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# Targeted disruption of the mouse β1-adrenergic receptor gene: Developmental and cardiovascular effects

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**ABSTRACT** **At least three distinct beta-adrenergic receptor (β-AR) subtypes exist in mammals. These receptors modulate a wide variety of processes, from development and behavior, to cardiac function, metabolism, and smooth muscle tone. To understand the roles that individual β-AR subtypes play in these processes, we have used the technique of gene targeting to create homozygous β1-AR null mutants (β1-AR −/−) in mice. The majority of β1-AR −/− mice die prenatally, and the penetrance of lethality shows strain dependence. β1-AR −/− mice that do survive to adulthood appear normal, but lack the chronotropic and inotropic responses seen in wild-type mice when β-AR agonists such as isoproterenol are administered. Moreover, this lack of responsiveness is accompanied by markedly reduced stimulation of adenylate cyclase in cardiac membranes from β1-AR −/− mice. These findings occur despite persistent cardiac β2-AR expression, demonstrating the importance of β1-ARs for proper mouse development and cardiac function, while highlighting functional differences between β-AR subtypes.**

Three distinct beta adrenergic receptor (β-AR) subtypes (β1, β2, and β3) have been defined by molecular cloning (1–3). All three β-ARs belong to the G-protein coupled receptor superfamily (4), and all three couple through the stimulatory G-protein ($G_s$) to activate adenylate cyclase in target tissues following activation by catecholamines (1–3). Traditionally, individual β-AR subtypes have been defined pharmacologically using subtype selective compounds, which are not absolute in their selectivity. The inability to pharmacologically isolate individual subtypes, especially in tissues where more than one subtype is expressed, makes it difficult to assign discrete functions to specific β-AR subtypes *in vivo*.

The heart exemplifies a tissue in which the functional contributions of individual β-AR subtypes are difficult to assess. Although β1-ARs predominate over β2-ARs in ventricular and atrial myocardium (5–7), β2-ARs appear to couple more efficiently to adenylate cyclase (8, 9), which may explain why this subtype contributes preferentially to cardiac cAMP production at submaximal agonist concentrations in humans (10). Although β-AR agonists are known to increase heart rate and contractility (11), there is controversy over the β-AR subtype(s) mediating these responses. Many studies have concluded that both β1- and β2-ARs are coupled to the tachycardic and inotropic effect of β-AR agonists (5, 12, 13), whereas others invoke a selective β1-AR involvement (14, 15).

The technique of targeted gene disruption is a powerful tool for examining the functional roles of β-AR subtypes. The technique not only allows one to assign functions to individual subtypes, but also serves as a test for functional redundancy between subtypes. We have observed that disruption of the β1-AR gene in mice results in impaired cardiac performance and significant prenatal lethality. These findings occur despite the apparently normal expression of β2-ARs, demonstrating that β1- and β2-AR functions are distinguishable.

## MATERIALS AND METHODS

**Gene Targeting.** A 1364-bp *Sac*II–*Sac*I fragment (−119 to +1245, relative to AUG initiation codon, containing all but the 3′ 153 bp of β1-AR coding sequence) was removed from a 6.4-kbp *Eco*RI–*Xba*I fragment of the mouse β1-AR gene (16) and replaced with a 1672-bp neomycin (neo) resistance gene cassette (17). This insertion leaves approximately 3.5 kbp of 5′ β1-AR flanking sequence, and a shorter 1.5 kbp arm of 3′ flanking sequence. A 2766-bp herpes simplex virus thymidine kinase cassette (17) was inserted downstream of the 1.5 kbp short arm. Homologous recombination resulting in a double-crossover event between the targeting construct and the endogenous β1-AR gene as diagrammed (Fig. 1) generates a β1-AR locus containing neo, but not thymidine kinase, and such targeted cells are G418- and gancyclovir-resistant by virtue of the neo inclusion and thymidine kinase exclusion, respectively (Fig. 1*A*). The wild-type β1-AR allele generates an ≈8-kbp *Eco*RI fragment visualized with either the downstream or coding probes, whereas the disrupted β1-AR gene generates an ≈5-kbp *Eco*RI fragment seen only with the downstream probe. Targeting construct (40 μg) linearized at a unique *Not*I site was electroporated into the R1 embryonic stem (ES) cell line (18) followed by selection in G418 and gancyclovir. This selection resulted in 300 doubly resistant ES cell colonies, 7 of which had a targeted insertion of PGK-neo in the β1-AR gene (2.3%). Targeted ES cell clones were used for microinjection of blastocyst-stage C57BL/6J mouse embryos, followed by uterine transfer into pseudopregnant CD-1 females. This generated seven chimeric mice based on coat color. Male chimeras were then mated to C57BL/6J × DBA/2 $F_1$ hybrids, and evidence of germ-line transmission monitored by agouti coat color contributed from the 129Sv-derived R1 ES cell genome (18). To obtain the β1-AR gene disruption on the 129Sv strain background, chimeric males were mated with wild-type 129Sv females.

