Decreased blood pressure response in mice deficient of the \(\alpha_{1b}\)-adrenergic receptor

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ABSTRACT To investigate the functional role of different \(\alpha_{1b}\)-adrenergic receptor (\(\alpha_{1b}\)-AR) subtypes in vivo, we have applied a gene targeting approach to create a mouse model lacking the \(\alpha_{1b}\)-AR (\(\alpha_{1b}^{-/-}\)). Reverse transcription–PCR and ligand binding studies were combined to elucidate the expression of the \(\alpha_{1b}\)-AR subtypes in various tissues of \(\alpha_{1b}^{-/+}\) and \(\alpha_{1b}^{-/-}\) mice. Total \(\alpha_{1b}\)-AR sites were decreased by 98% in liver, 74% in heart, and 42% in cerebral cortex of the \(\alpha_{1b}^{-/-}\) as compared with \(\alpha_{1b}^{-/+}\). Because of the large decrease of \(\alpha_{1b}\)-AR in the heart and the loss of the \(\alpha_{1b}\)-AR mRNA in the aorta of the \(\alpha_{1b}^{-/-}\), the \(\alpha_{1b}\)-AR is a mediator of the blood pressure and the aorta contractile responses induced by \(\alpha_{1b}\) agonists. Our findings provide strong evidence that the \(\alpha_{1b}\)-AR is a mediator of the blood pressure and the aorta contractile responses induced by \(\alpha_{1b}\) agonists. This was demonstrated by the finding that the mean arterial blood pressure response to phenylephrine was decreased by 45% in \(\alpha_{1b}^{-/-}\) as compared with \(\alpha_{1b}^{-/+}\). In addition, phenylephrine-induced contractions of aortic rings also were decreased by 25% in \(\alpha_{1b}^{-/-}\). The \(\alpha_{1b}\)-AR knockout mouse model provides a potentially useful tool to elucidate the functional specificity of different \(\alpha_{1b}\)-AR subtypes, to better understand the effects of adrenergic drugs, and to investigate the multiple mechanisms involved in the control of blood pressure.

The adrenergic receptors (ARs) mediate the physiological effects of the catecholamines epinephrine and norepinephrine by coupling to several of the signaling pathways modulated by G proteins. The AR family includes nine different gene products, three \(\beta\) (\(\beta_{1}, \beta_{2}, \beta_{3}\)), three \(\alpha_{2}\) (\(\alpha_{2a}, \alpha_{2b}, \alpha_{2c}\)), and three \(\alpha_{1}\) (\(\alpha_{1a}, \alpha_{1b}, \alpha_{1d}\)) receptor subtypes. The ARs share similar structural features characterized by the seven-transmembrane domain motif common to other G protein-coupled receptors.

A variety of physiological effects of catecholamines are mediated by the \(\alpha_{1a}\)-AR subtypes, including the control of blood pressure, glycogenolysis, and the contractility of the urinary tract (1). Heterogeneity of the \(\alpha_{1a}\)-AR initially was suggested by various pharmacological studies and confirmed by molecular cloning of three \(\alpha_{1a}\)-AR subtypes, as reviewed in ref. 2. The alignment of the cloned and pharmacologically defined \(\alpha_{1a}\)-AR subtypes has been the object of some controversy recently solved with the contribution of several studies (3, 4).

After the discovery of \(\alpha_{1a}\)-AR heterogeneity, a variety of studies have attempted to assess whether the different \(\alpha_{1a}\)-AR-mediated responses in various organs could be assigned to distinct subtypes that might differ in their signaling and/or regulatory properties. To address this question, the tissue distribution of the mRNA encoding the three \(\alpha_{1a}\)-AR subtypes has been investigated in various species, including humans and rat (3, 4), using Northern blot analysis, reverse transcription–PCR (RT-PCR), or RNase protection assay. The mRNA of different \(\alpha_{1a}\)-AR subtypes has been found in several organs, including brain, heart, liver, kidney, spleen, blood vessels, vas deferens, and prostate. However, the level of expressed mRNA does not necessarily reflect the expression of the receptor protein.

