Targeted Disruption of the β2 Adrenergic Receptor Gene*

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β-Adrenergic receptors (β-ARs) are members of the superfamily of G-protein-coupled receptors that mediate the effects of catecholamines in the sympathetic nervous system. Three distinct β-AR subtypes have been identified (β1-AR, β2-AR, and β3-AR). In order to define further the role of the different β-AR subtypes, we have used gene targeting to inactivate selectively the β2-AR gene in mice. Based on intercrosses of heterozygous knockout (β2-AR +/−) mice, there is no prenatal lethality associated with this mutation. Adult knockout mice (β2-AR −/−) appear grossly normal and are fertile. Their resting heart rate and blood pressure are normal, and they have a normal chronotropic response to the β-AR agonist isoproterenol. The hypotensive response to isoproterenol, however, is significantly blunted compared with wild type mice. Despite this defect in vasodilation, β2-AR −/− mice can still exercise normally and actually have a greater total exercise capacity than wild type mice. At comparable workloads, β2-AR −/− mice had a lower respiratory exchange ratio than wild type mice suggesting a difference in energy metabolism. β2-AR −/− mice become hypertensive during exercise and exhibit a greater hypertensive response to epinephrine compared with wild type mice. In summary, the primary physiologic consequences of the β2-AR gene disruption are observed only during the stress of exercise and are the result of alterations in both vascular tone and energy metabolism.

β-Adrenergic receptors (β-ARs)§ are members of the superfamily of G-protein-coupled receptors that are stimulated by the naturally occurring catecholamines, epinephrine and norepinephrine. As part of the sympathetic nervous system, β-ARs have been shown to have important roles in cardiovascular, respiratory, metabolic, central nervous system, and reproductive functions. Using techniques of molecular cloning, three distinct β-AR subtypes have been identified (β1-AR, β2-AR, and β3-AR) (1–3). All three of these β-AR subtypes are believed to signal by coupling to the stimulatory G-protein Gsα leading to activation of adenylyl cyclase and accumulation of the second messenger cAMP (1–3).

Because of the diverse physiological functions mediated by β-ARs, much effort has been spent in understanding the roles of individual β-AR subtypes. In the past, researchers have relied on pharmacological tools such as subtype-selective agonists and antagonists to probe the function of the different β-AR subtypes. The presence of multiple β-AR subtypes was first suggested by Lands and co-workers (4, 5) who divided β-ARs into β1-ARs and β2-ARs. According to Lands’ classification, β1-ARs mediate cardiac stimulation, and β2-ARs mediate smooth muscle relaxation in the peripheral vasculature and respiratory system. The presence of a third β-AR subtype was suggested when some of the effects of β-AR agonists could not be efficiently blocked by typical β-AR antagonists. This third β-AR subtype is now known as the β3-AR and has been shown to have important roles in adipose tissue and the gastrointestinal tract (6).

Although both β1-ARs and β2-ARs are expressed in the heart of most mammalian species, β1-ARs are expressed at higher levels and are recognized as playing the major role in regulating cardiac function. Functional studies have confirmed that activation of β1-ARs leads to increased heart rate and force of contraction (7). Although they represent a smaller population in the heart than β1-ARs, β2-ARs have also been shown to play a role in regulating cardiac function in a variety of species (7–9). In studies using subtype-selective agonists and antagonists in the human heart, β2-AR stimulation leads to activation of adenyl cyclase and contributes to both inotropic and chronotropic responses (7). In the murine heart, however, β2-ARs do not appear to couple to inotropic or chronotropic responses. When isolated cardiac muscle from β1-AR knockout mice is stimulated with the non-subtype-selective β2-AR agonist isoproterenol, neither inotropic nor chronotropic responses are observed (10).

In addition to their roles in the heart, β-ARs also regulate peripheral vascular tone. Stimulation of peripheral β-ARs leads to relaxation of vascular smooth muscle, thereby controlling the distribution of blood flow to different tissues. During exercise, for example, stimulation of β-ARs contributes to the increased blood flow to skeletal muscle. Based on the studies of Lands and co-workers (4, 5), the β-AR in the peripheral vasculature have been classified as the β2-AR. Some reports, however, have shown roles for the other β-AR subtypes, β1-ARs and β3-ARs, in the peripheral vasculature (11–13).

