EC_{50} value of isoproterenol did not differ between femoral arteries from wild-type and β_2-KO mice. However, in vessels from β_1-KO or $\beta_1\beta_2$-KO mice, the isoproterenol effect on vascular tone was abolished. A similar predominance of the β_1-subtype was observed in pulmonary artery segments (Fig. 3b). In these vessels, disruption of the β_1-receptor gene completely eliminated the vasorelaxant effect of isoproterenol whereas deletion of the β_2-receptor subtype did not affect β-adrenergic vasodilatation. Interestingly, in some murine blood vessels, β-adrenergic vascular relaxation had both a β_1- and a β_2-receptor component. In the carotid artery, disruption of either β_1- or β_2-receptor subtypes impaired isoproterenol-induced vasorelaxation, which was completely absent only in $\beta_1\beta_2$-KO vessels (Fig. 3c). In wild-type carotid arteries, isoproterenol reduced the vessel tone to a minimum of 49 ± 3% of the tension obtained after precontraction with $PGF_{2\alpha}$. In carotid arteries from knockout mice, isoproterenol decreased wall tension to 60 ± 4% in β_2-KO vessels and to 82 ± 5% in β_1-KO vessels. These results demonstrate that approximately 30% of the maximal β-adrenergic vasorelaxation of the carotid artery was mediated by the β_2-subtype and 70% was mediated by the β_1-receptor. For all vessels investigated, the EC_{50} values

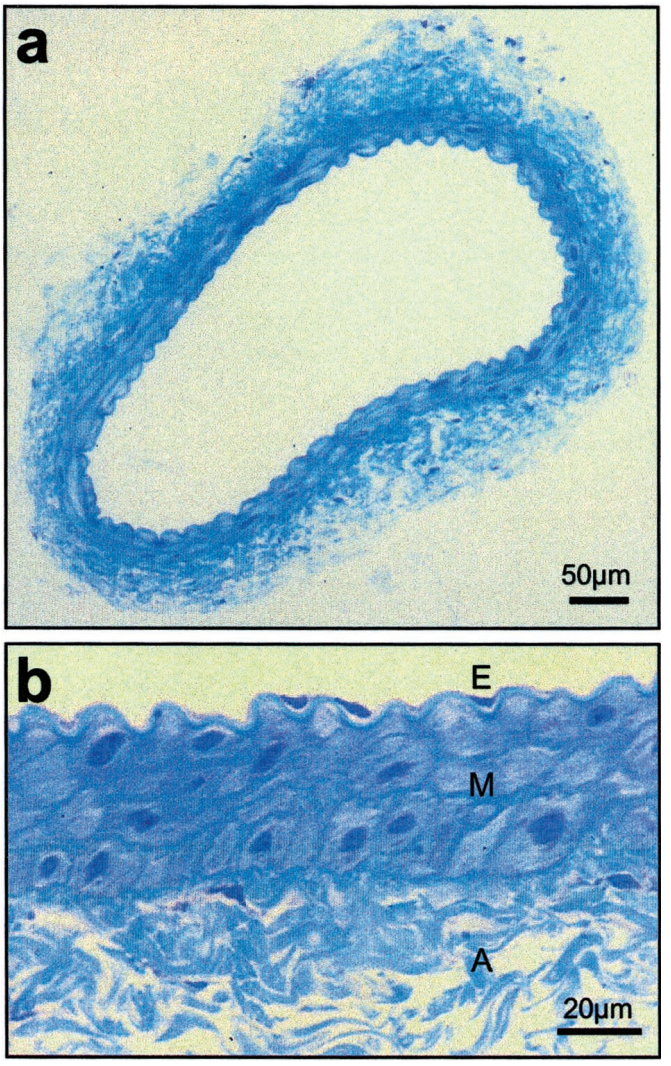

Fig. 1. Cross sections of femoral arteries β_1-receptor-deficient mice. Femoral arteries of 10- to 12-month-old mice were fixed in situ, embedded in plastic, and cut to obtain 0.5-μm cross-sections through the vessels. Overview (a) and larger magnification (b) of cross sections through a β_1-KO femoral artery. Endothelium (E), media smooth muscle cells (M), and adventitia (A) are normally developed and do not show any pathological alterations.

for isoproterenol-induced vasorelaxation were similar between the different genotypes (Table 2).