In vivo studies aiming to assess a specificity of the functional responses mediated by distinct \(\alpha_{1a}\)-AR subtypes have been hampered by the fact that the subtype-selective drugs are only moderately selective and might interact with other adrenergic as well as nonadrenergic receptors. Thus, the functional implications of \(\alpha_{1a}\)-AR heterogeneity and their physiological relevance remain largely unknown.

To contribute to the elucidation of the physiological role of the \(\alpha_{1a}\)-AR subtypes in vivo we have used gene targeting to create a mouse model lacking the \(\alpha_{1b}\)-AR. Recently, targeted gene disruption has been increasingly used to elucidate the in vivo functions of several receptors, including some AR subtypes (5–8). The potential functional changes occurring in the knockout mice might allow, on one hand, to assign distinct functions to the receptor that has been deleted, and on the other, to test the functional redundancy among receptor subtypes.

In this study, we describe the cloning and gene targeting of the mouse \(\alpha_{1b}\)-AR as well as the initial functional characterization of the knockout mice lacking this receptor subtype. Our findings identify the \(\alpha_{1b}\)-AR as a mediator of the vascular contractile and blood pressure responses.

MATERIALS AND METHODS

Cloning of the Mouse \(\alpha_{1b}\)-AR gene and cDNA. After the screening of a 129/Sv mouse genomic library (Stratagene) using the hamster \(\alpha_{1b}\)-AR cDNA as a probe, one positive clone was obtained. The HindIII restriction fragment of the genomic clone was subcloned in pBlueScript II SK and sequenced with primers derived from the hamster \(\alpha_{1b}\)-AR cDNA. The genomic clone contained the first exon of the mouse \(\alpha_{1b}\)-AR encoding amino acids 1–316 of the receptor. To obtain the full-length mouse \(\alpha_{1b}\)-AR cDNA, a BALB/c mouse brain cDNA library was screened with the hamster \(\alpha_{1b}\)-AR cDNA as a probe. The single cDNA clone obtained was missing the N terminus. Thus, the expression vector containing the full-
length mouse cDNA was constructed ligating the pRK5 with amino terminal and carboxyl terminal restriction fragments derived from the genomic and cDNA clones, respectively (further information is available upon request). Library screening and DNA sequencing were as described (9).

Gene Targeting. A ~7.5-kb restriction fragment of the mouse α1b-AR genomic clone (Fig. 1) was subcloned into pBlueScript. The ~2.6-kb NcoI-EcoRV restriction fragment including the first exon of the α1b-AR gene was replaced with a 1.6-kb cassette containing the neomycin resistance gene (neo) under the control of the phosphoglycerate kinase promoter, as described in ref. 10. In addition, the 1.8-kb herpes simplex virus thymidine kinase poly(A) cassette was inserted at the XbaI site to obtain the targeting vector α1bNeoTk (Fig. 1). After its linearization with XmnI, the targeting vector contained two regions of homology with the α1b-AR gene: ~0.9 kb and ~2.1 kb of the 5′ and 3′ untranslated sequences flanking the first exon, respectively. The linearized targeting vector was electroporated into 129 (HM-1) embryonic stem cells (ES), de

DNA was screened by Southern analysis, as described. The wild-type and disrupted allele, respectively. One of the two positive ES clones was expanded and microinjected into C57BL/6J mouse blastocysts (10), which then were transferred into pseudopregnant NMRI females. Two of seven chimeric mice that were mated gave rise to germ-line transmission of the hypoxanthine-phosphoribosyl-transferase (11). Phenylephrine (Sigma) and norepinephrine (Sandoz Pharmaceutical). Antagonists REC2739 and 3016 (Recordati, Milan, Italy) were dissolved in 10% polyethylene glycol 400 and administered in a volume of 10 μl.