Although much has been learned about the role of individual β-AR subtypes using classical pharmacological techniques, these studies are complicated by the fact that subtype-selective ligands are never perfectly selective. Moreover, at the doses required to block β-ARs in vivo, most β-AR ligands lose much of their subtype selectivity and may bind to other G-protein-coupled receptors such as serotonin receptors and dopamine receptors. Studies with β-AR ligands are especially difficult to interpret in vivo where it is hard to estimate the concentration.
of ligands and their metabolites in target tissues. In order to further investigate the roles of the different β-2AR subtypes in physiology, we have selectively inactivated the β2-AR gene in mice using gene-targeting techniques. The knockout (β2-AR −/−) mice appear grossly normal and are fertile. Resting cardiovascular physiology is remarkably unperturbed in β2-AR −/− mice. The major effects of β2-AR gene disruption were observed only during the stress of exercise. β2-AR −/− mice were able to exercise farther and with a lower respiratory exchange ratio at any given workload than wild type controls. However, they are hypertensive during exercise, suggesting an imbalance between the vasoconstrictive and vasorelaxant effects of endogenous catecholamines.

MATERIALS AND METHODS

Targeting Vector Construction—The targeting vector was constructed using sequence that had been cloned from a C57BL/6 mouse genomic library (14). In total, the targeting vector contained 11.4 kb of homology to the endogenous β2-AR genomic locus. The gene for the β2-AR was disrupted in the targeting vector by placing a neomycin (neo) resistance gene cassette into the coding sequence at a unique ClaI site (15). This insertion disrupts the β2-AR at the end of the fourth transmembrane segment and should produce a truncated receptor. The short arm of the targeting vector was a 2.6-kb fragment from a 5′ EcoRI site to the ClaI in the receptor. The long arm of the targeting vector (8.8 kb) extended from the ClaI site in the receptor to a downstream SaI site. Also included in the vector was the herpes simplex virus thymidine kinase cassette to allow for negative selection when isolating ES cell clones (15). In order to screen for homologous recombinants a 5′ external probe was used. This probe is a 300-base pair BamHI/EcoRI fragment that detects a 4.9-kb fragment after mouse genomic DNA is digested with BamHI and then subjected to Southern blot analysis. In cases where the targeting vector has homologously recombined with the endogenous locus, the same probe would detect an additional band at 6.6 kb.

Transfection of ES Cells—RI embryonic stem (ES) (16) cells were transfected using standard techniques (17). ES cells were grown on a monolayer of mouse embryonic fibroblasts in Dulbecco's modified Eagle's medium (UCSF tissue culture facility, San Francisco) supplemented with 20% fetal bovine serum (HyClone, Logan, UT), 1 mM sodium pyruvate (Life Technologies, Inc.), non-essential amino acids, and penicillin/streptomycin (UCSF Cell Culture Facility, San Francisco, CA). 10−4 M β-mercaptoethanol (Specialty Media, Lalavlette, NJ), and 2,000 units/ml of leukemia inhibitory factor (ESGRO; Life Technologies, Inc.). Cells were grown in an incubator at 37 °C in 95% air, 5% CO2, and 2,000 units/ml of leukemia inhibitory factor (ESGRO; Life Technologies, Inc.). Cells were grown in an incubator at 37 °C in 95% air, 5% CO2.

For the transfection, a 10-cm2 dish of ES cells was transfected via electroporation with 20 µg of targeting vector previously linearized with NotI. After selecting ES cells for 9 days in media containing G418 (Life Technologies, Inc.) and gancyclovir (Syntex, Palo Alto, CA), individual clones were picked and subcloned in 96-well plates. BamHI-digested DNA from clones was analyzed by Southern blot analysis with the 5′ external probe. Nine homologous recombinants were isolated from 300 ES cell clones. Homologous recombinants were also screened with a neo probe to confirm that a single integration of the targeting vector had occurred.