Several smaller arteries were investigated to determine whether they show a β-adrenergic vasorelaxation, including distal branches of the femoral artery, epigastric, and mesenteric arteries. Of these vessels, isoproterenol caused relaxation only in the mesenteric artery, whereas forskolin was capable of relaxing all of these vessels (data not shown). Vasorelaxation in the mesenteric artery was mediated solely by the β_1-receptor subtype, because the effect of isoproterenol was completely absent in vessels from β_1-KO mice (Table 1).

In addition to the arterial vessels, three types of veins were investigated: the femoral vein, the jugular vein, and the portal vein. In the portal vein, both β-receptor subtypes contributed to inhibition of vascular tone by isoproterenol (Fig. 4). After equilibrating in the organ bath, portal veins from wild-type and knockout mice displayed regular contractions that were enhanced in frequency and amplitude by $PGF_{2\alpha}$. When isoproterenol was added to the bath, the contractions were dramatically reduced in wild-type, β_1-KO, and β_2-KO portal veins (Fig. 4). Contractions in $\beta_1\beta_2$-KO portal veins showed no response to isoproterenol. However, forskolin was capable of relaxing the $\beta_1\beta_2$-KO portal vein (Fig. 4d). Studies on the murine portal vein, thus, suggest that both the β_1-AR and β_2-AR mediate vascular relaxation in this vessel. In wild-type femoral and jugular vein segments, isoproterenol decreased vessel tone by 51 ± 5% and 76 ± 9%, respectively (not shown). β-Adrenergic relaxation of these veins was mediated by the β_1-receptor subtype, as it could be observed in β_2-KO vessels but not in vessels lacking β_1- and β_2-receptors.

Relaxation could be elicited by direct activation of adenylyl cyclase in all blood vessels investigated (Fig. 5). For each vessel type, the degree of forskolin-mediated vascular relaxation did not differ between genotypes, demonstrating that signaling components downstream from the receptor were still functional in single or double β-receptor knockouts (Fig. 5). In contrast with the other large blood vessels, wild-type renal arteries did not display isoproterenol-induced relax-

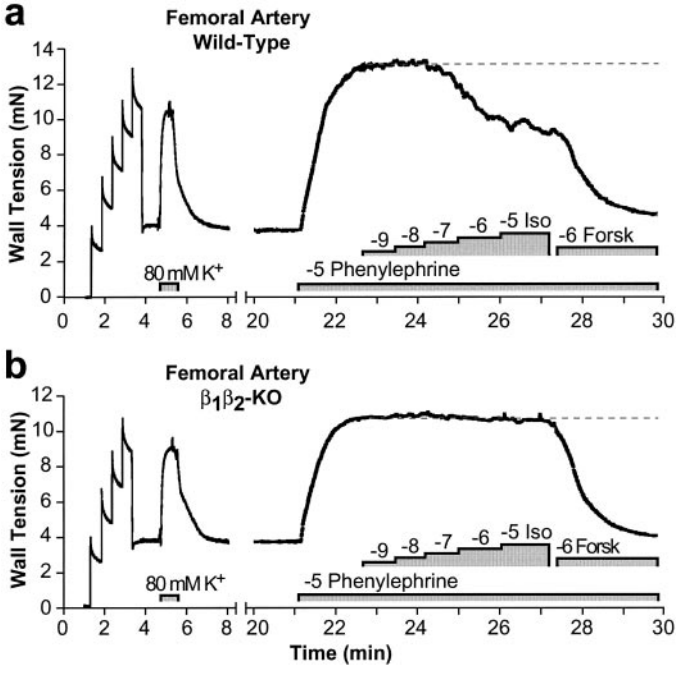

Fig. 2. Original trace recordings of wall tension of murine femoral arteries in a small vessel myograph. Segments of the femoral artery (2 mm long) from wild-type (a) or $\beta_1\beta_2$-KO mice (b) were mounted in a vessel myograph and vessel pretension was adjusted in steps to resemble an intraluminal pressure of 100 mm Hg (stepwise increases in wall tension between 1 and 4 min). Vessels were first contracted with 80 mM K^+, washed, and then contracted by 10^{-5} M phenylephrine. At the contraction plateau, isoproterenol (Iso) was added in increasing concentrations to the organ bath (10^{-9} to 10^{-5} M). Isoproterenol induced relaxation of wild-type but not $\beta_1\beta_2$-KO vessels. Addition of 10^{-6} M forskolin led to complete relaxation of wild-type and $\beta_1\beta_2$-knockout vessels.

