Alterations in dynamic heart rate control in the β₁-adrenergic receptor knockout mouse

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Rohrer, Daniel K., Eric H. Schauble, Kavin H. Desai, Brian K. Kobilka, and Daniel Bernstein. Alterations in dynamic heart rate control in the β₁-adrenergic receptor knockout mouse. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1184–H1193, 1998.—β₁-Adrenergic receptors (β₁-ARs) are key targets of sympathetic nervous system activity and play a major role in the beat-to-beat regulation of cardiac chronotropy and inotropy. We employed a β₁-AR gene knockout model to test the hypothesis that β₁-AR function is critical for maintenance of resting heart rate and baroreflex responsiveness and, on the basis of its important role in regulating chronotropy and inotropy, is also required for maximal exercise capacity. Using an awake unrestrained mouse model, we demonstrate that resting heart rate and blood pressure are normal in β₁-AR knockouts and that the qualitative responses to baroreflex stimulation are intact. Chronotropic reserve in β₁-AR knockouts is markedly limited, with peak heart rates ~200 beats/min less than wild types. During graded treadmill exercise, heart rate is significantly depressed in β₁-AR knockouts at all work loads, but despite this limitation, there are no reductions in maximal exercise capacity or metabolic indexes. Thus, in mice, the β₁-AR is not essential for either maintenance of resting heart rate or for maximally stressed cardiovascular performance.

MAMMALIAN β-ADRENERGIC RECEPTORS (β-AR) play critical roles in the regulation of blood pressure and smooth muscle tone, cardiac chronotropy and inotropy, and energy metabolism. Virtually all organ systems express at least one of the three known β-ARs (β₁, β₂, and β₃), which explains the pleiotropic effects that follow nonspecific β-AR agonist administration. From a therapeutic standpoint, there are many instances when β-AR subtype-selective stimulation or blockade is desired, and therefore, a detailed knowledge of subtype-specific functions is necessary. The traditional approach to the assignment of subtype-specific functions has been to use subtype-selective agents. Although these are indispensable for many in vitro or ex vivo applications, their relative selectivity in vivo can be compromised and may differ significantly from that observed in vitro. As an example, biodistribution of the well-known β₁-AR selective antagonist CGP-20712A in rats differs significantly from the true β₁-AR subtype distribution (48). The ability to selectively block or activate a given receptor subtype is thus dependent on the dose of such agents, as well as their relative selectivity for the "specific" vs. "nonspecific" subtypes in vivo. These variables must be taken into account when attempting to define subtype-specific functions, especially when more than one subtype is present in a given target tissue, or when ligand accessibility to distinct subtypes differs at the target site. The demonstration that most or all β-ARs are coexpressed in tissues such as heart (6), adipose (24), and vasculature (4, 32, 42) makes pharmacological isolation of subtype-specific functions of β-ARs a significant challenge.

The distribution of β-ARs in the heart has been determined both pharmacologically (6) and by quantitation of mRNA levels (46). In mammals, both β₁- and β₂-ARs are expressed in the heart, with β₁-ARs predominating at an ~75:25 ratio in ventricles and an ~60:40 ratio in atria and conduction tissue (6, 40). In human heart preparations studied with subtype-specific agonists and antagonists, both β₁- and β₂-ARs appear to couple to positive inotropic and chronotropic responses (6). In mice, the myocardial ratio of β₁- to β₂-AR and total β-AR density are similar to that found in humans. However, our recent studies in β₁-AR gene-targeted mice have demonstrated that cardiac β-AR inotropic and chronotropic responsiveness as well as adenylyl cyclase stimulation appear to be mediated solely through the β₁-AR (38). It is still not clear whether the differences noted between these studies are because of species-specific coupling behaviors or the inability of subtype-selective agents to effectively discriminate β-AR subtype functions in vivo.

Both exercise and stimulation of the baroreflex elicit physiological changes that are dependent on the integration of both sympathetic and parasympathetic nervous system activity. Whereas β-ARs are implicated as primary sympathetic nervous system targets in the reflex response to altered hemodynamics, their proportional contribution to the total baroreflex response and the subtype specificity are not clearly defined (3, 13, 18, 41, 49). Short-term adaptive responses to exercise also require an integrated response from multiple neuroeffector systems. Cardiac output increases commensurate with increasing exercise loads to meet peripheral oxygen and energy demands, and the well-known effects of β-AR agonists to regulate both heart rate and contractility suggest that these receptors may play an important role in this response. There is controversy, however, on the overall requirement for β-ARs during exercise. Whereas some studies have shown that maximal exercise capacity is reduced in the presence of β-AR antagonists (1, 9, 10, 19–21, 28, 44, 47, 50), others have failed to demonstrate this effect (5, 25, 29, 36). It is somewhat surprising that functional compensation can occur under β-AR blockade, given the fact that β-ARs have been invoked in virtually every aspect of the physiological response to exercise. Cardiac chronotropy and inotropy, skeletal muscle vasodilatation, lipid and...
carbohydrate mobilization, and airway conductance are all enhanced by \( \beta_1 \)-AR stimulation during exercise (2, 28, 51).