Morula Aggregation—Chimeric mice were generated using the morula aggregation technique described previously (18). Briefly, embryos at morula stage (2.5 days pc) were collected from oviducts of superovulated CD-1 mice by flushing the oviducts with M2 medium (Specialty Media, Lalavlette, NJ). After removing the zona pellucida with an acidic Tyrode's solution (Specialty Media, Lalavlette, NJ), the embryos were placed in depressions in a 6-cm tissue culture dish and covered with a droplet of M16 medium (Specialty Media, Lalavlette, NJ). A protective layer of mineral oil (Sigma) was placed over the droplets. Clumps of ES cells with the targeted disruption (10–20 cells) were then seeded into the depression and placed in contact with the embryos. After an overnight incubation at 37 °C in 95% air, 5% CO2, the chimeric embryos were transferred to the uteri of pseudopregnant CD-1 hosts (20–25 embryos per host). Chimeric mice were identified in the resulting litters by the presence of dark coat color patches. Chimeric males were then mated to FVB/N female mice to screen for germ line transmission of the ES cell DNA. After achieving germ line transmission, β2-AR −/− mice were intercrossed to generate β2-AR +/− mice for use in binding studies. For in vivo studies, the knockout allele was placed on a FVB/N background by backcrossing β2-AR +/− mice to wild type FVB/N mice for four additional generations (5 backcrosses to FVB/N in total).

Binding Assays—Whole lungs were dissected from wild type and knockout littermates, placed in a lysis buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4), and homogenized with a Polytron (4 × 20-s bursts). The membrane fraction was isolated by centrifuging at 10,000 × g and resuspending in binding buffer (75 mM Tris-HCl, 12 mM MgCl2, 1 mM EDTA, pH 7.4). Binding reactions were carried out by incubating membranes with the radiolabeled [125I]iodoaceptaminol (125I-CPY) (NEN Life Science Products) in 500-µl volumes. After a 2-h incubation at room temperature, vacuum filtration was performed, and the filters were counted in a gamma counter. For saturation experiments, 3 µg of membrane protein was incubated with increasing amounts of 125I-CPY (1–300 pM). Nonspecific binding was determined in the presence of 1 µM propanolol (Sigma). For competition experiments, binding reactions were set up with 50 µM 125I-CPY, 3–6 µg of membrane protein, and varying concentrations (50-13 µM) of the β2-AR-selective antagonist ICI 118,551 (Tocris Cookson, Ballwin, MO). Saturation and competition data were analyzed with GraphPAD software (GraphPAD Software Inc., San Diego, CA).

In Vivo Cardiovascular Physiology—In vivo studies were carried out as described previously (19). Adult male mice (12–16 weeks of age) were anesthetized with isoflurane using a vaporizer (Aircor Inc., Madison, WI), and a stretched Intramed PE10 polyethylene catheter (Clay Adams, Parsippany, NJ) was inserted into the left carotid artery. The catheter was tunneled through the neck and then placed into a subcutaneous pouch in the back. After the 16-h recovery, the saline-filled catheter was removed from the pouch and connected to a Spec-tramed DTX Plus pressure transducer (Spectramed, Oxnard, CA). Output from the pressure transducer was amplified using a Gould 8-channel recorder and digitized using a Data Translation Series DT2801 analog-digital converter (Marlboro, MA). The digital signal was analyzed using Crystal Biotech Dataflow data acquisition software (Crystal Biotech, Hopkinton, MA) on a Gateway 2000 486DX2 microcomputer (Sioux City, SD). Baseline heart rate and mean arterial blood pressure were recorded after a 1-h equilibration period when the animals were awake but not active. In order to examine drug responses, drugs were administered through the carotid artery catheter. (∗) Isoproterenol hydrochloride (3 µg/kg) and epinephrine bitartrate (3 µg/kg) were purchased from Sigma and dissolved in saline for injection. In order to measure heart rate and blood pressure during exercise, anesthetized mice were challenged with a graded treadmill exercise protocol (19) on a Simplex II rodent treadmill (Columbus Instruments, Columbus, OH). Treadmill activity was initiated at 3.5 m/min, 0° inclination, and increased to 5 m/min, 2° inclination 3 min later. Treadmill speed was then increased by 2.5 m/min and 2° inclination every 3 min thereafter. Exercise was terminated after the mice had completed 3 min at 20 m/min, 14° inclination. Mice that failed to complete the exercise protocol were excluded from the study.