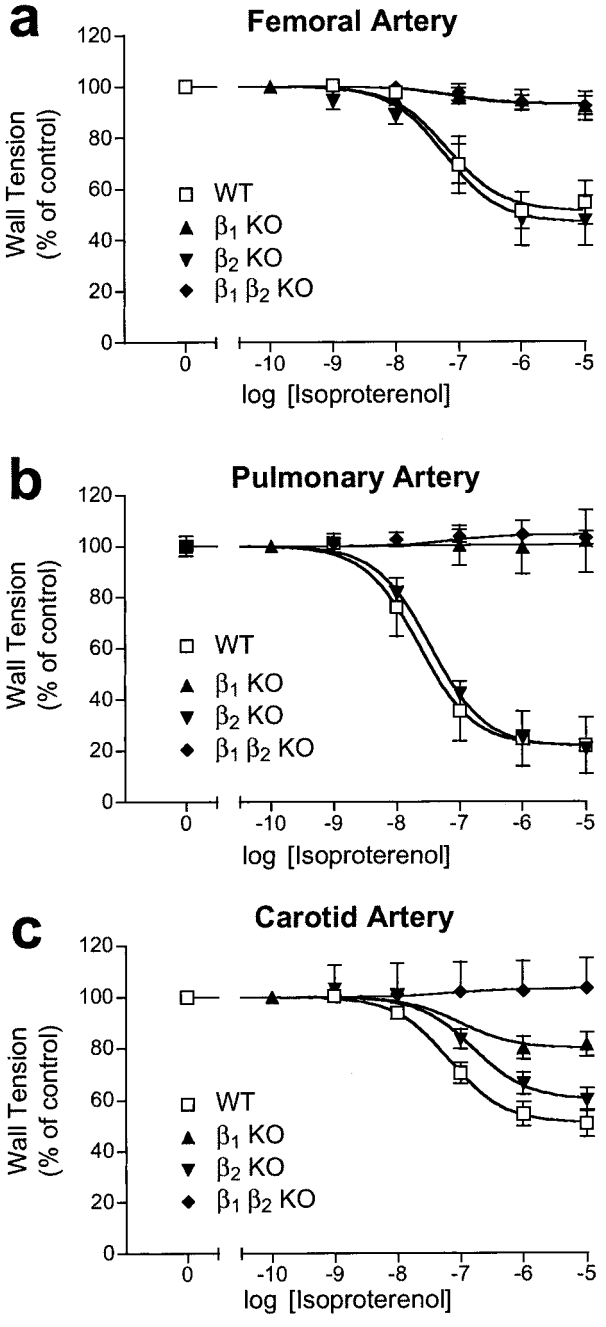

Fig. 3. Vasodilation induced by isoproterenol in femoral arteries (a), pulmonary arteries (b), and carotid arteries (c) from wild-type and β-AR knockout mice. Femoral and pulmonary arteries were precontracted with phenylephrine, carotid artery segments were stimulated with $PGF_{2\alpha}$ before addition of isoproterenol. Data shown are means ± S.E.M. for six to eight vessel segments per genotype.

ation (Fig. 5e). However, renal artery segments from all genotypes did relax when forskolin was added to the bath, even though the extent of relaxation was smaller than in all the other vessels (Fig. 5e).

To determine whether compensatory changes in remaining β-receptor subtypes might influence isoproterenol-induced vasorelaxation in vessels from mice lacking single β-receptor subtypes, we tested β-receptor subtype-selective antagonists in wild-type femoral arteries and in segments of the thoracic aorta (Fig. 6). In the femoral artery, relaxation was mediated solely by the β_1-subtype, both in specimens from KO mice (Fig. 5a) and in vessels with pharmacological inhibition of β-receptor subtypes (Fig. 6, c and d). Similar results were found for the contribution of β_1- and β_2-receptors to vasorelaxation in the aorta (compare Fig. 5d with Fig. 6, a and b). Taken together, these data indicate that there was no functional up-regulation of β-receptors in vessels from mice lacking single β-receptor subtypes.