In the present study, we have complimented what has traditionally been a pharmacological approach to studying receptor function with a genetic approach. Genetic ablation of \( \beta_1 \)-AR expression allows for an unambiguous test of \( \beta_1 \)-AR function in the mouse and, when combined with the pharmacological approach, can also provide information on other important cardiovascular control mechanisms. Knockout studies can also reveal novel or unexpected functions, such as the prenatal lethality observed in \( \beta_1 \)-AR-deficient mice (38), which had only weak support from prior pharmacological studies (23). On the basis of its predominant role in regulating cardiac inotropism and chronotropism, we hypothesized that \( \beta_1 \)-AR function would be critical for maintaining both resting and maximally stressed cardiovascular function. However, in mice lacking the \( \beta_1 \)-AR, we unexpectedly found that basal cardiovascular indexes were essentially unaltered and, furthermore, that the capacity to respond to stresses such as exercise was normal. The failure to impact exercise performance occurred despite the fact that chronotropic reserve was severely blunted in \( \beta_1 \)-AR knockout mice. The use of conscious and unrestrained animals is a critical component of these studies, since many aspects of reflex control and receptor function are lacking in isolated or anesthetized animal preparations. Moreover, the ability to observe conscious mice both at rest and under exercise stress provides additional opportunities to uncover phenotypic alterations relevant to \( \beta_1 \)-AR signaling.

**MATERIALS AND METHODS**

**Generation of \( \beta_1 \)-AR-Deficient Mice**

Creation of the \( \beta_1 \)-AR knockout mouse has been previously described (38). Eight- to 12-wk-old mice of both sexes were used for the studies here and were derived from a mixed strain background of 129Sv, C57Bl6/J, and DBA/2J. Wild-type control mice were either same-sex littermates or age- and sex-matched mice of the same strain background.

**Mouse Instrumentation**

Catheters were surgically implanted in either the left carotid artery or the left carotid artery plus the left jugular vein isolated by isoflurane anesthesia. Briefly, anesthesia was induced with 3% (vol/vol) isoflurane in oxygen using an isoflurane vaporizer (Airco, Madison, WI) and after induction was maintained at 1.25–1.75%. The vessels were cannulated with a stretched Intramedic PE-10 polyethylene catheter (Clay Adams, Parsippany, NJ), which was filled with heparinized normal saline, sutured in place, and tunneled to the back of the mouse. Blood pressure was measured using a DTX Plus pressure transducer (Spectramed, Oxnard, CA) amplified with a Gould eight-channel recorder, and the analog pressure was digitized using a Data Translation Series DT2801 analog-to-digital converter (Marlboro, MA). Digital signals were analyzed and stored using Crystal Biotech Dataflow data acquisition software (Crystal Biotech, Hopkinton, MA). Heart rate measurements were determined online, derived from the pressure recordings. Drugs were infused through the arterial catheter as a bolus (1–3 µl/g) except in the case of hexamethonium, which was infused at 60 mg·kg\(^{-1} \cdot h^{-1}\) over 15 min. L-isoproterenol hydrochloride (3 µg/kg), atropine sulfate (1 mg/kg), L-propranolol hydrochloride (3 mg/kg), and sodium nitroprusside (SNP; 30 µg/kg) were purchased from Sigma (St. Louis, MO). Phenylephrine (100 µg/kg), hexamethonium (15 mg/kg), and ICI-118,551 (1 mg/kg) were purchased from Research Biochemicals International (Natick, MA).