Blood Pressure Measurement. After cervical incision on mice anaesthetised with halothane (1–2% in oxygen), two catheters (PE-10 tubing) were inserted, one into the right carotid artery for blood pressure measurement and the other into the jugular vein for drug injection (16). The vessels then were ligated, and the catheters were tunnelled subcutaneously to exit at the back of the neck. The skin incision was closed, and mice were allowed to recover for 3 hr. After placing them in Plexiglas tubes for 30 min to partially restrict their movements, the arterial line was connected to a pressure transducer, and mean arterial blood pressure was recorded with a computerized data-acquisition system as described (17). Phenylephrine (Sigma) and norepinephrine (Sigma) were dissolved in saline, and increasing doses were administered in a volume of 100 μl at 15–20 min intervals to allow blood pressure and heart rate to return to baseline values. Angiotensin II from CIBA–Geigy and vasopressin from Sandoz Pharmaceutical. Antagonists REC2739 and 3016 (Recordati, Milan, Italy) were dissolved in 10% polyethylene glycol 400 and administered in a volume of 10 μl.
Aorta Contractility. Aortic rings were prepared as described (18). On the day of the experiment, the mice were weighed and decapitated. The thoracic aorta was excised and placed in cold Krebs–Henseleit bicarbonate buffer (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 25 mM NaHCO\(_3\), 5.6 mM glucose). The aorta was cleaned of adhering perivascular tissue and cut into 2-mm long rings. These rings were suspended in isolated tissue baths filled with 20 ml of the above buffer continuously bubbled with a mixture of 5% CO\(_2\)-95%O\(_2\) (pH 7.37–7.42) at 37°C. One end of the aortic ring was connected to a tissue holder and the other to an isometric force transducer. The signal was transmitted to a Gould (Cleveland) pressure processor and then into a computerized system by Gould’s data acquisition and signal analysis. The analysis of the generated curves was performed by the VIEW II software (Gould), and the sensitivity of the system was 5 ± 1 mg of tension generated. Rings were equilibrated for 90 min in the unstretched condition, and the buffer replaced every 20 min. The length of the smooth muscle was increased stepwise during the equilibration period to adjust passive wall tension to 0.5 g. This tension was found to be optimal in both the \(\alpha_{1}\a\) and \(\alpha_{1}\b\) mice for aorta contraction of 28–35 g mice induced by serotonin (10\(^{-6}\) M). Once basal tension was established, the length of the rings was not modified. Caution was made to avoid endothelial damage whose functional integrity was assessed using 10^{-7} M acetylcholine (results not shown).

RESULTS

Cloning of the Mouse \(\alpha_{1B}\)-AR gene and cDNA. To construct a targeting vector to disrupt the mouse \(\alpha_{1B}\)-AR gene we screened a 129/Sv mouse genomic library and isolated a single genomic clone containing the first exon of the mouse \(\alpha_{1B}\)-AR. The restriction and sequencing analysis of this clone has revealed that the genomic structure of the mouse \(\alpha_{1B}\)-AR gene is similar to that reported for the homologous gene in hamster (9) and humans (19), the bovine \(\alpha_{1A}\)-AR (13), and all three human \(\alpha_{1}\)-AR subtypes (20). Most of the receptor protein from its starting transmembrane domain VI. The 5\'-flanking sequence (750 bp) (sequence deposited in the EMBL database with accession no. Y12738) of the first exon containing the putative promoter and transcription initiation sites displays 85% identity with the homologous region of the human gene (19).