**Western Blotting.** Ventricular protein homogenates were prepared by polytron homogenization in 5 mM Tris·HCl, 5 mM EDTA (pH 7.4), followed by centrifugation at 200 × *g*. The resultant supernatant was centrifuged at 30,000 × *g*, and the pellet from this step was resuspended in 1× binding buffer (75 mM Tris·HCl/12.5 mM $MgCl_2$/1 mM EDTA, pH 7.4) and protein concentration determined. Ventricular membrane protein (80 or 160 μg) was electrophoresed on SDS/9%

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Abbreviations: β-AR, beta-adrenergic; ES, embryonic stem; NE, norepinephrine; E, embryonic day; PN, postnatal day; $^{125}$I-CYP, $^{125}$I-iodocyanopindolol; TH, tyrosine hydroxylase; DHPG, 3,4-dihydroxyphenylglycol.

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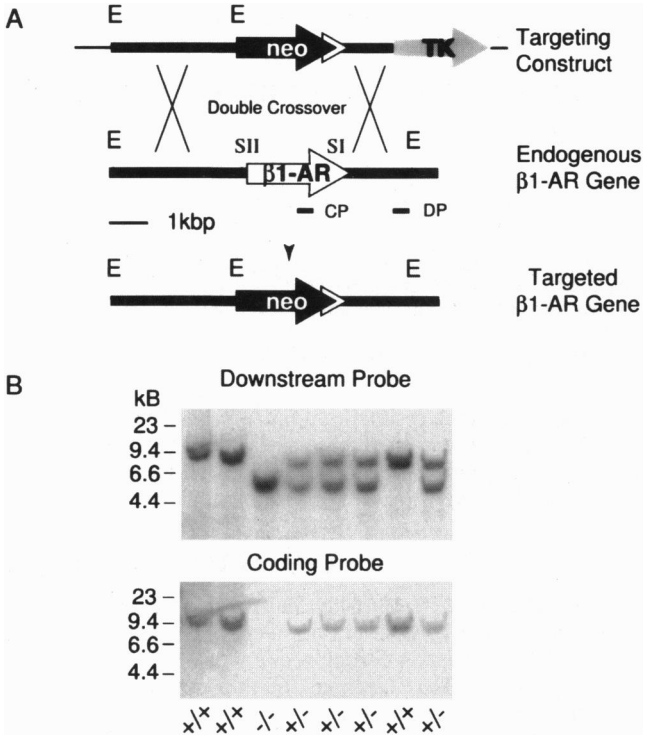


FIG. 1. β1-AR gene targeting. (*A*) Shown from the top is a schematic of the targeting construct used to transfect ES cells, the endogenous β1-AR gene, and the targeted β1-AR locus resulting from homologous recombination between targeting construct and endogenous gene. E, *Eco*RI; SI, *Sac*I; SII, *Sac*II restriction endonuclease sites. Arrows designate coding sequences, thick lines indicate the β1-AR noncoding sequence, thin lines designate the plasmid vector sequence. DP, downstream probe; CP, coding probe. (*B*) Southern blot of mouse tail DNA digested with *Eco*RI. Shown is a single litter derived from an intercross between β1-AR disruption heterozygotes. The upper blot was probed with a 500-bp DNA fragment downstream from the region immediately 3′ to the end of the targeting construct (DP). This blot was then stripped and reprobed with the 300-bp CP as illustrated in (*A*). Genotypes are indicated at the bottom. The wild-type β1-AR allele generates an ≈8-kbp *Eco*RI fragment visualized with either DP or CP, whereas the disrupted β1-AR gene generates an ≈5 kbp *Eco*RI fragment, seen only with the DP.

polyacrylamide gels and blotted to nitrocellulose. Affinity-purified rabbit polyclonal antiserum directed against the C-terminal 79 amino acids of the mouse β1-AR was reacted with the blotted nitrocellulose, and antibody-protein complexes visualized with the enhanced chemiluminescence kit (Amersham).

**Binding and Adenylate Cyclase Assays on Membrane Homogenates.** Ventricular or lung membrane homogenates were prepared by polytron homogenization of whole organs in 5 mM Tris·HCl, 5 mM EDTA (pH 7.4), followed by centrifugation at 10,000 × *g*. The resultant pellet was resuspended in 1× binding buffer (75 mM Tris·HCl/12.5 mM $MgCl_2$/1 mM EDTA, pH 7.4), and protein concentration determined. For saturation binding, 5–50 μg of homogenate protein was used in a 500 μl reaction containing 1–300 pM $^{125}$I-iodocyanopindolol ($^{125}$I-CYP), plus or minus 1 μM dl-propranolol, in 1× binding buffer. For competition studies, 5–50 μg of homogenate was added to a reaction containing 50 pM $^{125}$I-CYP, plus varying concentrations of CGP 26505 or ICI 118,551 (0–43 μM), in 1× binding buffer. All binding reactions were incubated at room temperature for at least 2 hr prior to vacuum filtration onto Whatman GF-C filters and determination of membrane bound $^{125}$I-CYP by gamma emission.