**Exercise Protocols and Metabolic Measurements**

Mice were subjected to either constant or graded treadmill exercise, using a Columbus Instruments Simplex II metabolic rodent treadmill, fitted with Oxymax oxygen and carbon dioxide gas analyzers (Columbus Instruments, Columbus, OH). For graded exercise, mice were placed in the exercise chamber and allowed to equilibrate (usually 30–60 min). Treadmill activity was initiated at 3.5 m/min, 0° inclination, and increased to 5 m/min, 2° inclination 3 min later. Treadmill speed and inclination were then increased by 2.5 m/min and 2° inclination every 3 min thereafter. Preoperative mice were initially subjected to this protocol, with regular stepwise increases until mice stopped running from exhaustion. Postoperative mice were run to a final end point of 20 m/min and 14° inclination. We have previously shown linear relationships among heart rate, oxygen consumption (VO\(_2\)), and carbon dioxide production (VCO\(_2\)) during graded treadmill exercise in mice (8). For constant treadmill exercise, the treadmill was fixed at 20 m/min, 14° inclination. Preoperative mice were run under these conditions to exhaustion, whereas postoperative mice were transitioned from rest to 20 m/min, 14° for 4 min. For constant treadmill studies under muscarinic blockade, mice were given atropine (1 mg/kg) after the first run cycle, allowed to equilibrate at rest for 10–20 min, and then subjected to another 4 min of 20 m/min, 14° work load. For \( \beta_2 \)-AR blockade studies, mice run under atropine blockade were allowed to rest 10 min, ICI-118,551 was administered at 1 mg/kg, and mice were allowed to equilibrate for 5–10 min before exercise was reinitiated.

**Physiological Measurements**

We have previously reported basal heart rate and blood pressure values of unrestrained wild-type and \( \beta_1 \)-AR knockout mice (38). These previous studies, however, were performed on mice that were operated on while under methoxyflurane anesthesia. For the studies reported here, isoflurane was used as the surgical anesthetic. Isoflurane was chosen for subsequent use because of its more rapid induction and recovery times, minimal long-term effects on cardiovascular indexes, and more reliable dosing (45). Postsurgical survival in our hands was also increased when using isoflurane as an anesthetic. For all studies, mouse recovery after surgery was at least 24 h. After recovery, arterial catheters were connected to the pressure transducer, and mice were allowed to equilibrate for a minimum of 20–30 min before drugs were administered or exercise was initiated. Basal heart rate and blood pressure were determined after this initial period. Basal values were taken as a 1-min average of mean blood pressure and heart rate during a period when the mouse was awake, neither grooming nor eating, immediately preceding the first drug or exercise challenge. Each single measurement represents a 10-s average of mean blood pressure and heart rate, so basal values represent an average of six individual measurements. When the percent change is reported, the time period is...
over which the average was calculated varies according to the
stimulus and is expressed relative to the 1 min just preceding
drug challenge. The number of single measurements aver-
gaged to determine a mean value is based on the duration of
effect, which differs substantially between these agents. Iso-
proterenol has a peak effect that persists for ~30 s, and
therefore, three measurements were used to determine the
mean. SNP and phenylephrine effects are more transient,
with peak effects lasting 10–20 s, and therefore, two mea-
surements were used to determine mean stimulated values.
Atropine, propranolol, and hexamethonium effects are stable
and long lasting; 1-min (6 measurements) means were deter-
mined for these agents 5 min (atropine) or 10 min (proprano-
lo and hexamethonium) after dosing. For the reported ex-
ercise values, 1-min averages of heart rate and blood pressure
were taken during the last minute of the exercise work load,
whether constant or graded. The recovery values reported
after exercise represent 1 min averages taken 10 min after
exercise termination.

Measurements of total hemoglobin (Hb) and percent satu-
ration of Hb in mixed venous and arterial blood samples were
determined both at rest and under constant treadmill exer-
cise (20 m/min, see Exercise Protocols and Metabolic Measure-
ments). Arterial or mixed venous blood (100 µl) was with-
drawn into heparinized 1-ml syringes, and determinations of
total Hb (in g/dl) and percent oxygen saturation were per-
formed by hemoximetry (model OSM 3; Radiometer, Copen-
hagen, Denmark). Total Hb values were corrected for dilution by
heparin sodium, which filled the dead space of the syringe.

Statistics

Statistical comparisons between groups are reported in the
legends to Figs. 1–4. Values are reported as means ± SE.
Statistical analysis was carried out using paired and un-
paired Student’s t-test and two-way analysis of variance, with
P < 0.05 considered as significant. Differences are assumed to
be nonsignificant unless otherwise noted.

RESULTS

Cardiovascular Indexes in β1-AR Knockout Mice at
Rest and After Pharmacological Manipulation

Resting values. Mice were instrumented under iso-
flurane anesthesia and allowed to recover for ~24 h before
measurements were taken. There was no difference in
the baseline heart rate and blood pressure of conscious,
unrestrained wild-type and β1-AR knockout mice (Fig.
1A), although the high variability in heart rate and
blood pressure seen in conscious, unrestrained mice (8)
may have obscured the slight trend toward lower values in β1-AR
knockouts.

Response to β-AR agonists and antagonists. Wild-
type mice given isoproterenol responded with a simulta-
nous drop in mean blood pressure and increase in
heart rate. Although β1-AR knockouts showed a qualita-
tively similar response, the percent increase in heart
rate after isoproterenol was significantly greater in
wild types than β1-AR knockouts, whereas the hypoten-
sive effect was comparable in magnitude (Fig. 1B).