To characterize the pharmacological properties of the mouse \(\alpha_{1B}\)-AR, we cloned its cDNA and expressed it in COS-7 cells. The mouse \(\alpha_{1B}\)-AR cDNA (sequence deposited in the EMBL database with accession no. Y12738) displays 99.3%, 99.3%, and 98.2% identity with its hamster (9), rat (21), and human (19) homologues, respectively. Both the maximal receptor expression and the binding affinities of prazosin, 5-methylurapidil, phenylephrine, and norepinephrine of the mouse \(\alpha_{1B}\)-AR were similar to that in \(\alpha_{1A}\)- and \(\alpha_{1C}\)-AR in various tissues of male \(\alpha_{1B}\)+/+ and \(\alpha_{1B}\)−/− mice. As shown in Fig. 2, in \(\alpha_{1B}\)+/+ mice the \(\alpha_{1B}\)-AR is expressed in all tissues investigated with apparently lower abundance in spleen and adipose tissue, whereas the expression of the \(\alpha_{1B}\)-AR seems uniform. The \(\alpha_{1B}\)-AR also is expressed in all tissues of the \(\alpha_{1B}\)−/+ mice, except in liver. Because our RT-PCR analysis is semiquantitative, the intensity of the signals corresponding to the amplified products can provide only an approximate measure of the abundance of each mRNA transcript. However, the complete lack of amplification of the \(\alpha_{1B}\)-AR mRNA in \(\alpha_{1B}\)−/− mice confirms that the knockout of the \(\alpha_{1B}\)-AR gene was successful. The expression of the \(\alpha_{1D}\)- and \(\alpha_{1A}\)-AR in the \(\alpha_{1B}\)−/− mice appeared similar to that in \(\alpha_{1B}\)+/+ (Fig. 2), suggesting that the inactivation of the \(\alpha_{1B}\)-AR gene does not have any dramatic compensatory effect on the expression of the other \(\alpha\)-AR subtypes.

Adrenergic Pharmacology in \(\alpha_{1B}\)+/+ and \(\alpha_{1B}\)−/−. Saturation binding analysis showed that the \(K_d\) value of the \(\alpha_{1}\)-antagonist \([\text{H}]\)prazosin was ~100 pM in all tissues explored for both \(\alpha_{1B}\)+/+ and \(\alpha_{1B}\)−/− mice (results not shown). On the other hand, receptor density (\(B_{max}\)) was significantly reduced in several tissues of the \(\alpha_{1B}\)−/− mice, except in kidney (Table 1). The loss of \(\alpha_{1B}\)-AR in \(\alpha_{1B}\)−/− mice was 98% in liver, 74% in heart, 42% in cerebral cortex, and 32% in cerebellum. These findings indicate that the inactivation of the \(\alpha_{1B}\)-AR gene results in the lack of the \(\alpha_{1B}\)-AR protein, which is reflected by the decrease of total \(\alpha\)-AR binding sites in various tissues of the \(\alpha_{1B}\)−/− mice. As shown in Table 1, the expression of the \(\alpha_{2}\) and \(\beta\)-AR was not significantly different between the \(\alpha_{1B}\)+/+ and \(\alpha_{1B}\)−/− mice as indicated by ligand binding studies using \([\text{H}]\)RX821002 and \([\text{I}^{25}\text{I}]\)yanodoilin in cerebellum and heart, respectively (the

Fig. 2. RT-PCR analysis of the RNA from different tissues of \(\alpha_{1B}\)+/+ (WT, wild type) and \(\alpha_{1B}\)−/− (KO, knockout) mice. (Left) Ethidium bromide staining of the RT-PCR fragments. The \(\alpha_{1A}\), \(\alpha_{1B}\), and \(\alpha_{1D}\) mRNAs were detected as 650-, 470-, and 450-bp fragments, respectively. RT-PCR analysis was controlled by detection of the 390-bp fragment of the hypoxanthine-phosphoribosyl-transferase message. The DNA size markers (M) are shown on the left. The positive control (C +) indicates the RT-PCR analysis of RNA derived from COS-7 cells expressing each \(\alpha\)-AR subtype. For the negative control (C −), RT-PCR analysis was performed on samples without RNA. (Right) Southern blots of the RT-PCR fragments shown on the left. The specificity of the amplified fragments was assessed using \([\text{I}^{32}\text{P}]\)-labeled probes specific for each receptor subtype (see Materials and Methods).
α2 and β-AR ranged from 150 to 180 and from 30 to 35 fmol/mg of protein, respectively, for both genotypes (results not shown).