For adenylate cyclase assays, the 10,000× *g* pellet was resuspended in 50 mM triethanolamine, 50 mM $MgCl_2$, 10 mM EGTA (pH 7.4) and protein concentration determined. Ventricular or lung homogenate protein (20–50 μg) was added to a reaction mixture containing 2.5 μCi [$\alpha$-$^{32}$P]ATP (1 Ci = 37 GBq), 9 mM phosphocreatine, 0.1 mM cAMP, 0.12 mM ATP, 0.4 mg/ml creatine kinase, 53 μM GTP, with 0–0.1 mM isoproterenol. Reactions were incubated at 37°C for 15 min, and terminated by addition of HCl to 0.6 N. cAMP was separated from ATP by single-step alumina chromatography, eluting [$^{32}$P]cAMP with 0.1 M $NH_4CH_3CO_2$, and quantifying by scintillation counting.

***In Vivo* Cardiovascular Physiology.** Adult mice (8–12 weeks of age) were anesthetized with methoxyflurane, and a stretched polyethylene (PE-10) catheter was inserted into the left carotid artery. After full recovery from anesthesia (12–24 hr), the saline filled catheter was connected to a Spectramed DTX Plus pressure transducer. The transducer was connected to a Gould 8-channel recorder and analog pressure data was digitized (Crystal Biotech Dataflow system, Hopkinton, MA) to obtain heart rate and blood pressure values. For electrocardiogram measurements of anesthetized mice, anesthesia was induced with Avertin, and electrocardiogram signals obtained from lead II.

***In Vitro* Cardiac Physiology.** Free right ventricles were dissected away from the left ventricle and interventricular septum and silk sutures tied at both ends of the long axis. Ventricles were placed in an oxygenated 32°C tissue bath containing modified Krebs solution (118 mM NaCl/5.4 mM KCl/2.5 mM $CaCl_2$/0.57 mM $MgSO_4$/1.0 mM $Na_2HPO_4$/2.5 mM $NaHCO_3$/11.1 mM D-glucose). Ventricles were paced at 3–3.3 Hz by use of a Grass stimulator (30 msec pulse duration, 8–15 V). Isoproterenol dose-response curves were cumulative. Signals from isometric force transducers were fed via a 4-channel Gould amplifier to the Crystal Biotech Dataflow system to determine twitch amplitude, dF/dt, and −dF/dt.

For isolated spontaneously beating atria, right and left atria were dissected free of ventricular tissue and both atrial appendages tied with a 4–0 silk suture. These were placed in an oxygenated, 32°C tissue bath and isometric force transduction monitored as above.

**Catecholamine Measurements.** Norepinephrine (NE) and a metabolite known to be an index of NE reuptake and deamination, 3,4-dihydroxyphenylglycol (DHPG), were measured from the supernatants of whole-heart homogenates using high-performance liquid chromatography coupled with electrochemical detection. Hearts were homogenized on ice by polytron in 0.1 M sodium phosphate (pH 7.4), and a small aliquot removed for protein determination. Perchloric acid was then added (0.6 M final concentration), and high speed supernatants used for analysis.

**Histological Analysis.** Hearts from adult β1-AR +/+ or −/− mice were perfusion fixed in PBS plus 10% formalin. Coronal sections of hearts were cut for gross morphological analysis, then paraffin embedded for thin sectioning followed by hematoxylin and eosin staining.

## RESULTS

**β1-AR Gene Targeting.** The mouse R1 ES cell line (18) was transfected with a β1-AR gene targeting construct (Fig. 1*A*). Homologous recombinants were identified and microinjected into C57BL6/J blastocyst stage embryos (see *Materials and Methods*). The resultant chimeric mice were bred with C57BL/6 × DBA2/J $F_1$ mice to obtain germ-line transmission of the disrupted allele. Genotype analysis of a typical litter derived from an intercross between β1-AR disruption heterozygotes (β1-AR +/−) is seen in Fig. 1*B*.