Discussion

Gene-targeted mouse models have been of great value for understanding the significance of receptor subtypes in vivo (Faraci and Sigmund, 1999). Genetic deletion of individual receptor genes in the mouse allows precise answers about the specific function of individual receptor subtypes at a level that usually cannot be achieved using pharmacological ligands because of the lack of sufficient subtype-selectivity. In this study, we have investigated 11 different blood vessel types from mice lacking β_1- or β_2-adrenergic receptors to identify the receptor subtype(s) responsible for mediating adrenergic vasodilation (Table 1). Surprisingly, the β_1-AR was found to predominate over the β_2-AR as the vasodilating receptor in isolated mouse blood vessels. In most vessel types, including the femoral, pulmonary, and superior mesenteric arteries and femoral and jugular veins, only the β_1-AR caused vasodilation. In large conduit arteries (thoracic aorta, carotid artery) and in the portal vein, β_1- and β_2-AR together mediated adrenergic vasodilation. In vascular segments from double $\beta_1\beta_2$-KO mice, the β-agonist isoproterenol did not cause any relaxation, suggesting that the β_3-AR did not contribute to adrenergic vasodilation in the vessel types investigated. Segments from the aorta of wild-type mice responded to stimulation with K^+, angiotensin II, $PGF_{2\alpha}$, and phenylephrine with a strong vasoconstriction as described before (Russell and Watts, 2000). Vessels from β-AR knockout mice did not differ in their vasoconstriction properties from wild-type control mice. Moreover, genotypes did not differ in vasodilation to activation of vascular adenylyl cyclase, indicating that the genetic modification was specific to the β-adrenergic receptors and did not lead to developmental alterations in vessel structure or contractile function.

However, the results obtained with isolated vascular segments differed from the in vivo experiments with β-AR knockout mice, in which all three β-AR subtypes were shown to mediate isoproterenol-induced hypotension (Rohrer et al., 1999). In mice lacking functional β_1-AR, hypotension after intravenous infusion of isoproterenol was attenuated by 20% compared with wild-type control mice (Rohrer et al., 1996). Isoproterenol-induced hypotension was further reduced by 35% and 71% in β_2-KO and double $\beta_1\beta_2$-KO animals, respectively (Chruscinski et al., 1999; Rohrer et al., 1999). Thus, based on in vivo experiments, all three β-AR subtypes contribute to the hypotensive effect of β-agonists in mice. It seems unlikely that these data are confounded by compensatory changes in the β-AR knockouts (see Fig. 6), even though some evidence suggests that the hypotensive β_3-AR response was enhanced in $\beta_1\beta_2$-KO mice compared with wild-type mice (Rohrer et al., 1999).

In vitro and in vivo experiments differ greatly in the types and sizes of blood vessels that can be investigated. In this study, large conduit arteries with a diameter of approximately 1100 μm and smaller muscular arteries down to a diameter of 140 μm were included. However, this size range covers only half of the total peripheral resistance; the other half is controlled by smaller sized precapillary resistance arteries. There may be a gradient of β-AR subtype distribution from larger to smaller vessels that cannot be investigated entirely with a small vessel myograph. In the feline skeletal muscle circulation, β-adrenergic effects were largely

TABLE 2

Contractile and relaxation parameters of isolated mouse vessel segments from wild-type mice or animals lacking β_1- or β_2-adrenergic receptors. Maximal wall tension was recorded in the presence of phenylephrine (femoral, pulmonary artery) or $PGF_{2\alpha}$ (carotid artery, portal vein). Maximal vessel relaxation by 10 μM isoproterenol (E_{max}) was determined after precontraction by phenylephrine or $PGF_{2\alpha}$. Concentrations of isoproterenol that caused 50% relaxation were determined by nonlinear regression analysis (log EC_{50}). Data shown are means ± S.E.M. for 6 to 10 vessels per genotype.