To better assess the expression of different α1-AR subtypes, competition binding experiments using 5-methylurapidil were performed in some tissues of α1b+/+ and −/− mice. The affinity of 5-methylurapidil for the α1b-AR subtypes from different species is high (Kd high = 10^−9 M) for the α1a subtypes and low (Kd low = 10^−7 M) for the α1b as well as for the α1d-AR (3). In agreement with these findings, 5-methylurapidil displayed low affinity (Kd = 2 × 10^−7 M) also for the mouse α1b-AR expressed in COS-7 cells (results not shown). Thus, competition binding experiments with 5-methylurapidil should allow us to discriminate the α1b-AR (measured as high-affinity binding sites) from the α1b and α1d-AR (measured together as low-affinity binding sites).

The monophasic low-affinity competition curve of 5-methylurapidil in the liver of the α1b+/+ mice strongly suggests the large prevalence of the α1b-AR in this tissue. This is in agreement with the almost complete loss of α1-AR binding sites in the α1b−/− mice (Table 1). On the other hand, the prevalence of high-affinity binding sites for 5-methylurapidil in the kidney of the α1b+/+ mice suggests that in this tissue the α1b-AR is the most abundant subtype. This also is in agreement with the observation that the α1b−/− mice do not display any significant loss of total receptors in the kidney (Table 1). In both cerebral cortex and cerebellum of the α1b+/+ mice the biphasic competition curves of 5-methylurapidil suggest the coexistence of the α1b-AR with one or both of the other subtypes. In the α1b−/− mice, a selective decrease of the low-affinity sites in cerebral cortex and cerebellum (Table 2) reflects the loss of the α1b-AR with no change in the α1d-AR number. On the other hand, the remaining low-affinity binding sites in both cerebral cortex and cerebellum of the α1b−/− mice (Table 2) might reflect the presence of the α1d-AR in these tissues.

The results of the ligand binding studies are in agreement with the mRNA levels as tested by RT-PCR. The presence of all three α1-AR subtype mRNA transcripts in brain (Fig. 2) agrees with the biphasic displacement curves of 5-methylurapidil in cerebral cortex and with the selective decrease of the low-affinity binding component in the α1b−/− mice (Table 2). However, the abundance of each α1-AR subtype in various tissues could have not been predicted from the results of the RT-PCR. For example, the important loss of α1-AR binding sites in the heart of the α1b−/− mice indicates that in this organ the number of α1b-AR is much greater than predicted from the RT-PCR studies, which detected apparently similar amounts of the three α1-AR mRNA.

**Measurement of Blood Pressure in α1b+/+ and −/− Mice.** The large decrease of α1-AR in the heart and the loss of the α1b-AR mRNA in the aorta of the α1b−/− mice prompted us to compare the cardiovascular regulation in α1b+/+ and −/− mice. Male mice between 12–18 weeks of age were sacrificed and analyzed for their heart weight/body weight ratios, which did not significantly differ between α1b+/+ and −/− mice (mean ± SE of 10 mice: 5.6 ± 0.5 and 5.1 ± 0.1 mg/g for α1b+/+ and −/−, respectively). Under basal conditions, either heart rate and blood pressure values were similar in the two groups of mice (mean ± SE of 20 mice: heart rate 551 ± 25 and 479 ± 20 bpm; mean arterial blood pressure 119.3 ± 6.6 and 118.5 ± 6.4 mmHg for α1b+/+ and −/−, respectively). Increasing doses of phenylephrine progressively increased the blood pressure over basal in both α1b+/+ and −/− mice.

As shown in Fig. 3, the blood pressure response induced by increasing doses of phenylephrine was considerably reduced in the α1b−/− mice. The maximal dose of phenylephrine used increased the blood pressure above basal by 40 mmHg in the α1b+/+, but only by 22 mmHg in the −/−. The effect induced by 2 μg/kg of phenylephrine was almost completely inhibited in mice of both genotypes by the coadministration of two α1-antagonists REC2739 and 3016 (22) administered intravenously at the dose of 10 μg/kg each 30 min before the agonist (results not shown). This supports the notion that the phenylephrine-induced response in vivo is mainly α1b-adrenergic.

Despite the diminished response to phenylephrine in α1b−/− mice, the increase of blood pressure induced by angiotensinII or vasopressin did not differ significantly between the α1b+/+ and −/− mice (Fig. 3). Altogether, these findings provide strong evidence that the decreased blood pressure response in the α1b−/− mice is truly resulting from the knockout of the α1b-AR.