We also tested the effects of propranolol, a nonspecific
β-AR antagonist, on cardiovascular function in wild-
type and β1-AR knockout mice. Propranolol markedly
slowed heart rate in wild-type mice, whereas in β1-AR
knockouts, heart rate did not change (Fig. 1B). Notably,
propranolol also elicited a modest hypertension in both
genotypes, which may reflect blockade of peripheral
β2-ARs to circulating catecholamines.

Baroreflex stimulation in β1-AR knockout mice. We tested
the hypothesis that the tachycardic effect of isoproter-
enol in β1-AR knockouts was because of an indirect,
baroreflex-mediated mechanism by administering the
direct vasodilator SNP, both alone (Fig. 1B) and in the
presence of atropine (see below). The baroreflex re-
response stimulated by SNP leads to a simultaneous

Fig. 1. Basal and stimulated cardiovascular measurements in wild-type and β1-
adrenergic receptor (AR) knockout mice. A: basal heart rate and mean blood pres-
sure, in beats/min and mmHg, respectively, are shown for wild-type mice (WT; solid bars, n = 24) and β1-AR knockouts (β1KO; hatched bars, n = 36). B: percent change in heart rate or blood pressure are shown after administration of various agents. Iso, isoproterenol (3 µg/kg), n = 17 for WT, n = 24 for β1KO; SNP, sodium nitroprusside (30 µg/kg), n = 11 for WT, n = 16 for β1KO; Prop, propranolol (3 mg/kg), n = 7 for WT, n = 14 for β1KO; Phen, phenylephrine (100 µg/kg), n = 4 for WT, n = 5 for β1KO. *P < 0.05, †P < 0.005 by unpaired Student’s t-test comparing WT and β1KO.
increase in sympathetic outflow and a decrease in parasympathetic outflow, which generally results in a rapid restoration of blood pressure and tachycardia. Both $\beta_1$-AR knockouts and wild-type mice respond to SNP with an initial drop in blood pressure and consequent increase in heart rate. Neither of these responses was statistically different comparing wild types and $\beta_1$-AR knockouts. The magnitude of the tachycardic response in $\beta_1$-AR knockouts was similar when comparing isoproterenol to SNP (22.5 $\pm$ 4.7 vs. 25.6 $\pm$ 3.9% increase in heart rate, respectively; P = NS), suggesting that the baroreflex arc is primarily responsible for the heart rate changes induced by isoproterenol in $\beta_1$-AR knockout mice.

The $\alpha_1$-AR agonist phenylephrine was also tested for its ability to stimulate the baroreflex, but in this case, the primary hypertension mediated by peripheral $\alpha_1$-ARs is followed by reflex bradycardia. Both wild-type mice and $\beta_1$-AR knockouts show the same qualitative response of hypertension and reflex bradycardia, and there was no statistical difference in this response between the two genotypes (Fig. 1B).

Role of vagal mechanisms in $\beta_1$-AR knockout cardiovascular regulation. We next tested the hypothesis that parasympathetic (i.e., vagal) mechanisms were playing a significant role in the tachycardic response of $\beta_1$-AR knockout mice to isoproterenol or SNP by administration of the muscarinic receptor antagonist atropine. The dose of atropine used (1 mg/kg) was determined by its ability to block the hypotensive response to intra-arterially administered carbachol (20 µg/kg). As can be seen in Fig. 2A and summarized in Fig. 2B, atropine significantly elevated heart rates in both wild-type and $\beta_1$-AR knockout mice. Heart rate at 5 min after atropine dosing was significantly different between the two genotypes (wild type, 572.5 $\pm$ 18.2 beats/min; $\beta_1$-AR knockout, 526.8 $\pm$ 6.6 beats/min; P < 0.02, n = 11 and 15, respectively). Of note, the qualitative response of
\(\beta_1\)-AR knockouts to atropine differs significantly from wild types (Fig. 2A). Wild-type mice showed an initial marked tachycardia, to rates in excess of 600 beats/min, immediately after atropine administration. This brief increase in heart rate partially attenuated over the next 2–3 min, which we suspected was through a withdrawal of sympathetic tone. \(\beta_1\)-AR knockouts also displayed an initial tachycardia in response to atropine; however, the response was sustained and stable and lacked the initial “overshoot” seen in wild-type mice. The normal fluctuations in heart rate observed in conscious mice while grooming, sleeping, or eating (8) were not seen in atropine-treated \(\beta_1\)-AR knockouts, suggesting that chronotropic regulation in these mice depends almost completely on their ability to regulate parasympathetic or vagal tone.

