Cardiovascular and Metabolic Alterations in Mice Lacking Both β1- and β2-Adrenergic Receptors*

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The activation state of β-adrenergic receptors (β-ARs) in vivo is an important determinant of hemodynamic status, cardiac performance, and metabolic rate. In order to achieve homeostasis in vivo, the cellular signals generated by β-AR activation are integrated with signals from a number of other distinct receptors and signaling pathways. We have utilized genetic knockout models to test directly the role of β1- and/or β2-AR expression on these homeostatic control mechanisms. Despite total absence of β1- and β2-ARs, the predominant cardiovascular β-adrenergic subtypes, basal heart rate, blood pressure, and metabolic rate do not differ from wild type controls. However, stimulation of β-AR function by β-AR agonists or exercise reveals significant impairments in chronotropic range, vascular reactivity, and metabolic rate. Surprisingly, the blunted chronotropic and metabolic response to exercise seen in β1/β2-AR double knockouts fails to impact maximal exercise capacity. Integrating the results from single β1- and β2-AR knockouts as well as the β1/β2-AR double knockout suggest that in the mouse, β-AR stimulation of cardiac inotropy and chronotropy is mediated almost exclusively by the β1-AR, whereas vascular relaxation and metabolic rate are controlled by all three β-ARs (β1-, β2-, and β3-AR). Compensatory alterations in cardiac muscarinic receptor density and vascular β3-AR responsiveness are also observed in β1/β2-AR double knockouts. In addition to its ability to define β-AR subtype-specific functions, this genetic approach is also useful in identifying adaptive alterations that serve to maintain critical physiological setpoints such as heart rate, blood pressure, and metabolic rate when cellular signaling mechanisms are perturbed.

The β-adrenergic receptors (β1-, β2-, and β3-AR)1 belong to the superfamily of G-protein-coupled receptors (1). Both sequence comparisons and functional studies suggest that these three receptors share many structural and mechanistic features (2). Agonist stimulation of cloned and exogenously expressed β-ARs has demonstrated that all three subtypes can couple through Gαs to stimulate adenylyl cyclase activity (3–5). Despite these common structural and functional properties, however, individual β-AR subtypes in vivo remain as distinct therapeutic targets due to a number of factors that actually serve to distinguish them. These distinctions include tissue-specific expression patterns, the ability to couple to different G-proteins, pharmacological heterogeneity, and differences in agonist-dependent desensitization (6, 7).

β-AR subtypes can be distinguished pharmacologically by synthetic as well as natural ligands. The β1-AR subtype shows little preference for epinephrine or norepinephrine, whereas the β2-AR preferentially interacts with epinephrine (8, 9). More recent experiments demonstrate that the β3-AR (previously termed “atypical”) preferentially interacts with norepinephrine over epinephrine. Synthetic subtype-selective agents have been developed which display much greater selectivity than these endogenous catecholamines. Some typical examples of these would include the antagonists CGP20712A (β1-AR-selective) and ICI118551 (β2-AR-selective) and the agonist CL316243 (β3-AR-selective). Such synthetic compounds have proven invaluable for studying β-AR pharmacology and function (2, 10).

In vivo, β-ARs are known to modulate a wide range of physiological processes, from cardiac chronotropy and inotropy to vascular and smooth muscle tone, metabolism, and behavior. Functional assignment of β-AR subtype functions using pharmacological tools suggests that the β1-AR is the predominant subtype regulating heart rate and contractility, although at least in the human, β2-ARs are also thought to participate. β2-ARs have been thought to be the predominant subtype mediating the vascular smooth muscle relaxant properties of β-AR agonists. The β3-AR was initially identified and proposed to be the major β-AR subtype controlling lipolysis in adipose tissue. Although these functional divisions are not absolute, they appear to be well conserved across species and serve as a convenient framework for β-AR classification. However, defining β-AR subtype-specific functions in vivo can present significant challenges. Some subtype-selective agents display non-ideal behavior in vivo, either due to poor biodistribution or cross-reactivity with unrelated receptors. Gene disruption, or “knockout” experiments, has proven to be a useful approach in defining adrenergic receptor function in vivo. To date, this technique has been used to disrupt expression of all three α2-AR subtypes, the α1b-AR, the β1-, and the β3-ARs (11–16), and most recently, the β2-AR (17). When the pharmacologic tools outlined above are used in conjunction with genetic techniques, the power to reveal novel functions and mechanisms of action can be greatly enhanced.