Similarly to what was observed for phenylephrine, the blood pressure response induced by the natural agonist norepineph-

### Table 1. Ligand binding in tissues of α1b+/+ and −/− mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>α1b+/+ Bmax, fmol/mg protein</th>
<th>α1b+/+ % high affinity</th>
<th>α1b−/− Bmax, fmol/mg protein</th>
<th>α1b−/− % high affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>94.3 ± 4.7</td>
<td>42.9 ± 2.1</td>
<td>54.0 ± 3.3*</td>
<td>80.2 ± 3.7*</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>76.7 ± 5.6</td>
<td>60.8 ± 1.2</td>
<td>51.8 ± 3.0*</td>
<td>90.7 ± 1.5*</td>
</tr>
<tr>
<td>Kidney</td>
<td>22.6 ± 1.5</td>
<td>76.2 ± 3.3</td>
<td>21.2 ± 1.2</td>
<td>86.1 ± 4.5*</td>
</tr>
<tr>
<td>Liver</td>
<td>39.4 ± 9.6</td>
<td>0</td>
<td>0.6 ± 0.2*</td>
<td>n.d.</td>
</tr>
<tr>
<td>Heart</td>
<td>4.7 ± 1.1</td>
<td>n.d.</td>
<td>1.2 ± 0.3</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

[1] prazosin binding in saturation and competition experiments was measured as described in Materials and Methods. Receptor number in heart is lower than reported in other studies (26) probably because a crude membrane preparation has been used. The Kd high and Kd low of 5-methylurapidil ranged 0.5–1 and 80–200 nM in various tissues, respectively. The results are the mean ± S.E. of 4–7 independent experiments, n.d., not determined. *, P < 0.05 as compared to α1b+/+ mice in a paired two-tailed t test.

### Table 2. Pharmacological profile of the α1-AR subtypes in tissues of the α1b+/+ and −/− mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>High affinity, fmol/mg protein</th>
<th>Low affinity, fmol/mg protein</th>
<th>High affinity, fmol/mg protein</th>
<th>Low affinity, fmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>40</td>
<td>54</td>
<td>43</td>
<td>11</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>47</td>
<td>30</td>
<td>47</td>
<td>5</td>
</tr>
<tr>
<td>Kidney</td>
<td>17</td>
<td>6</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>39</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

The binding sites (fmol/mg of protein) displaying high- and low-affinity for 5-methylurapidil have been calculated from the results shown in Table 1. n.d., not determined.
blood pressure response in \( \alpha_{1b}^-/- \) mice also was reduced as compared with that observed in the ++/+ mice. However, the reduction in the blood pressure response in \( \alpha_{1b}^-/- \) mice was significant only for the higher doses of norepinephrine tested and overall smaller than that observed for phenylephrine. This might be explained by the fact that the blood pressure response induced by norepinephrine results from multiple mechanisms via the activation of different ARs, whereas the effect of phenylephrine is almost exclusively mediated by the \( \alpha_1 \)AR. Thus, the lack of the \( \alpha_{1b} \)-AR in \( \alpha_{1b}^-/- \) mice has a more pronounced effect on the blood pressure response induced by \( \alpha_1 \)-selective phenylephrine than by norepinephrine.

**Aorta Contractility in \( \alpha_{1b} \)-selective Phenylephrine.**

To assess the functional consequences due to the loss of the \( \alpha_{1b} \)-AR mRNA in the aorta of \( \alpha_{1b}^-/- \) mice, we measured the effect of \( \alpha_1 \)-agonist phenylephrine on the contraction of isolated aortic rings from male \( \alpha_{1b} \)-selective phenylephrine in \( \alpha_{1b}^-/- \) mice. Phenylephrine induced a concentration-dependent contractile response of the aortic rings of \( \alpha_{1b}^-/- \) mice ranging from 42 to 421 mg of tension above basal at 10\(^{-9}\) and 10\(^{-6}\) M phenylephrine, respectively (Fig. 4).

