Differential Distribution of β-Adrenergic Receptor Subtypes in Blood Vessels of Knockout Mice Lacking β₁- or β₂-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 β₁- and/or β₂-adrenergic receptor subtypes (β₁-KO, β₂-KO, β₁β₂-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 β₁-KO mice. In aortic and carotid arteries and in portal veins, the vasodilating effect of isoproterenol was reduced in mice lacking β₁- or β₂-receptors. However, in these vessels the vasodilating effect was only abolished in double KO mice lacking both β₁- and β₂-receptors. Vessel relaxation induced by forskolin did not differ between wild-type and KO mice. Similar contributions of β₁- and β₂-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 β₁-adenrenergic receptors play a dominant role in the murine vascular system to mediate vasodilation. Surprisingly, β₂-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 (β₁-AR, β₂-AR, β₃-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 β₂-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 β₂-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 β₂-ARs (Abdelrahman et al., 1990; Zwaveling et al., 1996). Recent reports also demonstrate that β₂-AR activation can lead to hypotensive responses caused by peripheral vasodilation (Eknoksson et al., 1995). Most importantly, one report suggests that also in the human vascular system, β₁-adrenergic receptors may play a dominant role over the β₂-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 β₁-AR knockout, β₂-AR knockout, and β₁β₂-AR double knockout mice have implicated all three β-AR subtypes in mediating hypotensive responses to exogenous catecholamines. In β₂-AR knockout mice the hypotensive blood pressure response to the β-recept-

ABBREVIATIONS: β-AR, β-adrenergic receptor; KO, knockout; PG, prostaglandin.
tor agonist isoproterenol was significantly blunted, demonstrating a role for β₂-ARs in mediating vascular relaxation (Chruscinski et al., 1999). The fact that a hypotensive response remained in β₂-AR knockout mice, however, suggests that additional β-AR subtypes can mediate vascular relaxation. In β₁β₂-AR double knockout mice, the hypotensive response to isoproterenol was further attenuated, demonstrating a role for the β₁-AR in mediating vascular relaxation (Rohrer et al., 1999). Residual hypotensive responses to isoproterenol in β₁β₂-AR double knockout mice are presumably caused by β₁-AR activation. Interestingly, hypotensive responses to the β₁-receptor agonist CL316243 were exaggerated in β₁β₂-AR double-knockout mice, suggesting up-regulation of β₁-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 β₁-adrenergic receptor subtype dominates over the β₂-subtype in mediating vasorelaxation in the murine vasculature.

Materials and Methods

Generation of Knockout Mice. Mice lacking functional β₁- and/or β₂-adrenergic receptors have been generated previously (Rohrer et al., 1996, 1999; Chruscinski et al., 1999). All mice were maintained under specific 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). β₁-Receptor KO chimeric mice were originally crossed with C57BL/6J × DBA/2 F₁ hybrid mice (Rohrer et al., 1996), whereas the β₂-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 × 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 CaCl₂, 1.18 mM MgSO₄, 1.18 mM KH₂PO₄, 24.9 mM NaHCO₃, 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% CO₂/95% O₂ 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 (L₁₀₀) 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 × L₁₀₀. 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 then added to the bath to block the activation of α₁ adrenergic receptors. After the equilibration period, prostaglandin F₂α (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 adenyl 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.