In Vivo Metabolic Responses to Exercise and Total Exercise Capacity—In order to measure metabolic responses to exercise and exercise capacity, non-instrumented mice were challenged with the graded treadmill exercise protocol described above. Treadmill activity was initiated after the mice had equilibrated in the exercise chamber for 30–60 min. During the exercise protocol, oxygen consumption and carbon dioxide production were continuously monitored with an Oxymax gas analyzer (Columbus Instruments, Columbus, OH). Stepwise increases in treadmill speed and inclination were made every 5 min until the mice stopped running from exhaustion. Exercise capacity was calculated as the total distance run by the animals during the exercise protocol.

Body Weight, Epididymal Fat Pad Weight, Density, FFA Levels, and Glycerol Levels—Male mice, 12–13 weeks old, were used for these studies. Mice were maintained in 12-h light/dark cycles. On the day of study, food was removed from the cage at the beginning of the light cycle, and mice were studied 3–5 h later. Each mouse was weighed and then anesthetized with 5% isoflurane for 45 s in an anesthesia induction box. The mouse was quickly removed from the box, and blood was collected by cardiac puncture with a 22-gauge needle. The mouse was then sacrificed via cervical dislocation. The volume of the mouse was determined by attaching a weight to the mouse and measuring the water displacement. Density was calculated as the body weight divided by the volume. After the volume measurement, both epididymal fat pads were removed, and the total weight of the fat pads was calculated by dividing the fat pad weight by the total body weight. After the blood samples had clotted in serum separator tubes (Becton Dickinson, Franklin Lakes, NJ), the samples were spun at 17,000 × g for 5 min to isolate the serum. Free fatty acid levels were determined with an enzymatic colorimetric kit (Wako Chemicals, Germany). Glycerol levels were determined with an enzy-
Fig. 1. Gene targeting strategy for the β2-AR gene. A, shown from the top is the targeting vector, the endogenous locus of the β2-AR gene, and the result of homologous recombination. B, BamHI site; C, ClaI site; E, EcoRI site; N, NotI site; S, SalI site; Neo, neomycin resistance cassette; HSVTK, thymidine kinase cassette. The black box represents coding sequence of resistance cassette; HSVTK, thymidine kinase cassette. The 5′ external probe and the expected fragments from Southern blot analysis are also shown. B, Southern blot of tail DNA from offspring of a heterozygous knockout intercross. Wild type (+/+), heterozygous knockout (+/−), and homozygous knockout (−/−) mice are recovered from this mating.

**RESULTS**

β2-AR Gene Targeting—Using standard ES cell techniques, the R1 ES cell line was transfected with the β2-AR targeting vector shown in Fig. 1A. Homologous recombinants were identified by performing Southern blot analysis using the 5′ external probe. Targeted clones were rescreened with a probe to the neomycin resistance gene to ensure that a single integration of the targeting vector had occurred (data not shown). Chimeric mice were generated with the targeted ES clones using the morula aggregation technique. Following germ line transmission of the knockout allele, heterozygous knockout pairs were intercrossed to generate β2-AR +/+ , β2-AR +/− , and β2-AR −/− mice. Shown in Fig. 1B is a Southern blot using DNA from the offspring of a β2-AR +/− intercross.