Given the prominent role of β-AR signaling in the maintenance of normal physiology in vivo, we sought to test the functional consequences of β-AR gene disruption via a combi-
natatorial approach. In the companion article (17), the functional consequences of β2-AR disruption are described. We have previously described the functional consequences of β1-AR gene disruption (13, 18). We have now produced mice that lack both β1- and β2-ARs. The role of these two β-AR subtypes and the inferred role of the remaining β3-AR subtype in cardiovascular physiology and metabolism are reported here.

**Materials and Methods**

**Generation of β-AR Knockout Mice**—The generation of β1-AR knockout mice has been previously described (13). Briefly, disruption of the β1-AR gene was achieved using a positive-negative selection strategy to effect homologous recombination in the R1 embryonic stem cell line, using a targeting construct in which over 90% of the coding sequence was deleted. The strain background of β1-AR knockout mice was a mixture of 129SvJ, C57Bl/6J, and DBA/2 which is less prone to the prenatal mortality previously described (13). The targeting strategy used to create β2-AR knockout mice is described in the companion article (17) and is based on a similar positive-negative selection scheme and homologous recombination in the R1 embryonic stem cell line. Combination β1/2-AR double knockouts were generated by mating β2-AR homozygous knockouts (on a combined 129SvJ and FVB/N mouse strain background) to homozygous β1-AR knockouts. The resulting F1 generation of compound heterozygotes was subsequently intercrossed with wild-type mice to produce the resultant double knockouts used in our experiments. The wild type F1 generation of compound heterozygotes was subsequently intercrossed to create homozygous β1- and β2-AR knockouts. The strain background of β2-AR knockout mice is bred onto a multiple strain background. As described by our previous studies, all progeny were predicted to be wild type for both β1- and β2-AR (see Table I). The β2/β2-AR knockout double knockouts were bred to produce double knockouts used in our experiments. The wild type F2 mice were bred to produce wild type controls. Thus, the overall surviving F1 generation of compound heterozygotes was subsequently intercrossed to create homozygous β1- and β2-AR double knockouts were equivalent. Mice were genotyped for both β1- and β2-AR disruptions by Southern blotting of mouse tail biopsies (13, 17).

**Mouse Instrumentation**—Catheters were surgically implanted in either the left carotid artery or the left carotid artery plus the left jugular vein under isoflurane anesthesia. Briefly, anesthesia was induced with 3% (v/v) isoflurane in oxygen using an isoflurane vaporizer (Airco Inc., Madison, WI), and then induction was maintained at 1.25–1.75% while monitoring the responsiveness of the animal. The vessels were cannulated with a stretched Intramedic PE10 polyethylene catheter (Clay Adams, Parsippany, NJ), which was filled with heparinized normal saline, sutured in place, and tunneled to the back. Blood pressure was measured using a DTX Plus pressure transducer (Spectramed, Oxnard, CA) amplified with a Gould 8-channel recorder, and the analog pressure was digitized using a Data Translation Series DT2801 analog-digital converter (Marboro, MA). Digital signals were analyzed and stored using Crystal Biotech Dataflow data acquisition software (Crystal BioTech, Hopkinson, MA). Heart rate measurements were determined on-line and were derived from the pressure recordings. Drugs were infused through the internal catheter again in a volume of 1–3 μl/g. Isoproterenol, a-agonist (1,2-β-[4-(hydroxyphenyl)-ethyl]-aminomethyl)tetralone or 300 pm [3H]-methyl scopolamine (both from NEN Life Science Products). Nonspecific binding was performed in duplicate with 20 μM prazosin (Research Biochemicals, Natick, MA) or 5 μM atropine sulfate, respectively (Sigma). All binding reactions were carried out at room temperature for 2–3 hours prior to vacuum filtration onto Whatman GF-C filters and determination of membrane-bound radioactivity.