<table>
<thead>
<tr>
<th>Blood Vessel</th>
<th>Internal Diameter (μm)</th>
<th>Precontracting Agent</th>
<th>Relaxation to Isoproterenol</th>
<th>β-Adrenergic Receptor Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large arteries</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Thoracic aorta</td>
<td>1102 ± 24</td>
<td>PGF₂α</td>
<td>Y</td>
<td>β₁ &gt; β₂</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>829 ± 48</td>
<td>Phenylephrine</td>
<td>Y</td>
<td>β₁</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>492 ± 22</td>
<td>PGF₂α</td>
<td>Y</td>
<td>β₁ &gt; β₂</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>412 ± 29</td>
<td>Phenylephrine</td>
<td>Y</td>
<td>β₁</td>
</tr>
<tr>
<td>Renal artery</td>
<td>411 ± 10</td>
<td>Phenylephrine</td>
<td>N</td>
<td>β₁</td>
</tr>
<tr>
<td>Small arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epigastric artery</td>
<td>162 ± 06</td>
<td>Phenylephrine</td>
<td>N</td>
<td>β₁</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>143 ± 14</td>
<td>PGF₂α</td>
<td>Y</td>
<td>β₁</td>
</tr>
<tr>
<td>Distal femoral artery</td>
<td>105 ± 13</td>
<td>Phenylephrine</td>
<td>N</td>
<td>β₁</td>
</tr>
<tr>
<td>Veins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal vein</td>
<td>1500 ± 34</td>
<td>PGF₂α</td>
<td>Y</td>
<td>β₁ = β₂</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>375 ± 27</td>
<td>PGF₂α</td>
<td>Y</td>
<td>β₁</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>278 ± 34</td>
<td>PGF₂α</td>
<td>Y</td>
<td>β₁</td>
</tr>
</tbody>
</table>
the organ bath to determine the contribution of β₁- and β₂-receptors to isoproterenol-induced relaxations. For these experiments, 40 nM CGP-20712A (β₂-receptor antagonist) or 14 nM ICI-118,551 (β₂-receptor antagonist) were used to inhibit β₁- or β₂-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 β₁- or β₂-adrenergic receptor subtypes did not affect vascular structure.

As illustrated in Fig. 2, isolated femoral arteries from wild-type and β₁β₂-KO mice showed similar increases in wall tension upon stimulation with 80 mM K⁺ or the α₁-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 β₁-KO, β₂-KO, or β₁β₂-KO mice (data not shown), indicating that contractile function was not affected by deletion of the β-adrenergic receptor genes. However, deletion of both β₁- and β₂-receptors (β₁β₂-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 adenyl cyclase by forskolin led to a similar decrease in wall tension in wild-type and in β₁β₂-KO femoral arteries (Fig. 2).

Surprisingly, vascular relaxation of the murine femoral artery was dependent solely on the β₁-receptor subtype (Fig. 3a). Maximal vasorelaxation and the EC₅₀ value of isoproterenol did not differ between femoral arteries from wild-type and β₂-KO mice. However, in vessels from β₁-KO or β₁β₂-KO mice, the isoproterenol effect on vascular tone was abolished. A similar predominance of the β₁-subtype was observed in pulmonary artery segments (Fig. 3b). In these vessels, disruption of the β₁-receptor gene completely eliminated the vasorelaxant effect of isoproterenol whereas deletion of the β₂-receptor subtype did not affect β-adrenergic vasodilatation. Interestingly, in some murine blood vessels, β-adrenergic vascular relaxation had both a β₁- and a β₂-receptor component. In the carotid artery, disruption of either β₁- or β₂-receptor subtypes impaired isoproterenol-induced vasorelaxation, which was completely absent only in β₁β₂-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 PGF₂α. In carotid arteries from knockout mice, isoproterenol decreased wall tension to 60 ± 4% in β₂-KO vessels and to 82 ± 5% in β₁-KO vessels. These results demonstrate that approximately 30% of the maximal β-adrenergic vasorelaxation of the carotid artery was mediated by the β₂-subtype and 70% was mediated by the β₁-receptor. For all vessels investigated, the EC₅₀ values

![Fig. 1. Cross sections of femoral arteries β₁-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 β₂-KO femoral artery. Endothelium (E), media smooth muscle cells (M), and adventitia (A) are normally developed and do not show any pathological alterations.](image-url)
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 β₁-receptor subtype, because the effect of isoproterenol was completely absent in vessels from β₂-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 PGF₂α. When isoproterenol was added to the bath, the contractions were dramatically reduced in wild-type, β₁-KO, and β₂-KO portal veins (Fig. 4). Contractions in β₁β₂-KO portal veins showed no response to isoproterenol. However, forskolin was capable of relaxing the β₁β₂-KO portal vein (Fig. 4d). Studies on the murine portal vein, thus, suggest that both the β₁-AR and β₂-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 β₁-receptor subtype, as it could be observed in β₂-KO vessels but not in vessels lacking β₁ and β₂-receptors.