**Genotype Analysis of β1-AR +/− Intercrosses.** β1-AR +/− mice of mixed strain background (contributed from 129/Sv, C57BL6/J, and DBA2/J mice, see *Materials and Methods*) were intercrossed, and progeny analyzed at embryonic days

(E) 10.5 and 18.5, as well as postnatal days (PN) 10–17, whereas intercrosses set up between β1-AR disruption heterozygotes on a fixed 129/Sv background were analyzed at PN 10–17 only. Fig. 2 shows that at E10.5, β1-AR +/+, +/−, and −/− mice are recovered in the expected ≈1:2:1 ratio (25%:50%:25%). At this stage there were no obvious defects observable in β1-AR −/− mice, and all mice analyzed had beating hearts. Analysis of later stage embryos (E18.5) and PN mice (10–17) revealed a dramatic loss of β1-AR −/− embryos (Fig. 2). Assuming that equal numbers of β1-AR +/+ and −/− mice should be recovered, these data suggest that ≈70% of the β1-AR −/− mice die *in utero*. Analysis of β1-AR genotype frequencies on the 129/Sv background reveals an ≈90% mortality rate among β1-AR −/− mice, significantly higher than the mixed strain background. $\chi^2$ analysis of genotype frequencies among the four groups analyzed indicates that E10.5 embryos do not deviate from Mendelian expectations, whereas E18.5 and PN mice of either strain background deviate significantly from expected ratios (Fig. 2).

**Heart Weight, Catecholamine Content, and Histology of β1-AR −/− Hearts.** β1-AR +/+ and −/− mice were sacrificed between 8–16 weeks of age and analyzed for heart-weight/body-weight ratios, cardiac NE and DHPG content (see *Materials and Methods*), and gross morphology. As seen in Table 1, heart-weight/body-weight ratios are unaltered between the two groups. NE content is decreased, and the DHPG/NE ratio is increased in β1-AR −/− mice compared with β1-AR +/+, though these differences did not reach statistical significance. There were no obvious differences between β1-AR +/+ and −/− hearts with respect to gross morphology or myocyte appearance (data not shown), though the possibility of more subtle differences remains.

**β-AR Expression and Pharmacology in β1-AR +/+ and −/− Mice.** Cardiac β-AR expression was analyzed to verify the lack of β1-AR protein and examine the effect of β1-AR gene disruption on the expression of β2-ARs. Western blot analysis with antisera raised against the C terminus of the mouse β1-AR confirms the loss of β1-AR protein expression in the hearts of β1-AR −/− mice (Fig. 3*A*). Likewise, saturation binding of cardiac homogenates with the nonspecific β-AR antagonist $^{125}$I-CYP reveals that maximal binding ($B_{max}$, Fig. 3*B*, Table 2) is reduced by ≈80% in β1-AR −/− cardiac homogenates compared with +/+ homogenates. The (−) isomer of the β1-AR specific antagonist CGP20712A (CGP26505) was used in competition-binding experiments to reveal the β-AR subtype distribution in cardiac homogenates.

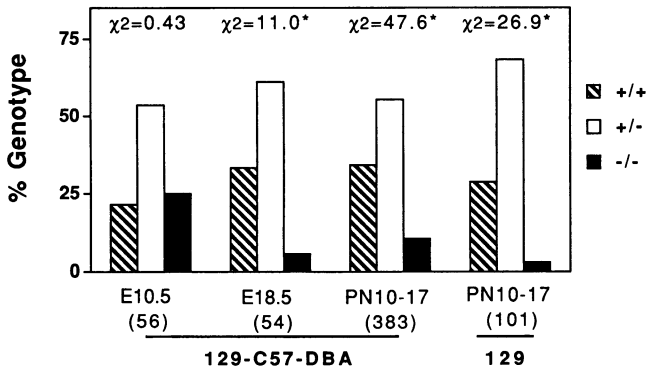


FIG. 2. Genotype analysis. β1-AR heterozygote matings were set up and the resultant offspring from either E10.5 or E18.5, or PN 10–17 were genotyped for the β1-AR allele by Southern blotting as per Fig. 1. 129-C57-DBA and 129 indicate the strain background, and the numbers in parentheses indicate the number of animals genotyped. Mendelian expectations predict 25% +/+, 50% +/−, 25% −/− genotypes from such a cross. $\chi^2$ analysis: $\chi^2 = \Sigma\, d^2/E$, where d is expected number − observed number and E is expected number, with two degrees of freedom. *, $P < 0.005$

Table 1. Heart weight and catecholamine levels

| | HW/BW, mg/g | NE, pmol/mg protein | DHPG/NE, % |
|---|---|---|---|
| +/+ | 5.35 + 0.18 (13) | 12.25 + 0.27 (4) | 5.77 + 1.07 (4) |
| −/− | 5.20 + 0.23 (11) | 10.82 + 0.55 (4) | 6.47 + 0.62 (4) |

Heart-weight/body-weight ratios (HW/BW) were calculated for β1-AR +/+ and −/− mice and expressed as milligram of heart weight/gram of body weight. Both NE content (pmol NE/mg protein), and the percent ratio between DHPG, an NE metabolite, and NE are also displayed. Values are expressed as mean ± SEM, the number of determinations in parentheses.