Interestingly, the efficacies of the contractile responses to 10\(^{-9}\) and 10\(^{-6}\) M phenylephrine were significantly reduced of 40% and 28%, respectively, in aortic rings from \( \alpha_{1b}^-/- \) as compared with ++/+ mice. On the other hand, the contractile effect induced by increasing concentrations of serotonin was similar in \( \alpha_{1b} \)-selective phenylephrine in the mouse aorta contractility induced by \( \alpha_{1b}^-/- \) mice (Fig. 4). The effect of 10\(^{-7}\) M phenylephrine was completely inhibited by the \( \alpha_{2} \)-antagonist prazosin (10\(^{-6}\) M) in both \( \alpha_{1b} \)-selective phenylephrine in \( \alpha_{1b}^-/- \) and ++/+ mice (results not shown). In addition, stimulation with the \( \alpha_{2} \)-agonist UK14,304 (10\(^{-6}\) M) did not induce any significant increase in tension, thus excluding the involvement of an \( \alpha_{2} \)-AR in the mouse aorta contractile response (results not shown). An involvement of the \( \alpha_{2} \)-AR in the murine aortic contraction has been recently ruled out using different antagonists (23). Altogether these findings provide strong evidence that the contractile effect of phenylephrine in mouse aorta is \( \alpha_1 \)-adrenergic and that the \( \alpha_{1b} \)-AR contributes to mediate the aortic contractions induced by \( \alpha_1 \)-agonists. This is in agreement with recent findings suggesting that, despite the apparently predominant role of the \( \alpha_{1d} \)-AR in a number of rat vessels, vascular contractility cannot be mediated by a single receptor subtype (24, 25).

**DISCUSSION**

In this study, we describe the targeted inactivation of the mouse \( \alpha_{1b} \)-AR gene and its consequences on the blood pressure response. Our findings provide strong evidence that the \( \alpha_{1b} \)-AR is a mediator of the blood pressure response and of the aorta contractility induced by \( \alpha_1 \)-agonists. This work contributes to unravel the functional role in vivo of different \( \alpha_1 \)-AR subtypes for which a knockout has not been described so far.

**Cardiovascular Implications of the \( \alpha_{1b} \)-AR Knockout.**

Clinical efficacy of \( \alpha_1 \)-agonists as antihypertensive drugs reflects the important physiological role of \( \alpha_1 \)-ARs in vascular function and in the maintenance of arterial blood pressure. Thus, this study was exclusively focused on the functional characterization of the \( \alpha_{1b} \)-AR knockout model on the in vivo blood pressure response and in vitro vascular contractility.

The fact that the increase of the mean arterial blood pressure induced by phenylephrine is reduced by \( \sim 45\% \) in \( \alpha_{1b} \)-selective phenylephrine in \( \alpha_{1b}^-/- \) as compared with ++/+ mice suggests that the \( \alpha_{1b} \)-AR can mediate a large portion of the vasopressor response to \( \alpha_1 \)-agonists. The blood pressure response mediated by the \( \alpha_{1b} \)-AR might involve, at least in part, its effect on the control of the vascular tone. This is supported by the observation that in \( \alpha_{1b} \)-selective phenylephrine-induced contractions also was reduced as compared with the ++/+ mice. Future studies will attempt to investigate the role of the \( \alpha_{1b} \)-AR in the contractility of other small resistance mouse vessels that might be more directly involved in the regulation of blood pressure.

The role of the \( \alpha_{1b} \)-AR in the control of the vascular tone was not clearly anticipated by previous pharmacological studies. In vivo studies on blood pressure responses in various species have been difficult to interpret because the available pharmacological agents have limited selectivity for a single \( \alpha_1 \)-AR subtype and some of them have additional properties. Recent studies suggest a predominant role of the \( \alpha_{1d} \)-AR in the vascular contractions induced by \( \alpha_1 \)-agonists in the rat (24). However, the profile of the \( \alpha_{1d} \)-selective antagonist BMY7735 in antagonizing norepinephrine-induced contractions does not suggest competitive antagonism at a single receptor (25). Thus, even in isolated vessels the results deriving from the use of selective antagonists might be difficult to interpret. Therefore, the \( \alpha_{1b} \)-AR knockout represents an useful model to further investigate the properties of \( \alpha_{1d} \)- and \( \alpha_{1d} \)-selective drugs in an \( \alpha_{1b} \)-lacking background.