Isoproterenol, SNP, and phenylephrine were then administered while mice were under muscarinic receptor blockade with atropine (Fig. 2A, summarized in Fig. 2B). Wild-type mice retain chronotropic responsivity to these agents, whereas \(\beta_1\)-AR knockout responsiveness is severely attenuated. This again suggests that chronotropic responsiveness is largely governed by vagal tone in \(\beta_1\)-AR knockout mice. The chronotropic effect of isoproterenol in \(\beta_1\)-AR knockout mice is reduced by 85% under atropine blockade (130.8 ± 35.6 beats/min increase over basal before blockade vs. 20.2 ± 2.2 beats/min increase over basal after blockade; P < 0.02), whereas the chronotropic effect of SNP in \(\beta_1\)-AR knockouts is reduced by 90% under atropine blockade (115.1 ± 16.2 beats/min increase over basal before blockade vs. 11.7 ± 7.1 beats/min increase over basal after blockade; P < 0.01). Despite the fact that the vast majority of chronotropic responsiveness in \(\beta_1\)-AR knockouts appears to be vagal in nature, the residual chronotropic response to isoproterenol during atropine blockade (3.7 ± 0.4%) was statistically significant (Fig. 2B). This residual chronotropic reserve seen in \(\beta_1\)-AR knockouts under atropine blockade is not the result of incomplete muscarinic receptor blockade, since the baroreflex response to phenylephrine is completely abolished at this dose of atropine in both wild types and \(\beta_1\)-AR knockouts (Fig. 2B, right). The effect of atropine (alone and in conjunction with these other agents) on blood pressure was virtually identical in wild types and \(\beta_1\)-AR knockouts (Fig. 2B, bottom).

Effect of ganglionic blockade with hexamethonium. We tested the effects of hexamethonium, a ganglionic blocking agent, in wild types and \(\beta_1\)-AR knockouts. This agent serves to block autonomic nervous system input at the nicotinic ganglia, which are common to both sympathetic and parasympathetic nervous systems. Hexamethonium (15 mg/kg) was infused slowly over a 10- to 20-min period, and after the infusion, mice were allowed to stabilize for at least 10 min before we administered other agents. There were small but nonsignificant decreases in the heart rates of wild-type and \(\beta_1\)-AR knockout mice after hexamethonium infusion in six wild-type mice and six \(\beta_1\)-AR knockouts (wild type, 528.6 ± 20.5 beats/min pre- vs. 465.8 ± 32.3 beats/min posthexamethonium; \(\beta_1\)-AR knockouts, 463.2 ± 16.9 beats/min pre- vs. 439.2 ± 21.6 beats/min posthexamethonium), whereas mean blood pressure was reduced significantly in both genotypes after hexamethonium administration (wild type, 117.2 ± 6.3 mmHg pre- vs. 98.0 ± 6.2 mmHg posthexamethonium; \(\beta_1\)-AR knockouts, 104.9 ± 6.5 mmHg pre- vs. 91.5 ± 6.4 mmHg posthexamethonium; P < 0.05 for both by paired t-test). These data suggest that when autonomic outflow is blocked, the intrinsic heart rates and blood pressures are comparable between the two genotypes. Under hexamethonium blockade, the baroreflex heart rate response to SNP was blocked by 70% in wild-type mice and 61% in \(\beta_1\)-AR knockouts (data not shown).

Cardiovascular Response to Exercise

Given the important role that \(\beta_1\)-ARs play in the regulation of cardiac inotropy and chronotropy, we tested the hypothesis that exercise performance would be decreased in \(\beta_1\)-AR knockout mice. We used two different exercise protocols, one graded and the other constant (see MATERIALS AND METHODS). In the first phase of exercise protocols, we tested maximal exercise capacity in noninstrumented animals running either the graded or constant protocol until physical exhaustion, while we measured \(\dot{V}O_2\) and \(\dot{V}CO_2\). In the second phase, intra-arterial catheters were surgically implanted in mice to monitor heart rate and blood pressure during the exercise regimen, and mice were run on either graded or constant exercise protocols.

The first phase of exercise experiments, which tested maximal exercise capacity, revealed that wild-type and \(\beta_1\)-AR mice achieved essentially the same work load before exhaustion. On both the graded and constant treadmill protocols, mice ran approximately the same distance, 450–500 m, with no significant differences between wild types and \(\beta_1\)-AR knockouts in either exercise regimen (Fig. 3A). On average, both wild-type and \(\beta_1\)-AR knockout mice terminated their run on the graded protocol during the 27.5 m/min, 20° inclination phase of their run (see MATERIALS AND METHODS). We have determined from other experiments that 27.5 m/min is well below the maximum acute sprinting speed for untrained mice (data not shown), indicating that when grading exercise, stoppage was from exhaustion, not an intrinsic limitation on running speed.