	Wild-Type	β_1-KO	β_2-KO	$\beta_1\beta_2$-KO
Femoral Artery				
Max. wall tension (mN/mm)	2.1 ± 0.3	1.9 ± 0.2	2.2 ± 0.2	1.8 ± 0.2
Relaxation by Iso (E_{max}, %)	48.8 ± 2.4	7.2 ± 1.4*	53.2 ± 2.8	6.8 ± 0.5*
Isoproterenol log EC_{50}	−7.2 ± 0.1	−7.1 ± 0.2	−7.2 ± 0.1	−7.1 ± 0.3
Pulmonary Artery				
Max. wall tension (mN/mm)	1.7 ± 0.3	1.7 ± 0.3	1.2 ± 0.2	1.3 ± 0.3
Relaxation by Iso (E_{max}, %)	78.1 ± 1.7	−0.6 ± 2.5*	78.5 ± 1.2	−4.6 ± 2.1*
Isoproterenol log EC_{50}	−7.6 ± 0.1	N.D.	−7.4 ± 0.1	N.D.
Carotid Artery				
Max. wall tension (mN/mm)	2.3 ± 0.2	2.0 ± 0.2	2.0 ± 0.1	2.5 ± 0.3
Relaxation by Iso (E_{max}, %)	49.1 ± 0.6	40.0 ± 2.0*	20.0 ± 0.9*	−3.2 ± 0.4*
Isoproterenol log EC_{50}	−7.2 ± 0.1	−6.8 ± 0.4	−7.0 ± 0.2	N.D.
Portal Vein				
Max. wall tension (mN/mm)	0.19 ± 0.02	0.20 ± 0.02	0.21 ± 0.01	0.18 ± 0.02
Relaxation by Iso (E_{max}, %)	77.6 ± 3.4	84.1 ± 2.7	86.1 ± 1.2	−1.5 ± 2.4*
Isoproterenol log EC_{50}	−7.4 ± 0.1	−7.7 ± 0.1	−7.8 ± 0.0	N.D.

N.D., not determined.
* $p < 0.05$ vs. wild type.

confined to the microcirculation, causing dilation of the precapillary sphincters and the small resistance vessels (Lundvall et al., 1982). To test the hypothesis that smaller resistance arterioles contain additional β_2-receptors, alternative methods of measuring tissue perfusion (e.g., microspheres) would be required.

Alternatively, the β-adrenergic hypotension observed in vivo may be caused by venodilation, leading to reduced preload and cardiac output. Indeed, in the mouse portal vein, β_1-AR and β_2-AR contributed equally to the inhibition of venous tone and spontaneous contractions. Furthermore, there was no defect in β-AR mediated vasodilation in femoral and jugular veins from β_2-KO mice, suggesting that β-AR mediated venodilation is intact in β_2-KO mice. Additional factors may influence the difference between receptor subtype contributions observed in vivo and in vitro. In vivo studies usually measure blood flow or resistance of small arteries, whereas in vitro studies have generally examined larger arteries. In addition, potential metabolic and/or blood flow-dependent effects after systemic administration of drugs complicate in vivo experiments.

Based on early pharmacological studies, the β_2-AR has been classified as the smooth muscle β-AR and the β-AR subtype that mediates relaxation in the peripheral vasculature (Lands et al., 1967; Ahlquist, 1976). This concept was largely based on the observation that epinephrine and norepinephrine are essentially equipotent at β_1-AR whereas epinephrine is 10- to 50-fold more potent at the β_2-AR (Lands et al., 1967). Although this hypothesis has been verified in several species, there is also evidence that the other β-AR subtypes (β_1-AR and β_3-AR) can mediate vascular relaxation in humans and in other animal species (for review, see Bülbring and Tomita, 1987). In conscious dogs, administration of norepinephrine or endogenous norepinephrine elicited potent peripheral vasodilation in the presence of α-adrenergic blockade (Vatner et al., 1985). These experiments demonstrate that norepinephrine's vasodilatory action, which is mediated by the β_1-AR, is usually masked by the strong activation of constricting α-AR. β_1-AR contribute significantly to vasodilation in bovine, canine, and rat coronary arteries (Vatner et al., 1984, 1986; Nakane et al., 1988; Abdelrahman et al., 1990; Young et al., 1990), rat superior mesenteric and renal arteries (Taira et al., 1977; Zwaveling et al., 1996), and rat mesenteric and portal veins (Kaumann and Groszmann, 1989).