Relaxation could be elicited by direct activation of adenyl 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 β₁β₂-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 10⁻⁶ M). Isoproterenol induced relaxation of wild-type but not β₁β₂-KO vessels. Addition of 10⁻⁶ M forskolin led to complete relaxation of wild-type and β₁β₂-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 PGF₂α 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 β1-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 β1- and β2-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 β1- or β2-adrenergic receptors to identify the receptor subtype(s) responsible for mediating adrenergic vasodilation (Table 1). Surprisingly, the β1-AR was found to predominate over the β2-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 β1-AR caused vasodilation. In large conduit arteries (thoracic aorta, carotid artery) and in the portal vein, β1- and β2-AR together mediated adrenergic vasodilation. In vascular segments from double β1,β2-KO mice, the β1-agonist isoproterenol did not cause any relaxation, suggesting that the β2-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, PGF2α, 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 adenyl 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 β1-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 β2-KO and double β1β2-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 β2-AR response was enhanced in β1,β2-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 β1- or β2-adrenergic receptors. Maximal wall tension was recorded in the presence of phenylephrine (femoral, pulmonary artery) or PGF2α (carotid artery, portal vein). Maximal vessel relaxation by 10 µM isoproterenol (Emax) was determined after precontraction by phenylephrine or PGF2α. Concentrations of isoproterenol that caused 50% relaxation were determined by nonlinear regression analysis (log EC50). Data shown are means ± S.E.M. for 6 to 10 vessels per genotype.

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th>β1-KO</th>
<th>β2-KO</th>
<th>β1,β2-KO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Femoral Artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. wall tension (mN/mm)</td>
<td>2.1 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Relaxation by Iso (Emax, %)</td>
<td>48.8 ± 2.4</td>
<td>7.2 ± 1.4*</td>
<td>53.2 ± 2.8</td>
<td>6.8 ± 0.5*</td>
</tr>
<tr>
<td>Isoproterenol log EC50</td>
<td>−7.2 ± 0.1</td>
<td>−7.1 ± 0.2</td>
<td>−7.2 ± 0.1</td>
<td>−7.1 ± 0.3</td>
</tr>
<tr>
<td><strong>Pulmonary Artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. wall tension (mN/mm)</td>
<td>1.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Relaxation by Iso (Emax, %)</td>
<td>78.1 ± 1.7</td>
<td>−0.6 ± 2.5*</td>
<td>78.5 ± 1.2</td>
<td>−4.6 ± 2.1*</td>
</tr>
<tr>
<td>Isoproterenol log EC50</td>
<td>−7.6 ± 0.1</td>
<td>N.D.</td>
<td>−7.4 ± 0.1</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>Carotid Artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. wall tension (mN/mm)</td>
<td>2.3 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>2.5 ± 0.3</td>
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<tr>
<td>Relaxation by Iso (Emax, %)</td>
<td>48.1 ± 0.6</td>
<td>40.0 ± 2.0*</td>
<td>20.0 ± 0.9*</td>
<td>−3.2 ± 0.4*</td>
</tr>
<tr>
<td>Isoproterenol log EC50</td>
<td>−7.2 ± 0.1</td>
<td>−6.8 ± 0.4</td>
<td>−7.0 ± 0.2</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>Portal Vein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. wall tension (mN/mm)</td>
<td>0.19 ± 0.02</td>
<td>0.20 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0.18 ± 0.02</td>
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<tr>
<td>Relaxation by Iso (Emax, %)</td>
<td>77.6 ± 3.4</td>
<td>84.1 ± 2.7</td>
<td>86.1 ± 1.2</td>
<td>−1.5 ± 2.4*</td>
</tr>
<tr>
<td>Isoproterenol log EC50</td>
<td>−7.4 ± 0.1</td>
<td>−7.7 ± 0.1</td>
<td>−7.8 ± 0.0</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

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 $\beta_2$-receptors, alternative methods of measuring tissue perfusion (e.g., microspheres) would be required.