Figure 3B displays the \(\dot{V}O_2\) and \(\dot{V}CO_2\) of wild-type and \(\beta_1\)-AR knockout mice during the graded exercise protocol. There were no differences in \(\dot{V}O_2\) and \(\dot{V}CO_2\) between wild types and \(\beta_1\)-AR knockouts over the range of work loads, although there was a nonsignificant trend toward lower values in \(\beta_1\)-AR knockout mice. Of note, the general shape of both curves is well conserved, suggesting common response pathways during physical exertion. The disparity between the number of wild types and \(\beta_1\)-AR knockouts at the highest work loads reflects the dropout of animals during the highest exercise work loads because of exhaustion. The ratio between \(\dot{V}CO_2\) production and \(\dot{V}O_2\), or respiratory exchange ratio (RER), is one indicator of the transition between aerobic and anaerobic metabolism. In our hands, maximum RER is achieved usually 1–2 min after cessation of
exercise. A comparison of basal RER as well as the maximum achievable RER reveals no significant differences between wild-type and β1-AR knockout mice (basal RER, 0.82 ± 0.02 vs. 0.78 ± 0.02, respectively; maximum RER, 1.04 ± 0.03 vs. 1.05 ± 0.03, respectively; \( P = NS \)).

Although lack of the β1-AR does not appear to affect total exercise performance or the metabolic response to exercise, an examination of the cardiovascular response to exercise reveals striking differences. In the second phase of the exercise protocol where mice were catheterized, blood pressure and heart rate could be directly monitored during all phases of the regimen. Figure 3C displays the chronotropic responses of wild type and β1-AR knockout mice to the graded treadmill exercise protocol. Wild-type mice showed a robust and work load-dependent increase in heart rate during the run, whereas the β1-AR knockout displayed little overall tachycardia and almost no work load-dependent increases in heart rate once the run had been initiated. At the maximum work load (20 m/min and 14° inclination), there was an ~200 beats/min difference between wild-type mice and β1-AR knockout. Exercise had little influence on mean blood pressure in either genotype, consistent with previous results in wild-type mice of various strains (8).

We also tested the effects of atropine on the chronotropic responses of wild types and β1-AR knockout to constant exercise (Fig. 4A). Naive mice of both genotypes were monitored at rest and at 20 m/min, 14°, then given atropine, and the experiment was repeated. Before atropine, wild-type mice showed a robust tachycardic response to the increased treadmill speed, whereas knockout mice showed a modest heart rate increase. After atropine administration, basal heart rates were elevated in both genotypes as expected. Atropine-treated wild-type mice had increased heart rate in response to the increased work load, and β1-AR knockout mice were still able to mount a small but significant increase in heart rate under muscarinic blockade (Fig. 4A). These studies suggest that in both genotypes vagal withdrawal accounts for ~50% of the tachycardic response to exercise, although the absolute increase in heart rate is much smaller for β1-AR knockout. Wild-type mice had increased heart rate 232.7 beats/min before atropine vs. 110.7 beats/min after atropine, whereas β1-AR knockout mice had increased heart rate 54.3 beats/min before atropine vs. 28.1 beats/min after atropine.

To test the hypothesis that the chronotropic reserve present in β1-AR knockout mice under atropine blockade is because of β2-AR activation, we administered the β2-AR selective antagonist ICI-118,551 to atropine-blocked mice and stimulated chronotropy through the constant treadmill exercise protocol. The results, shown in Fig. 4B, demonstrate that β2-AR blockade has no effect on...
DISCUSSION

The β1-AR plays a central role among G protein-coupled receptors in its ability to regulate both resting and stressed cardiac function. The purpose of these experiments was to test the hypothesis that β1-AR expression would be critical for maintenance of resting heart rate as well as the response to autonomic stimuli and exercise. We have demonstrated that the β1-AR is an important mediator of the in vivo chronotropic response and have used this model to demonstrate the importance of parasympathetic pathways in murine homeostatic control mechanisms. The use of awake and unrestrained mice is a critical component to the success of these studies, since reflex pathways can be obscured in anesthetized mice and are absent in in vitro preparations.