Results of Intercrosses—After backcrossing β2-AR +/+ mice to wild type FVB/N mice for 5 generations, β2-AR +/+ mice were intercrossed. From 171 intercross progeny screened at weaning, 36 β2-AR +/+ mice, 91 β2-AR +/− mice, and 44 β2-AR −/− mice were identified. These results are consistent with the ratio predicted by Mendelian genetics (χ-squared = 1.45, p > 0.4). Thus, there is no embryonic or postnatal lethality associated with disruption of the β2-AR gene in mice. After maturing into adults, β2-AR −/− mice appear grossly normal and do not exhibit overtly abnormal behavior. Both β2-AR −/− males and females are fertile.

β2-AR Expression and Pharmacology in β2-AR −/− Mice—In order to verify that the genetic modification prevents expression of the β2-AR gene, ligand binding experiments were performed using lung tissue isolated from β2-AR +/+ and −/− mice. Saturation binding experiments were performed using the β2-AR selective antagonist ICI 118,551. Nonspecific binding was determined in the presence of 1 μM DL-propranolol and subtracted from total binding. Values shown represent the mean ± S.E. for three individual experiments. A, saturation binding on membranes prepared from lungs of β2-AR +/+ and −/− mice. Studies were performed by incubating membranes with varying concentrations of the β2-AR antagonist 125I-CYP. Nonspecific binding was determined in the presence of 1 μM DL-propranolol and subtracted from total binding. Values shown represent the mean ± S.E. for three individual experiments.
Cardiovascular Physiology—In order to examine the effects of the gene disruption on whole animal physiology, $\beta_2$-AR +/+ and $\beta_2$-AR −/− mice were instrumented with carotid catheters to allow measurements of mean arterial blood pressure and heart rate in awake, non-anesthetized, and non-restrained mice. Under baseline resting conditions, mean blood pressure and heart rate were not significantly different between $\beta_2$-AR +/+ and $\beta_2$-AR −/− mice (Table I). In order to examine the effects of $\beta$-AR stimulation, $\beta_2$-AR +/+ and $\beta_2$-AR −/− mice were given an intra-arterial bolus of 3 μg/kg of the non-selective $\beta$-AR agonist isoproterenol, a dose previously shown to produce maximal increases in heart rate and maximal reductions in blood pressure in wild type mice. Fig. 3 shows the typical response of a $\beta_2$-AR +/+ and a $\beta_2$-AR −/− mouse to isoproterenol. In $\beta_2$-AR +/+ mice, isoproterenol produced a rapid onset tachycardia and hypotension. In $\beta_2$-AR −/− mice, the tachycardic response to isoproterenol was preserved, but the hypotensive response was significantly blunted (Fig. 3 and Table I).

Responses to the endogenous catecholamine, epinephrine (a combined $\alpha$-AR and $\beta$-AR agonist), were also significantly different between $\beta_2$-AR −/− and wild type mice (Fig. 4). In both $\beta_2$-AR −/− and wild type mice, administration of epinephrine produced a transient hypertensive response (blood pressure typically returned to baseline within 1 min). However, the hypertensive response was significantly greater in $\beta_2$-AR −/− mice than in wild types (Table I and Fig. 4). Heart rate responses in both $\beta_2$-AR −/− and wild type mice to epinephrine were variable (Table I). Although there was a trend for wild type mice to show heart rate increases while $\beta_2$-AR −/− showed heart rate decreases, these heart rate responses were not significantly different between genotypes.

The effects of exercise on heart rate and blood pressure are shown in Fig. 5. For these experiments, catheterized mice were tested using a graded exercise treadmill protocol. $\beta_2$-AR −/− and wild type mice showed similar heart rate increases during the exercise protocol. A significant difference, however, was observed in the blood pressure response to exercise. During the exercise protocol, $\beta_2$-AR −/− mice became hypertensive compared with wild type mice. At the peak exercise level of 20 m/min, $\beta_2$-AR −/− mice had a mean blood pressure of 139.3 ± 4.4 mm Hg (mean ± S.E.), whereas wild type mice had a mean blood pressure of 126.3 ± 3.3 mm Hg (mean ± S.E.).