As seen in Fig. 3*C* and summarized in Table 2, competition with CGP26505 for $^{125}$I-CYP binding clearly reveals two receptor populations in β1-AR +/+ cardiac homogenates (71.9 ± 1.8% β1-AR and 28.1 ± 1.8% β2-AR; mean ± SEM), whereas cardiac homogenates from β1-AR −/− mice reveal only a single low- affinity class of receptor, consistent with β2-AR pharmacology. The $^{125}$I-CYP bound by β1-AR −/− cardiac homogenates can also be effectively competed for by 30 nM of the β2-AR specific antagonist ICI 118,551 (data not shown). β2-AR density was calculated either from $B_{max}$ (−/− homogenates), or $B_{max}$ × fraction of β2-AR sites (+/+ homogenates), revealing a small but significant decrease in β2-AR density in β1-AR −/− hearts (Table 1). $^{125}$I-CYP-binding experiments performed on lung membrane preparations also show that β1-AR −/− mice display a reduced $B_{max}$ value and β2-AR density relative to β1-AR +/+ mice (Table 2).

We also examined the ability of isoproterenol to stimulate adenylate cyclase activity in β1-AR +/+ and −/− cardiac homogenates (Fig. 3*D*). Basal adenylate cyclase activity is equivalent between +/+ and −/− preparations, as is forskolin-stimulated adenylate cyclase activity (data not shown). Whereas +/+ ventricular homogenates exhibits a robust stimulation of adenylate cyclase activity in response to $10^{-4}$M isoproterenol (152.5 ± 5.0% over basal; mean ± SEM; $P <$ 0.001 versus basal; $n = 3$), β1-AR −/− homogenates show only weak stimulation under the same conditions (16.7 ± 3.6% over basal; $P < 0.005$ versus basal; $n = 3$). This does not represent a generalized depression of cyclase stimulation in knockout mice, however, as lung homogenates from β1-AR −/− mice show no deficit in maximum isoproterenol- stimulated adenylate cyclase activity (94.5 ± 15.8% increase over basal for β1-AR −/−, versus 64.8 ± 14.6% increase over basal for β1-AR +/+; mean ± SEM, $n = 4$).

**Cardiovascular Physiology in β1-AR −/− Mice.** *In vivo* heart rate and blood pressure were monitored in awake, unrestrained mice by the use of in-dwelling carotid arterial catheters. Under basal conditions, neither heart rate nor mean blood pressure differed significantly between β1-AR +/+ and −/− mice (Table 3). However, when β1-AR −/− mice were challenged with the nonspecific β-AR agonist isoproterenol, the increase in heart rate normally seen with this drug was absent (Fig. 4 and Table 3), whereas the β2-AR mediated hypotensive response was preserved in both β1-AR +/+ and −/− mice (Fig. 4 and Table 3). Additional experiments performed on anesthetized mice and in isolated atria confirm the lack of isoproterenol-stimulated chronotropy in β1-AR −/− mice (Table 3).

Cardiac contractility (inotropy) was monitored by the isometric twitch response of isolated, paced right ventricles. β1-AR +/+ right ventricles respond to isoproterenol by increased twitch amplitude, increased rate of contraction (dF/dt), and increased rate of relaxation (−dF/dt) (Fig. 5). Twitch amplitude is increased up to a maximum of 8.9-fold by isoproterenol in +/+ ventricles (Fig. 5 *A* and *B*), whereas β1-AR −/− ventricles display absolutely no increase in twitch amplitude, dF/dT, or −dF/dT in response to isoproterenol.