The finding that resting heart rates are not significantly altered in β1-AR knockout mice is somewhat surprising given the fact that both nonselective and β1-selective β-AR antagonists cause significant bradycardia in humans and other mammals (16). Despite this apparent lack of difference in heart rate between the two genotypes at rest, experiments using propranolol and atropine suggest that the β1-AR does play some role in the control of resting heart rate in the mouse. First, propranolol caused a significant bradycardia in wild-type but not in β1-AR knockout mice, suggesting that β1-AR signaling does partially regulate resting heart rate in normal mice. Second, the heart rates of β1-AR knockouts were significantly lower than wild-type mice after atropine administration. Both experiments reveal that a tonic level of β1-AR signaling is present and contributes to resting heart rate in wild-type but not in β1-AR knockout mice. Our inability to document statistically significant baseline differences in resting heart rate may result from the high degree of heart rate variability in nonanesthetized animals (8), which can be significantly reduced by either propranolol or atropine (17, 33). With the blockage of parasympathetic outflow with atropine, variability was reduced to the point where differences in heart rate between wild-type mice and β1-AR knockout mice became discernible.

Whereas we have previously demonstrated that wild-type and β1-AR knockout mice did not statistically differ with respect to basal heart rate and blood pressure values (38), we report these values here as well since the surgical anesthetic agent was altered. Of interest, this change allowed us to observe a chronotropic responsiveness to isoproterenol that was lacking in our earlier studies (38). We attribute this to the variable effect of different anesthetic agents on cardiac reflexes, even after a 12- to 24-h recovery period. In the present study, chronotropic responses could be observed in β1-AR knockouts to a variety of stimuli, including isoproterenol, SNP, and exercise. Of note, the basal heart rate value we previously obtained after surgery with methoxyflurane (542.6 ± 28.9 beats/min) is comparable to the peak heart rate value obtained after isoproterenol administration in mice instrumented under isoflurane studied here (547.3 ± 10.0 beats/min), suggesting that tachycardic reserve was limited or absent in mice studied within 24 h of methoxyflurane anesthesia.

The importance of vagal or parasympathetic mechanisms in the control of murine heart rate is clearly
shown by these studies. Whereas β₁-AR knockouts display a qualitatively normal baroreflex response, the chronotropic component following a hypotensive stimulus can be almost totally blocked by pretreatment with atropine. Wild-type mice under the same conditions, while exhibiting an attenuated chronotropic response, retain significant chronotropic reserve in comparison with β₁-AR knockouts. The difference in chronotropic response noted between wild types and β₁-AR knockouts occurs despite the fact that the magnitude of the hypotensive stimulus is equivalent between the two genotypes. This suggests that vascular responsiveness and peripheral β₂-AR function is intact in β₁-AR knockouts. Given the extremely high heart rates in comparison with other species, a common assumption has been that sympathetic drive predominates in maintaining resting heart rate in the mouse (17). However, the demonstration that a significant portion of chronotropic reserve, whether stimulated by drugs or exercise, is blocked by atropine reveals the importance of vagal mechanisms. The relative contributions of parasympathetic vs. sympathetic control in the mouse are reminiscent of that found in larger mammals and humans (26), with the exception that the set point for intrinsic rate is considerably higher in the mouse. The phenomenon of high set points with balanced antagonism between parasympathetic and sympathetic input is also reminiscent of that seen in neonates of many species. The range for heart rate in normal mice can extend to a low of ~300 beats/min, and under significant stress such as exercise can exceed ~800 beats/min. On the basis of our observations, the upper end of this heart rate spectrum, above ~550 beats/min, is mediated by sympathetic stimulation through the β₁-AR. In comparison, in the human, the transition between vagal and sympathetic control occurs at a heart rate of ~100 beats/min (37, 39). In the mouse, vagal outflow can also increase under some conditions, such as the baroreflex response to phenylephrine, and serves to drive heart rate downward to the ~300 beats/min range in both wild-type mice and β₁-AR knockouts. Maximum achievable heart rates, whether stimulated by drugs or exercise, were unaffected by atropine within a given genotype. This suggests that under conditions of maximal stress, vagal input is small or nonsignificant.