Metabolic Response to Exercise—In a separate set of experiments, metabolic responses to exercise and exercise capacity were measured in uncatheterized mice. Oxygen consumption and carbon dioxide production were continuously monitored while the mice exercised according to a graded treadmill exercise protocol (Fig. 6). Oxygen consumption and carbon dioxide production were not significantly different between the two genotypes. However, there was a trend for $\beta_2$-AR −/− mice to have greater levels of oxygen consumption at any given workload. $\beta_2$-AR −/− mice had a significantly lower respiratory exchange ratio during exercise than did wild type mice (Fig. 6C). There was also a significant difference between $\beta_2$-AR +/+ mice and $\beta_2$-AR −/− mice in exercise capacity. Interestingly, $\beta_2$-AR −/− mice exercised significantly longer than wild type control mice (Fig. 6D). Wild type mice covered 471 ± 22 meters (mean ± S.E.), whereas $\beta_2$-AR −/− mice covered 582 ± 15 meters (mean ± S.E.) during the graded exercise protocol.

Body Weight, Body Fat, and Serum Free Fatty Acids—To investigate possible mechanisms for the greater exercise capacity in $\beta_2$-AR −/− mice, we examined body weight, epididymal fat pad weight, body density, and serum levels of free fatty acid (FFA) and glycerol in wild type and $\beta_2$-AR −/− mice. As shown in Table II, $\beta_2$-AR −/− mice weigh significantly less than wild type mice. Epididymal fat pads from $\beta_2$-AR −/− mice also represent a smaller proportion of total body weight than fat pads from wild type mice. Previous studies have shown that the epididymal fat pad weight as a proportion of total body weight is highly correlated with total body fat in mice (20, 21). Body density, serum FFA levels, and serum glycerol levels were not significantly different between the two genotypes under baseline conditions.

Locomotor Activity—Activity studies were performed to determine if the observed differences in exercise capacity, body fat, and body weight in $\beta_2$-AR +/+ and $\beta_2$-AR −/− mice can be explained by differences in the level of daily activity. As shown in Fig. 7, there was no significant difference in locomotor activity between the two genotypes over a 48-h period. No significant differences were observed when the 48-h period was broken down into day and night segments (data not shown).

DISCUSSION

Using gene targeting techniques we have generated mice that have a disruption in the $\beta_2$-AR gene. Based on ligand binding data this mutation blocks expression of the $\beta_2$-AR. $\beta_2$-ARs do not appear to play a critical role in prenatal development since there is no embryonic lethality associated with the mutation. Furthermore, $\beta_2$-AR −/− mice appear grossly normal and are fertile, demonstrating that $\beta_2$-ARs are not required for postnatal development or for normal reproductive function. There is no apparent compensatory up-regulation of $\beta_1$-ARs in the lungs of $\beta_2$-AR −/− mice.

Cardiovascular Effects of the $\beta_2$-AR Gene Disruption—One of the goals in generating $\beta_2$-AR −/− mice was to define further the roles of the three different $\beta$-AR subtypes in the cardiovascular system. A genetic approach has been used previously to show that $\beta_1$-AR and not $\beta_2$-AR stimulation regulates cardiac inotropy and chronotropy in the murine heart (10). Even though $\beta_2$-ARs are present in the myocardium of $\beta_1$-AR −/− mice, stimulation of $\beta_2$-ARs does not lead to improvements in
cardiac function either in vivo or in vitro. In contrast, in β2-AR −/− mice, normal heart rate responses are observed in response to isoproterenol and to exercise, further demonstrating that the β1-AR plays the major role in regulating cardiac function in the mouse.

Classical pharmacological studies have suggested that the β2-AR is the β-AR subtype that mediates vascular smooth muscle relaxation (4, 5). Vascular relaxation leads to a decrease in total peripheral resistance and is manifested by a hypotensive blood pressure response. In β2-AR −/− mice, the hypotensive response to isoproterenol is significantly blunted compared with wild type mice, confirming that β2-ARs play a significant role in mediating peripheral vascular relaxation. The fact that hypotensive responses are still present in β2-AR −/− mice, however, suggests that other β-AR subtypes also play a role, albeit a smaller one, in regulating peripheral vascular tone in the mouse. This response could also be due to an up-regulation of other β-AR subtypes in response to deletion of the β2-AR gene; however, there was no evidence for compensatory up-regulation of the β1-AR in the lung, the tissue with the highest density of β2-ARs in wild type mice.