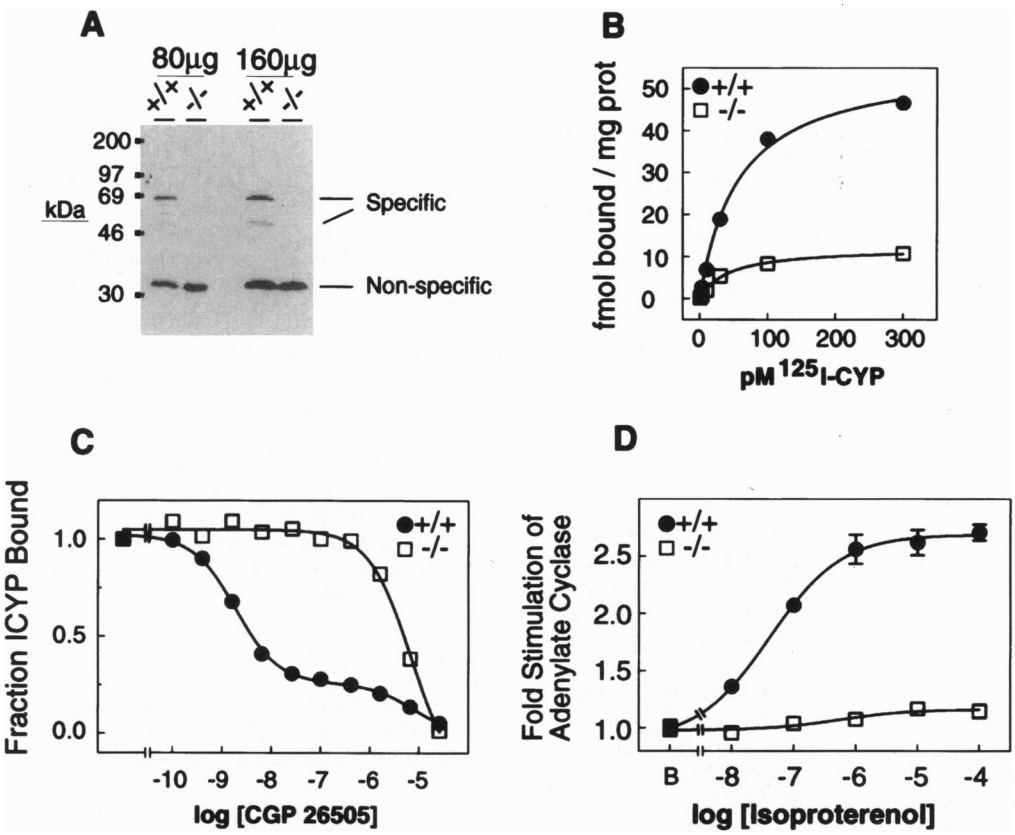


FIG. 3. β-AR expression and pharmacology in β1-AR +/+ and −/− ventricles. (*A*) Western blot of ventricular protein homogenates from β1-AR +/+ and −/− mice. Homogenate (80 or 160 μg) was electrophoresed on an SDS/9% PAGE gel, transferred to nitrocellulose, and blotted with polyclonal antiserum directed against the mouse β1-AR C terminus. Specific and nonspecific antibody-protein complexes were visualized by the enhanced chemiluminescence kit (Amersham). (*B*) Saturation binding of ventricular homogenates from β1-AR +/+ and −/− mice with β-AR antagonist $^{125}$I-CYP. Homogenates were incubated with 1–300 pM $^{125}$I-CYP in the presence or absence of 1 μM dl-propranolol to determine nonspecific and specific binding (specific binding displayed). (*C*) Competition binding of $^{125}$I-CYP with the β1-AR specific antagonist CGP 26505 (− isomer of CGP 20712A). +/+ and −/− homogenates were incubated with 50 pM $^{125}$I-CYP plus varying concentrations of CGP 26505 from 0 to 26 μM. (*D*) Activation of adenylate cyclase by isoproterenol in β1-AR +/+ and −/− ventricular homogenates. Stimulation of adenylate cyclase activity was assessed by production of [$^{32}$P]cAMP from [$^{32}$P]ATP using ventricular homogenates in the presence of $10^{-8}$ to $10^{-4}$M (−) isoproterenol. Values represent mean ± SD; error bars not shown when smaller than symbol. B, basal (no isoproterenol).

β1-AR −/− ventricles do respond to postreceptor activators of adenylate cyclase such as forskolin in both twitch amplitude, dF/dt, and −dF/dt (Fig. 5*B*), and the level of stimulation is similar to that observed for +/+ ventricles.

## DISCUSSION

Analysis of mice lacking the β1-AR has shown that this receptor is critical for proper mouse development, and appears to be a central mediator of the positive chronotropic and inotropic responses following catecholamine stimulation. Despite the high degree of relatedness between β1- and β2-AR members and their common coupling behaviors to $G_s$ and adenylate cyclase, these studies show that β-AR functions are not redundant, even in tissues where subtypes are coexpressed.

The embryonic lethality observed in β1-AR −/− mice is not currently understood, though there may be a common etiology between β1-AR −/− mice and mice with a targeted disruption of the gene encoding tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. Two groups have reported on the phenotype of TH −/− mice, which lack the ability to produce the endogenous ligands for β1-ARs, epinephrine, and NE (19, 20). Zhou *et al.* (19) report that TH −/− mice die between E11.5 and E15.5, presumably of a

Table 2. Summary of cardiac and pulmonary β-AR pharmacology using $^{125}$I-CYP

| | $K_d$, pM | $B_{max}$, fmol/mg protein | % β1:β2 | β2-AR density, fmol/mg protein |
|---|---|---|---|---|
| +/+ Heart | 70.5 ± 8.1 | 50.0 ± 5.2* | 72:28 | 13.9 ± 0.7† |
| −/− Heart | 51.3 ± 6.7 | 9.8 ± 1.3 | 0:100 | 9.8 ± 1.3 |
| +/+ Lung | 74.1 ± 12.3 | 2538 ± 441 | 26:74 | 1873 ± 325 |
| −/− Lung | 45.7 ± 3.0 | 1261 ± 261 | 0:100 | 1261 ± 261 |

Shown are the $^{125}$I-CYP dissociation constant ($K_d$), β-AR binding sites/milligram of protein ($B_{max}$), percentage of β1-AR vs. β2-AR as determined by CGP 26505 and ICI 118,551 competition, and the β2-AR binding sites/milligram of protein. β2-AR density in +/+ mice is calculated from $B_{max}$ and the percent of β2 sites, whereas β2-AR density in −/− mice is determined directly from $B_{max}$. Values are expressed as mean ± SEM from the results of three independent experiments. *, significance at $P < 0.005$. †, Significance at $P < 0.05$ between +/+ and −/− for Student's *t* test. Pharmacological analysis was performed with GRAPHPAD software.