Perhaps the most striking phenotype of the β₁-AR knockout mouse is the fact that, despite the large chronotropic deficit seen during exercise, total exercise capacity is not different from wild types. Treadmill exercise is one of the most effective means to increase heart rate, and, coupled with our previous findings which demonstrated a lack of inotropic response in β₁-AR knockouts, we expected that this disparity between wild types and β₁-AR knockouts would produce a functional deficit. Surprisingly, neither exercise capacity nor the metabolic response to exercise is significantly altered in these mice. The possible mechanisms by which the β₁-AR knockout mouse can compensate for this chronotropic deficit are revealed by an examination of the Fick equation, which yields the following relationship: \( \dot{V}O_2 = \text{heart rate} \times \text{stroke volume} \times (A-V O_2 \text{ difference}) \), where A-V O₂ difference represents the difference in oxygen content between arterial and venous blood. At all work loads, \( \dot{V}O_2 \) is essentially equivalent between wild types and β₁-AR knockouts, whereas the heart rate response is markedly attenuated in β₁-AR knockout mice. To achieve the same \( \dot{V}O_2 \) as wild types, β₁-AR knockouts must compensate by either larger increases in stroke volume or larger increases in oxygen extraction than wild types. On the basis of our examination of arterial and venous oxygen content, our data do not support the hypothesis that β₁-AR knockouts compensate through increased oxygen extraction, but rather that compensation must be occurring through increases in stroke volume. In support of the idea that stroke volume changes underlie the compensatory changes in β₁-AR knockouts, their lower heart rates would allow for increases in diastolic filling time (15). This is further supported by human studies where subjects under β-AR blockade maintained equivalent cardiac outputs by increasing stroke volume via the Frank-Starling mechanism (5), whereas chronotropic and inotropic responses remained largely suppressed. Pharmacological approaches to study the impact of β-AR blockade on exercise have either shown deficits (1, 9, 10, 19, 20, 28, 47, 50) or no effect (5, 25, 29, 36) on exercise performance. Our attempt to test the effect of genetic ablation of the β₁-AR has shown that acute exercise performance is not affected by the congenital absence of β₁-ARs and also suggests that the chronotropic component of the exercise response may be less critical than previously thought.

Despite the central role that we have attributed to β₁-AR function in the control of heart rate, several lines of evidence indirectly suggest that other chronotropic regulators may play a minor role in both resting and stimulated heart rate. For example, there is a small but significant increase in heart rate after isoproterenol administration or exercise in atropine-blocked β₁-AR knockouts (atropine blockade is complete based on the failure of phenylephrine to produce bradycardia). Also, full autonomic blockade with hexamethonium results in lower heart rates than that achieved by parasympathetic blockade with atropine, suggesting that sympathetic nervous system-derived neuroeffectors may be modulating heart rate. On the basis of the failure of propranolol to produce bradycardia in β₁-AR knockouts at rest, and the inability of the β₁-AR-selective antagonist ICI-118,551 to block chronotropic responses to exercise, we assume that the β₂-AR is not fulfilling this role. Our previous study also supports the lack of β₂-AR involvement in direct chronotropic responses (38). Whereas overexpression of the β₂-AR in mice (30) results in hyperfunctioning hearts (showing increases in rate, inotropy, and adenylate cyclase activation), it appears that the endogenous β₂-ARs show little if any functional coupling in the heart. These differences may be reconciled by the differences in methodology between the studies: supraphysiological expression of the β₂-AR by a nonhomologous promoter can clearly give rise to constitutive signaling; however, one cannot
extrapolate from this transgenic model to the function of endogenous β₁-ARs expressed at physiological levels. Of the several receptor subtypes whose activity may be affected by ganglionic blockade but not by β-blockade, both α₁-ARs (34) and the receptors for neuropeptide Y (11) have shown direct chronotropic coupling in isolated atria. Other potential chronotropic modulators that could be indirectly affected by ganglionic blockade include angiotensin II (27), serotonin (31), and endothelin (35). Further studies will be necessary to clarify the role of these mediators in controlling resting mouse heart rates and whether they can functionally compensate for β₁-AR signaling defects in mice lacking the β₁-AR.

Together with our previous characterization of the β₁-AR knockout, these studies suggest that, at least postnatally, loss of the β₁-AR has little functional impact on either resting or stressed cardiovascular performance. These results are striking given the central role that has been attributed to β₁-AR function in the heart. The loss of β₁-AR density and function in many forms of heart failure has been hypothesized to be a primary pathophysiological mechanism underlying the functional decline in patients suffering from this disease (6). Our studies show that whereas decreased β₁-AR function may lead to some functional deficits, downregulation of this receptor cannot be the primary cause of pump failure in a structurally normal heart. Such alterations in β₁-AR density and/or function may be an “epiphenomenon,” playing a minor role in the etiology of heart failure. It is clear, however, that inappropriate stimulation of the β₁-AR system in chronic heart failure is detrimental (43), whereas β₁-AR blocker therapy has beneficial effects (12, 14, 22). Once born, β₁-AR knockout mice appear normal in all respects in comparison with their wild-type counterparts and clearly show the proper response to stresses such as exercise. Cardiac histology (38) and echocardiographic studies (7) also fail to uncover any defects in β₁-AR knockout mice. This genetic model of β₁-AR ablation will allow future studies to address the importance of β₁-AR signaling to the development and progression of heart failure.

In summary, our studies indicate that within the murine sympathetic nervous system, β₁-ARs are probably the largest single contributor to cardiac chronotropic responses. In addition to the utility of the β₁-AR knockout in determining a direct role for β₁-ARs in cardiovascular function, this knockout model is also valuable for its ability to confirm the role of other homeostatic control mechanisms that are important for mammalian cardiovascular regulation.

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