Similar data exist for β-adrenergic vasodilation in human blood vessels. Precontracted human coronary arteries respond to norepinephrine and to epinephrine and isoproterenol with a pronounced vasodilation, indicating that the β_1-AR is the major vasodilating β-AR subtype in these vessels despite the presence of β_2-AR (Monopoli et al., 1993). In isolated human cerebral arteries, isoproterenol was approximately 1000 times more potent than the β_2-agonist terbutaline in producing relaxation, suggesting that β_1-AR mediate adrenergic vasodilation in human cerebral arteries (Edvins-

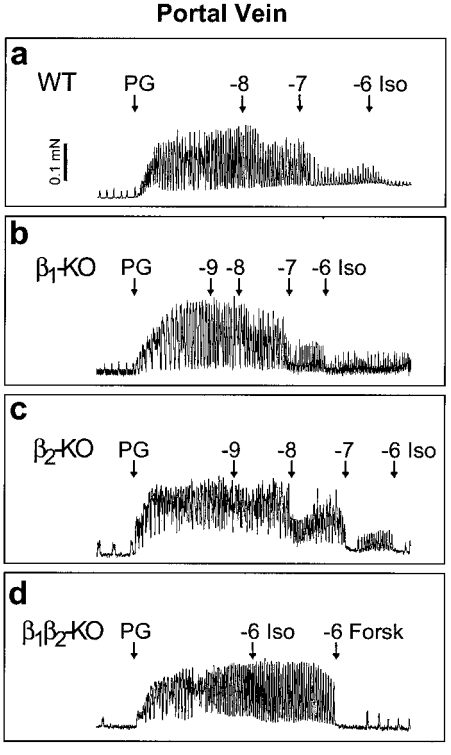

Fig. 4. Effect of isoproterenol on vascular tone and contractions of portal veins from wild-type (a), β_1-KO (b), β_2-KO (c), or $\beta_1\beta_2$-KO mice (d). Portal vein segments were stimulated with $PGF_{2\alpha}$ before addition of isoproterenol (10^{-9} to 10^{-6} M Iso). In $\beta_1\beta_2$-KO portal veins, isoproterenol did not attenuate vascular contractions, but 10^{-6} M forskolin (Forsk) completely inhibited vein contractions (d). Results show trace recordings representative for four to six vessels per genotype.

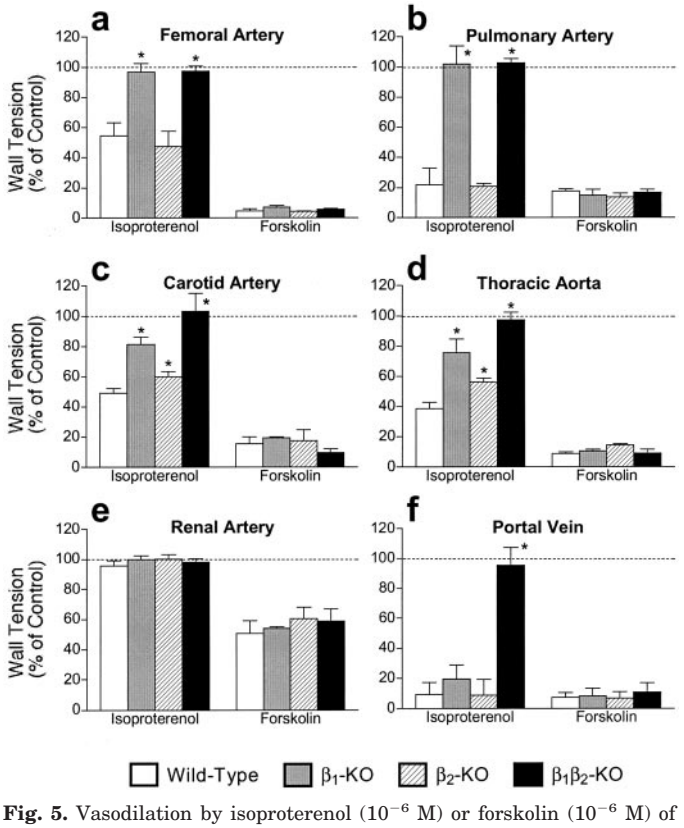

Fig. 5. Vasodilation by isoproterenol (10^{-6} M) or forskolin (10^{-6} M) of femoral (a), pulmonary (b), carotid (c), and renal arteries (e), and thoracic aorta (d) and portal vein segments (f) from wild-type and β-AR knockout mice. In the femoral and pulmonary arteries, isoproterenol-mediated vasodilation was solely mediated by the β_1-AR subtype; in carotid arteries, thoracic aorta, and portal vein, relaxation was induced by activation of both β_1- and β_2-AR. In the renal artery, no β-adrenergic relaxation was observed. All vessels responded similarly to forskolin. Data shown are mean ± S.E.M. for six to eight vessel segments per genotype. *$p < 0.05$ versus wild-type relaxation.