**Results**

**Generation and Recovery of β-AR Knockout Mice**—The generation and viability of β1-AR knockout mice (β1-AR−/−) have been described previously (13). Briefly, homozygous β1-AR knockout mice derived from heterozygote:heterozygote matings (β1-AR+/− × β1-AR+/−) are recovered at an unexpectedly low frequency as predicted from Mendelian inheritance, although this effect can be ameliorated if the β1-AR gene disruption is bred onto a multiple strain background. As described by Chruscielski et al. (17), the recovery of homozygous β2-AR knockout mice (β2-AR−/−) is in accord with expected Mendelian frequencies.

Crossties were carried out between homozygous β1-AR knockout mice and homozygous β2-AR knockout mice to generate compound heterozygote (β1-AR+/− × β2-AR−/−, see Materials and Methods), and these in turn were intercrossed to generate homozygous β1- and β2-AR double knockout mice (β1-AR−/− × β2-AR−/−). The expected frequency of recovering double knockout mice from compound heterozygote matings is 1 out of 16 or 6.25%. The observed frequency among weanlings was 7.23%, well within the expected range. Table I lists the expected and observed frequencies among the nine possible genotypes arising from the compound heterozygote intercrosses. The χ² distribution suggests that there are no significant deviations from Mendelian expectations either among individual genotypes or the group as a whole (χ² = 9.38 with 8 degrees of freedom).
freedom, $p = 0.29$, although $\beta_1$-AR knockouts ($\beta_1$-AR $-/ - ; \beta_2$-AR $+/ +$) appear to be less well represented, in accord with our previous findings (13). Double knockout:double knockout matings were subsequently performed to generate mice for the studies reported here. Litter size, maternal behavior, and pup viability all appeared to be normal in this group.

**Basal Cardiovascular Function**—Basal cardiovascular parameters were measured in awake, unrestrained mice by use of indwelling carotid arterial catheters. As seen in Fig. 1, neither baseline heart rate (range 400–470 beats/min) nor mean arterial blood pressure (range 115–125 mm Hg) are significantly different when comparing wild type mice ($\beta_1$-AR $+/ + ; \beta_2$-AR $+/ +$) to double knockouts ($\beta_1$-AR $-/ -; \beta_2$-AR $-/ -$).

**Response to Catecholamines**—Both isoproterenol and epinephrine were administered to wild type and $\beta_1$/$\beta_2$ double knockout mice. The grouped response to these agents is shown in Fig. 2A. Whereas the non-selective $\alpha$-AR agonist isoproterenol (5 $\mu$g/kg) elicits robust chronotropic and hypertensive responses in wild types, both of these responses are severely attenuated in $\beta_1$/$\beta_2$ double knockout mice. Of note, both responses are also significantly time-delayed in $\beta_1$/$\beta_2$ double knockouts in comparison to wild type responses. Furthermore, the small but significant increase in heart rate seen in double knockout mice in response to isoproterenol was attenuated by 93% in mice pretreated with the muscarinic antagonist atropine (1 mg/kg, data not shown), suggesting that the majority of this effect is due to the baroreflex, mediated by the vagus in response to the drop in blood pressure.

The effect of epinephrine (3 $\mu$g/kg) on $\beta_1$/$\beta_2$ double knockouts is seen in the right-hand panel of Fig. 2A. This endogenous catecholamine is a mixed, non-selective $\alpha$-AR and $\beta$-AR agonist. In wild types, this dose of epinephrine elicits tachycardia and a biphasic blood pressure response consisting of an initial brief hypertension followed by a more prolonged hypertensive response. In contrast, ablation of $\beta_1$- and $\beta_2$-AR signaling in the double knockout appears to convert this mixed $\alpha$- and $\beta$-AR agonist into a selective $\alpha$-AR agonist; these mice display concomitant bradycardia and a monophasic hypertensive blood pressure response. Again, the heart rate response to epinephrine seen in double knockouts appears to be predominantly due to baroreflex stimulation, as atropine pretreatment blocks 60% of this response (data not shown).