Although β1-ARs are considered to be the cardiac β-AR, some previous studies support our conclusion that β1-ARs can mediate vascular relaxation. β1-ARs have been shown to be involved in vascular relaxation in the isolated rat aorta and rat pulmonary artery (22). β1-ARs have also been implicated in vascular relaxation in the coronary arteries from a variety of species (23–26). Although these larger vessels are not expected to be attenuated (28). It is not clear from these studies, however, suggests that other β-AR subtypes also play a role, albeit a smaller one, in regulating peripheral vascular tone in the mouse. This response could also be due to an up-regulation of other β-AR subtypes in response to deletion of the β2-AR gene; however, there was no evidence for compensatory up-regulation of the β1-AR in the lung, the tissue with the highest density of β2-ARs in wild type mice.

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FIG. 6. Metabolic response to exercise and exercise capacity in β2-AR +/+ and −/− mice. During a graded treadmill exercise protocol, O₂ consumption (A), CO₂ production (B), and the respiratory exchange ratio (C) were determined for each step in the exercise protocol. Values shown represent the mean ± S.E. for β2-AR +/+ (n = 5) and β2-AR −/− (n = 5) mice. O₂ consumption and CO₂ production are reported in units of ml/min/kg. The RER represents the ratio of CO₂ production to O₂ consumption. Exercise capacity (D) was measured as the total distance covered during the exercise protocol. Values shown represent the mean ± S.E. for β2-AR +/+ (n = 5) and β2-AR −/− (n = 6) mice. ¥, significance at p = 0.0062 for comparing exercise RER curves from β2-AR +/+ mice to β2-AR −/− mice by 2-way analysis of variance with repeated measures (treadmill speed × RER interaction); *, significance at p = 0.0031 for comparing β2-AR +/+ to −/− mice using an unpaired t test.

FIG. 7. Locomotor activity of β2-AR +/+ and −/− mice. The total number of beam breaks for β2-AR +/+ (n = 5) and β2-AR −/− (n = 5) mice is shown. Values shown represent the mean ± S.E. The total number of beam breaks is not significantly different between the two genotypes (p > 0.05 using an unpaired t test).

TABLE II

| Genotype      | Weight (g) | % weight | Density (g/ml) | FFA (μmol/liter) | Glycerol (mg/dl) |
|---------------|------------|----------|----------------|------------------|-----------------|
| β2-AR +/+     | 32.31 ± 0.65 | 3.01 ± 0.27 | 0.942 ± 0.006 | 2190 ± 207 | 39.2 ± 8.4 |
|               | (11)        | (11)     | (11)           | (11)            | (11)            |
| β2-AR −/−     | 29.12 ± 0.51 | 2.22 ± 0.12 | 0.943 ± 0.005 | 2365 ± 422 | 34.1 ± 7.8 |
|               | (12)        | (12)     | (12)           | (12)            | (12)            |

*Significance at p = 0.0008 for comparing β2-AR +/+ to −/− mice.

*Significance at p 0.0118 for comparing β2-AR +/+ and −/− mice.

In generating the β2-AR −/− mouse we had the opportunity to test whether or not defective β2-AR signaling can lead to a hypertensive state. Under baseline conditions we found that β2-AR −/− mice are normotensive compared with wild type control mice. Thus, β2-AR stimulation may not be involved in regulating resting blood pressure in mice. Given that β2-AR −/− mice have had the disruption of the β2-AR gene since conception, it is also possible that there have been other compensatory changes that allow the maintenance of resting blood pressure homeostasis. During the stress of treadmill exercise, however, β2-AR −/− mice become hypertensive compared with wild type mice. As an endogenous stimulus for catecholamine release, exercise may lead to unopposed α-adrenergic mediated vasoconstriction in the β2-AR −/− mice. In support of this hypothesis, administration of epinephrine to the β2-AR −/− mice reproduced the hypertensive phenotype. As a result of the disruption of the β2-AR gene, α-adrenergic receptor stimulation may predominate and predispose the animals to develop hypertension in states where endogenous catecholamines are elevated. In humans, hypertension worsens with the process of aging. In the present study, we studied young adult animals at 12–16 weeks of age. Future studies will be required to examine whether the β2-AR −/− mice develop hypertension as they age.