Alternatively, the $\beta$-adrenergic hypotension observed in vivo may be caused by venodilation, leading to reduced preload and cardiac output. Indeed, in the mouse portal vein, $\beta_1$-AR and $\beta_2$-AR contributed equally to the inhibition of venous tone and spontaneous contractions. Furthermore, there was no defect in $\beta$-AR mediated vasodilation in femoral and jugular veins from $\beta_2$-KO mice, suggesting that $\beta$-AR mediated venodilation is intact in $\beta_2$-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 $\beta_2$-AR has been classified as the smooth muscle $\beta$-AR and the $\beta$-AR subtype that mediates relaxation in the peripheral vasculature (Land et al., 1967; Ahluquist, 1976). This concept was largely based on the observation that epinephrine and norepinephrine are essentially equipotent at $\beta_1$-AR whereas epinephrine is 10- to 50-fold more potent at the $\beta_2$-AR (Land et al., 1967). Although this hypothesis has been verified in several species, there is also evidence that the other $\beta$-AR subtypes ($\beta_1$-AR and $\beta_2$-AR) can mediate vascular relaxation in humans and in other animal species (for review, see Bülbbring and Tomita, 1987). In conscious dogs, administration of norepinephrine or endogenous norepinephrine elicited potent peripheral vasodilation in the presence of $\alpha$-adrenergic blockade (Vatner et al., 1985). These experiments demonstrate that norepinephrine’s vasodilatory action, which is mediated by the $\beta_2$-AR, is usually masked by the strong activation of constricting $\alpha$-AR. $\beta_1$-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 $\beta$-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 $\beta_1$-AR is the major vasodilating $\beta$-AR subtype in these vessels despite the presence of $\beta_2$-AR (Monopoli et al., 1993). In isolated human cerebral arteries, isoproterenol was approximately 1000 times more potent than the $\beta_2$-agonist terbutaline in producing relaxation, suggesting that $\beta_1$-AR mediate adrenergic vasodilation in human cerebral arteries (Edvins-

![Image](attachment://image.png)

**Fig. 4.** Effect of isoproterenol on vascular tone and contractions of portal veins from wild-type (a), $\beta_1$-KO (b), $\beta_2$-KO (c), or $\beta_1\beta_2$-KO mice (d). Portal vein segments were stimulated with PGF$_2\alpha$ before addition of isoproterenol (10$^{-8}$ 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.

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**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 $\beta$-AR knockout mice. In the femoral and pulmonary arteries, isoproterenol-mediated vasodilation was solely mediated by the $\beta_1$-AR subtype; in carotid arteries, thoracic aorta, and portal vein, relaxation was induced by activation of both $\beta_1$- and $\beta_2$-AR. In the renal artery, no $\beta$-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 isoproteanol 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 β1-receptor antagonist CGP-20712A (40 nM) as well as by the β2-receptor antagonist, ICI-118,551 (14 nM), indicating that β1- and β2-receptors mediate vasodilation in wild-type aorta. In the femoral artery, only the β1-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.

In other human vascular beds, β2-AR predominate over β1-AR-mediated vasorelaxation, including internal mammary artery and saphenous vein (Ikezono et al., 1987; Ferro et al., 1993), and arteries supplying abdominal subcutaneous tissue (Blak et al., 1995; Barbe et al., 1996). However, in the human forearm vasculature and in gastrocnemius muscle, only β2-AR are responsible for adrenergic vasodilation (Dawes et al., 1997; Hagström-Toft et al., 1998). In vivo, both β1- and β2-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 β1-AR and only 23% is caused by the β2-receptor. Thus, in humans, the contribution of the β1-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-adrenergic receptor subtype diversity. Genetic polymorphisms of the β2-AR have been shown to affect blood pressure regulation, vasodilation, and cardiac responses to β-agonists in humans (Grazete et al., 1999; Cockcroft et al., 2000; Brodde et al., 2001; Hein, 2001). The relevance of β1-AR polymorphisms for vascular regulation has not yet been investigated in humans. These studies suggest that distribution of β1- and β2-adrenergic receptor subtypes may play an important role in redirection of tissue perfusion.

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References


Cockcroft JR, Gazis AG, Cross DJ, Wheatley A, Dewar J, Hall IP, and Noon JP (2000) CGP-20712A (40 nM) as well as by the β2-receptor antagonist, ICI-118,551 (14 nM), indicating that β1- and β2-receptors mediate vasodilation in wild-type aorta. In the femoral artery, only the β1-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.


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