Table 3. Summary of cardiovascular measurements

| | *In vivo* | | | | | *In vitro* |
|---|---|---|---|---|---|---|
| | Basal | | Isoproterenol stimulated | | | |
| | Mean BP | HR | % Δ BP | % Δ HR | % Δ HR, anesthetized | % Δ atrial rate |
| +/+ | 101.1 ± 9.5 (6) | 520.6 ± 24.8 (6) | −21.9 ± 3.1 (6) | +18.6* ± 5.9 (6) | +40.3† ± 9.4 (4) | +58.1† ± 11.7 (6) |
| −/− | 91.9 ± 5.3 (5) | 542.6 ± 28.9 (5) | −23.5 ± 4.9 (5) | +1.8 ± 0.5 (5) | −0.7 ± 3.1 (5) | −2.6 ± 2.9 (6) |

*In vivo* refers to the measurement of basal blood pressure (BP, mmHg) and heart rate (HR, beats/min), and the percent change in BP and HR following an intra-arterial dose of 1 μg/kg isoproterenol in awake, unrestrained animals. Another *in vivo* measurement of chronotropic responsiveness is the percent change in HR seen in anesthetized animals given a 10 μg/kg isoproterenol dose (intraperitoneal). Anesthetized mouse heart rates were measured by electrocardiogram (lead II), expressed as maximum percent change 2–10 min after isoproterenol administration. *In vitro* refers to the percent change in spontaneous atrial rate following 1 μM isoproterenol administration. Values are mean ± SEM, the number of independent measurements is given in parentheses. *, Significance at $P < 0.05$; †, significance at $P < 0.01$ for Student's $t$ test comparing +/+ and −/− mice.

structural cardiac defect, whereas Kobayashi *et al.* (20) find that lethality occurs primarily in the perinatal period, and though lacking structural cardiac defects, these TH −/− mice have markedly slowed heart rates. In both cases, it appears that TH −/− mice may be incapable of maintaining sufficient cardiac output. Also of interest is the finding that β-AR antagonists possess cardiac teratogenic activity (21), suggesting that disruption of the β1-AR gene could lead to cardiac defects. Further delimiting the phase of development where death occurs in β1-AR −/− mice will make it possible to examine cardiac anatomy and function in embryos immediately preceding intrauterine death.

The finding that basal heart rate is normal in β1-AR −/− mice is surprising, given both the effectiveness of β-AR antagonists in reducing heart rate in normal animals (5, 11–15), and the apparent lack of β2-AR mediated tachycardia in this model. There may be compensatory mechanisms to ensure that basal heart rate is maintained, such as decreased vagal tone (11) or altered α-AR signaling (22), but in either case the β1-AR gene disruption model may be useful for examining alternative mechanisms of heart rate control.

Previous studies (8, 9) have shown that the β2-AR couples more efficiently to the effector adenylate cyclase than does the β1-AR. Moreover, recent studies have shown that cardiac-specific overexpression of the β2-AR results in increased basal and isoproterenol-stimulated heart rate and inotropic state (23). It is thus somewhat surprising that isoproterenol does not stimulate heart rate or contractility in β1-AR −/− mice. The lack of responsiveness of β2-ARs in β1-AR −/− mice might be explained in several ways. Xiao *et al.* (24) have recently reported that β2-ARs can also couple to inhibitory G proteins in cardiac myocytes. Thus, cardiac β2-ARs may be coupled to a different signaling pathway than the β1-AR. It is also possible that β2-ARs normally couple to $G_s$, but are desensitized or downregulated in β1-AR −/− mice. Support for this hypothesis comes from the small but significant decrease in β2-AR density seen in β1-AR −/− hearts. Desensitization and downregulation of β-ARs is known to occur in human heart failure where early demands for increased output are met by increased sympathetic drive. This state of compensation eventually leads to depleted catecholamine stores and decreased β-AR density and coupling (25). Although the reduced NE content and

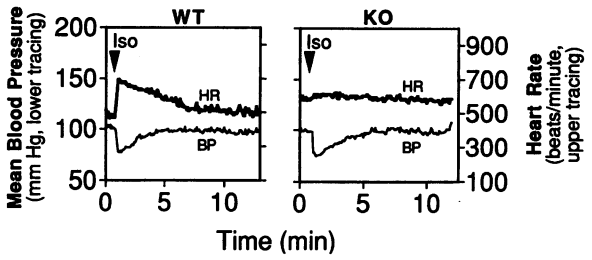


FIG. 4. *In vivo* cardiovascular responses in β1-AR +/+ and −/− mice to isoproterenol. Catheters chronically implanted in the left carotid artery were used to measure blood pressure and heart rate in awake, unrestrained mice. Measurements were taken approximately every 10 sec. Arterially administered (−) isoproterenol (1 μg/kg) was given as indicated by arrows to either β1-AR +/+ (WT), or β1-AR −/− (KO) mice. A representative experiment is shown.