Metabolic Effects of the β2-AR Receptor Gene Disruption—β2-ARs are known to play a role in the metabolic response to stress (32). To investigate the effects of the knockout mouse on physiologic stress, metabolic responses to exercise and exercise capacity were measured in β2-AR −/− and wild type mice.
Surprisingly, β2-AR −/− mice exercised for a longer duration than wild type mice did. One explanation for this difference in exercise capacity is that there are alterations in energy metabolism secondary to ablation of the β2-AR gene. In support of this hypothesis is the finding that β2-AR −/− mice have a lower body fat content, and the respiratory exchange ratio (RER) was significantly lower in β2-AR −/− mice at any given workload than in wild type mice during exercise. Although carbon dioxide production was similar between wild type and β2-AR −/− mice, there was a trend toward higher oxygen consumption in the β2-AR −/− mice. This trend for increased oxygen consumption during exercise may be explained by the lower body fat in β2-AR −/− mice. Fat can be considered to be metabolically inert; therefore, changes in body fat content can also have an impact on oxygen consumption and carbon dioxide production when these parameters are normalized to total body weight. A change in body composition, however, does not explain the decrease in exercise RER in the β2-AR −/− mice. RER is a ratio of oxygen consumption to carbon dioxide production; therefore, body composition factors are cancelled out by taking a ratio.

RER is an indicator of metabolic state and substrate utilization. Since more oxygen is required to burn fat than carbohydrate, a lower RER in β2-AR −/− mice suggests that they may use a greater ratio of fat to carbohydrate than do wild type mice during exercise. This could also explain the lower body fat content in β2-AR −/− mice. Previous studies (33, 34) have demonstrated that β-AR stimulation leads to glycogenolysis during exercise. If the knockout mice were to result in a defect in β2-AR-mediated mobilization of glycogen, β2-AR −/− mice may preferentially metabolize fat during exercise. Reduced utilization of the glycogenolysis pathway during exercise would conserve muscle glycogen and may be responsible for increasing the duration of exercise before glycogen depletion. We did not observe significant differences in serum-free fatty acids and glycerol in β2-AR −/− mice under basal conditions; however, it is possible that differences in fat metabolism occur during exercise. Lactate production may also be influenced by the β2-AR gene disruption. It has been shown that catecholamines stimulate lactate production, possibly through β2-AR receptors (33, 34). Thus, β2-AR −/− mice may have lower lactate levels for a given workload.

Other mechanisms may contribute to the increase in exercise capacity of β2-AR −/− mice. There may be differences in the redistribution of cardiac output between visceral and peripheral muscular beds during exercise because of alterations in β2-AR-mediated vasorelaxation. In the β2-AR −/− mouse, the defect in β2-AR-mediated vasorelaxation may attenuate the increase in flow to non-exercising tissues thereby allowing a larger percentage of the cardiac output to be diverted to skeletal muscle. The increased exercise capacity and reduced body fat content of β2-AR −/− mice could reflect an elevated basal level of activity in these mice. However, β2-AR +/+ and β2-AR −/− mice displayed similar 48-h activity levels (Fig. 7).

In summary, we have generated mice that have a targeted disruption in the β2-AR gene. These mice have normal resting heart rate and blood pressure but manifest hypertension in response to epinephrine infusion or to the cardiovascular stress induced by exercise. β2-AR knockout mice should prove to be a useful model for further defining the roles of β-AR subtypes in cardiovascular, respiratory, metabolic, central nervous system, and reproductive functions.

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