**Heart rate (bpm)**

**Mean Blood Pressure (mm Hg)**

**TABLE I**

| Genotype | Observed | Expected | $\chi^2$ | $p$ |
|----------|----------|----------|----------|-----|
| $\beta_1$ | $\beta_2$ | $+/+$ | $+/+$ | $+/+$ | $+/+$ | $+/+$ | $+/+$ | $+/+$ | $+/+$ | $+/+$ | $+/+$ | Totals |
| $+/+$ | 9 | 5.19 | 5.19 | 2.80 |
| $+/+$ | 9 | 10.38 | 10.38 | 0.15 |
| $+/+$ | 5 | 10.38 | 10.38 | 0.01 |
| $+/+$ | 13 | 20.75 | 14 | 0.66 |
| $+/+$ | 19 | 20.75 | 1 | 12.7 |
| $+/+$ | 14 | 10.38 | 5.19 | 3.38 |
| $+/+$ | 1 | 10.38 | 5.19 | 1.10 |
| $+/+$ | 1 | 10.38 | 5.19 | 0.13 |
| $+/+$ | 1 | 10.38 | 5.19 | 9.67 |

**Fig. 1. Basal cardiovascular indices in wild type and $\beta_1$/$\beta_2$ knockout mice.** Conscious, unrestrained mice instrumented with carotid arterial catheters were monitored for both heart rate (in beats per min [bpm]), and mean arterial blood pressure (mm Hg). 12 mice of each genotype were studied. $\beta_1$$\beta_2$KO refers to $\beta_1$/$\beta_2$ double knockout mice.

**Fig. 2B** is a compilation of the chronotropic and hemodynamic effects of isoproterenol on conscious and unrestrained $\beta_1$-AR knockouts, $\beta_2$-AR knockouts, and $\beta_1$/$\beta_2$ double knockouts. These are all displayed relative to the response seen in wild type mice (dotted line at 100%) and represent the peak chronotropic and vasodilatory responses obtained in each genotype, respectively. Based on these data, $-$50% of the chronotropic response to isoproterenol is lost when the $\beta_1$-AR is knocked out, whereas there is no detrimental effect on heart rate in $\beta_2$-AR knockouts. The combined $\beta_1$- and $\beta_2$-AR deficiency reduces the chronotropic response by over 85%. In terms of the vasodilatory response to isoproterenol, there appears to be a graded and additive attenuation of the hypertensive response with loss of the $\beta_1$-AR (20% reduction), $\beta_2$-AR (35% reduction), and combined $\beta_1$-$\beta_2$-AR (71% loss).
Cardiovascular response to catecholamines. A, isoproterenol (3 μg/kg) or epinephrine (3 μg/kg) were injected intra-arterially as a bolus to conscious unrestrained wild type mice (squares) or β1/2 knockouts (filled circles) at the 2.5-min time point. The effects on heart rate and blood pressure are shown for these two agents, expressed as the change (Δ) in beats/min (bpm) or mm Hg, respectively. Wild type, n = 10; β1β2KO, n = 9. B, the percentage contribution of individual β-AR subtypes is inferred from a comparison of β1-, β2-, and β1/2-AR knockouts. As above, the response to isoproterenol (1–3 mg/kg intra-arterially) is shown as a percentage of the wild type response for either the increase in heart rate or the decrease in mean blood pressure. The dotted line indicates 100% of the wild type response (n, β1 knockouts, n = 24; ■, β2 knockouts, n = 16; □, β1/2 knockouts, n = 9). Data for β1 knockouts were adapted from Rohrer et al. (18); data for β2 knockouts were adapted from Chrucinski et al. (17). Responses in all groups except heart rate in β2 knockouts are significantly decreased (p ≤ 0.01) in comparison to the wild type response. For heart rate responses, all genotypes display significantly different heart rate responses from each other (p ≤ 0.02). For blood pressure response, both β1 and β2 knockouts are significantly different from β1/β2 knockouts (p ≤ 0.01).

and β2-AR signaling on the response to the physical stress of exercise. Knowing that β-ARs are recruited during exercise to modulate heart rate, hemodynamics, airway conductance, and metabolic rate, we hypothesized that mice lacking both β1- and β2-ARs would be compromised in both exercise capacity as well as the cardiovascular and metabolic response to exercise. Using graded treadmill exercise (GTE) as a stimulus, where both speed and angle of inclination are progressively increased, both wild type mice and β1/2-AR double knockouts were tested for total exercise capacity as well as the physiological response to fixed end point GTE. Total exercise capacity was measured as cumulative distance run in non-instrumented mice, with treadmill speed and angle of inclination increasing by 2.5 m/min and 2° every 3 min until mice stopped running from exhaustion. Physiological responses to fixed end point GTE were obtained by running instrumented mice to a final end point of 20 m/min and 14° inclination (see “Materials and Methods”).