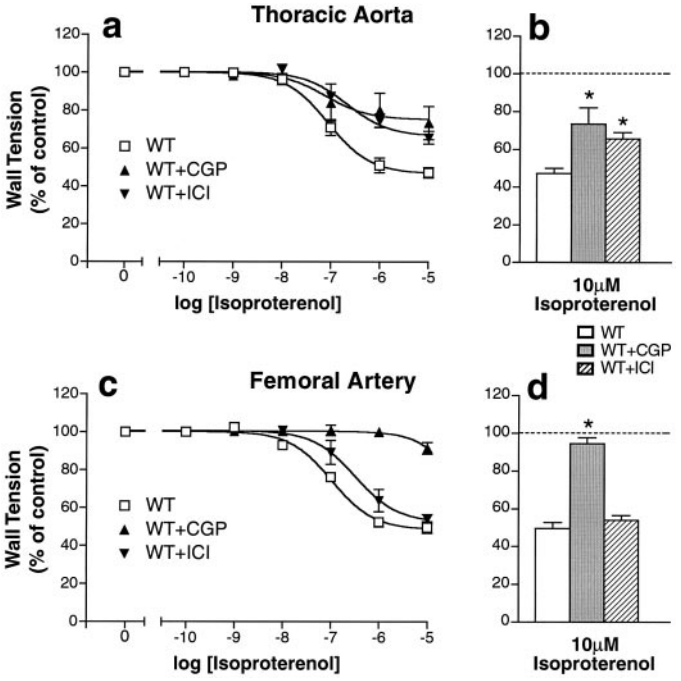

Fig. 6. Vasodilation by isoproterenol of wild-type femoral arteries (a, b) or thoracic aorta segments (c, d) in the presence of β-receptor subtype-selective antagonists. In vessel segments from the aorta, maximal isoproterenol-induced relaxation was reduced by the β_1-receptor antagonist CGP-20712A (40 nM) as well as by the β_2-receptor antagonist, ICI-118,551 (14 nM), indicating that β_1- and β_2-receptors mediate vasodilation in wild-type aorta. In the femoral artery, only the β_1-receptor antagonist, CGP-20712A, inhibited the isoproterenol-induced vasorelaxation. Data shown are mean ± S.E.M. for six to eight vessels per experiment. $^*p < 0.05$ versus control.

son and Owman, 1974). In other human vascular beds, β_2-AR predominate over β_1-AR-mediated vasorelaxation, including internal mammary artery and saphenous vein (Ikezono et al., 1987; Ferro et al., 1993), and arteries supplying abdominal subcutaneous tissue (Blaak et al., 1995; Barbe et al., 1996). However, in the human forearm vasculature and in gastrocnemius muscle, only β_2-AR are responsible for adrenergic vasodilation (Dawes et al., 1997; Hagström-Toft et al., 1998). In vivo, both β_1- and β_2-AR mediate the isoproterenol-induced hypotension in humans. In a thorough in vivo analysis, Wellstein et al. (1988) estimate that 77% of the β-adrenergic hypotension is mediated by the β_1-AR and only 23% is caused by the β_2-receptor. Thus, in humans, the contribution of the β_1-AR to β-adrenergic vasodilation may be even greater than in the mouse. Further studies are required to dissect the physiological and pathophysiological significance of vascular β-adrenoceptor subtype diversity. Genetic polymorphisms of the β_2-AR have been shown to affect blood pressure regulation, vasodilation, and cardiac responses to β-agonists in humans (Gratze et al., 1999; Cockcroft et al., 2000; Brodde et al., 2001; Hein, 2001). The relevance of β_1-AR polymorphisms for vascular regulation has not yet been investigated in humans. These studies suggest that distribution of β_1- and β_2-adrenergic receptor subtypes may play an important role in redirection of tissue perfusion.

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