The important decrease of \( \alpha_1 \)-AR binding sites in the heart of the \( \alpha_{1b}^-/- \) mice (Table 1) suggests that the \( \alpha_{1b} \)-AR plays an...
important functional role in cardiac function. The observations that the heart weight/body weight ratios as well as the basal values of both heart rate and mean arterial blood pressure do not differ between 1b/+/+ and −/− mice seem to exclude a major role of the 1-AR in the normal development and function of the heart. However, the role of the 1-AR in catecholamine-induced increase of cardiac inotropy remains to be investigated with direct measurements of the heart contractile function in 1b/+/+ and −/− mice. At present, we cannot exclude that the role of the 1-AR in mediating the blood pressure response to phenylephrine involves both vascular and cardiac mechanisms. Interestingly, overexpression of a constitutively active 1-AR mutant in the heart of transgenic mice resulted in cardiac hypertrophy with increased heart weight/body weight ratios (26). These findings suggest that the 1-AR can activate biochemical mechanisms that contribute to the development of cardiac hypertrophy and related cardiac diseases. This also is supported by in vitro studies on cardiomyocyte systems showing that Gq-coupled receptors may mediate cardiac hypertrophy (27, 28). In future studies, it might be interesting to investigate whether the inactivation of the 1-AR can protect mice from the development of the cardiac hypertrophy induced by pressure overload or other conditions.

Although our data clearly demonstrate that the 1-AR can participate in the regulation of vasoconstriction and hence blood pressure, we did not observe alterations of basal blood pressure in the knockout mice. This apparent discrepancy may be explained by several mechanisms. First, nonadrenergic mechanisms, e.g., the renin-angiotensin or nitric oxide systems, may compensate for the potential decrease of a tonic response. Second, it should be considered that our blood pressure experiments were performed in conscious mice, i.e., most likely under conditions of low sympathetic tone. Under these experimental conditions, the contribution of any AR to blood pressure maintenance will be underestimated by measurements of basal blood pressure. Future studies with hemodynamic stress models may be more informative in this respect.

The finding that 1-AR knockout mice displayed a decreased blood pressure response to 1 agonists was not expected because of the potential compensatory effects of the other 1-AR subtypes. Our results suggest that, despite the presence of multiple 1-AR subtypes in the same tissue, only partial functional redundancy is at the cardiovascular level, i.e., multiple 1-AR subtypes can mediate the vasopressor response, but cannot compensate each other. This has important implications for better understanding the cardiovascular effects of drugs acting at the 1-AR and for more precisely defining the goals linked to the development of 1-AR subtype-selective ligands.

Conclusions. In this study, our characterization of the 1b-AR knockout model has focused on the cardiovascular system. Future studies will extend the functional investigation to other organs of the knockout mice in which the 1b-AR normally is expressed. The 1b-AR knockout model provides a useful tool to elucidate the functional specificity of different 1-AR subtypes and to further elucidate the pharmacological effects of adrenergic drugs. A full understanding of the functional implications of AR heterogeneity awaits the knockout of all AR subtypes and the intercross among these different knockout models.

This study also might provide a contribution to the investigation of the mechanisms involved in the control of blood pressure and its regulation. Human hypertension is caused by the interplay of several “risk” genes and environmental factors. An important line of investigation in hypertension concerns the mechanisms of integration among different homeostatic systems, including the sympathoadrenomedullary, the renin-angiotensin, the nitric oxide as well as those involved in the control of sodium balance. It could be envisaged that intercrosses between the 1b-AR knockout and other mouse models mutated in various genes potentially involved in the control of blood pressure might contribute to investigate the role of the sympathoadrenomedullary system in hypertension.

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