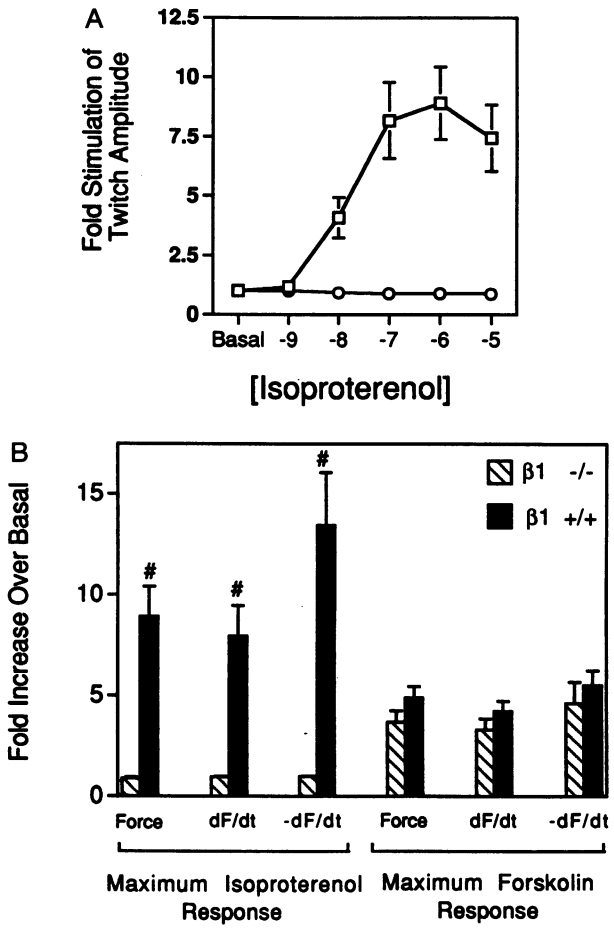


FIG. 5. Twitch response of isolated, paced right ventricle. (*A*) The response of right ventricles to doses of isoproterenol ranging from $10^{-9}$ M to $10^{-5}$ M is expressed as fold-stimulation of twitch amplitude, comparing β1-AR +/+ (□, $n = 5$) and −/− (○, $n = 5$) right ventricles. (*B*) Comparison of maximal isoproterenol versus maximal forskolin-stimulated contractile function. dF/dt and −dF/dt, the first derivatives of force for contraction and relaxation phases, respectively, are illustrated along with twitch amplitude. Exposure to 100 μM of forskolin results in significant inotropic stimulation of both β1-AR +/+ ($n = 3$) and −/− ($n = 5$) ventricles. All values are expressed as mean ± SEM; error bars are not shown when smaller than symbol. #, Significance at $P < 0.005$ between +/+ and −/− for Student's $t$ test.

increased DHPG/NE ratios do not reach statistical significance between β1-AR −/− and β1-AR +/+ mice (Table 1), the trend is consistent with elevated sympathetic tone in β1-AR −/− mice. Finally, it is possible that mouse myocytes express low or physiologically nonfunctional levels of β2-ARs. Although β1- and β2-AR coexpression on rat myocytes has been observed (26), the areas of highest expression of β2-ARs in the heart are thought to be in connective tissue and arterioles (7). Thus, whereas a number of studies have demonstrated chronotropic and inotropic responses to stimulation of cardiac β2-ARs in humans (5, 10, 27, 28) and rats or dogs (12, 13), cardiac β2-AR function in mice may be restricted primarily to connective tissue and blood vessels. Future studies will examine the distribution of β2-ARs in the hearts of β1-AR −/− mice, and attempt to test whether such receptors can be supersensitized to reveal functional coupling (29, 30). Nevertheless, our results suggest that in the β1-AR knockout model, the β2-AR is a minor contributor to the high output state normally induced by catecholamines in the heart.

In summary, targeted disruption of the mouse β1-AR gene has helped to clarify β-AR subtype-specific roles in development and cardiovascular function. These mice may also serve as a model system that can be used to study the determinants of basal heart rate, to test subtype selective β-AR compounds, or to mimic various pathological states where cardiovascular dynamics have been compromised.

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