Experiments designed to test total exercise capacity showed no significant differences between wild types and β1/2-AR double knockouts with respect to cumulative distance run. Wild type mice ran a total distance of 578.8 ± 33.3 m (n = 7), whereas β1/2-AR double knockouts ran a total distance of 545.2 ± 30.0 m (n = 5). The metabolic response to GTE in the maximal exercise capacity experiment is shown in Fig. 4B, demonstrating that whereas both wild types and β1/β2-AR double knockouts have virtually identical levels of O2 consumption and CO2 production at rest, consistent deficits in both of these indices are revealed at all exercise levels in the double knockout. This metabolic deficit appears to result from the combined deficiency of β1- and β2-ARs, since neither the β1-AR knockout nor the β2-AR knockout display such deficits (17, 18). Interestingly, however, there are differences in total exercise capacity between wild type mice and β2-AR knockouts, with the β2-AR knockout demonstrating a slight enhancement of total exercise capacity and reduced respiratory exchange ratios (17). In contrast, β1-AR ablation has no effect on total exercise capacity (18).

The physiological response to GTE is seen in Fig. 4A. Both blood pressure and heart rate were monitored in resting and exercising mice by use of indwelling carotid arterial catheters. The normal response to increasing exercise workloads is a corresponding increase in heart rate (up to the maximally achievable rate of ~800 beats/min in the mouse). Both wild type and β1/2-AR knockouts show workload-dependent increases in heart rate; however, the heart rate of β1/2-AR mice was lower than the heart rate of wild type mice at all exercise levels (at rest, heart rate differences are not statistically significant between the two genotypes). The effect of exercise on mean peripheral arterial blood pressure is not different between these two groups. The loss of exercise-induced tachycardia is most likely the result of β1-AR ablation, as a virtually identical behavior is seen in β1-AR knockout mice (18), whereas β2-AR knockouts show no deficit in exercise-induced tachycardia (17).

Muscarinic and α1-Adrenergic Receptor Density in Cardiac Membranes—There is a well known functional interdepend-
mented mice, which were run to their voluntary limit. O2 consumption post-exercise). Wild type, Both wild type and 54.1

FIG. 4. Cardiovascular and metabolic response to exercise. Both wild type and β1/β2-AR knockouts were subjected to a GTE regimen (see "Materials and Methods"). A, the cardiovascular response to GTE was determined in instrumented mice, which were run to a final end point of 20 m/min and 14° inclination. Rec, recovery (10 min post-exercise). Wild type, n = 7. β1/β2 knockouts, n = 6. For heart rate, wild type versus β1/β2 knockouts is significantly different (p ≤ 0.01 by two-way analysis of variance with repeated measures, excluding recovery). B, the metabolic response to GTE was monitored in non-instrumented mice, which were run to their voluntary limit. O2 consumption and CO2 production are expressed as ml/min/kg. Wild type, n = 7. β1/β2 knockouts, n = 5. Wild type mice are significantly different than β1/β2 knockouts in both O2 consumption and CO2 production (p ≤ 0.01 by two-way analysis of variance with repeated measures, up to 27.5 m/min treadmill speed).

ence between β-ARs and muscarinic receptors in the heart, which represent the two major targets of cardiac sympathetic and parasympathetic stimulation, respectively. Additionally, the role of α1-ARs either alone or in combination with β-ARs is thought to be critical for both acute responsiveness to catecholamines, as well as in longer term adaptive or remodeling responses in the heart. We thus sought to test whether any gross alterations in either of these receptor families was apparent in β1/β2-AR double knockouts in comparison to wild types. Whereas muscarinic receptor density displayed a mild but significant reduction in double knockouts in comparison to wild types (28.9 ± 1.6 fmol/mg protein versus 33.6 ± 1.0 fmol/mg protein; n = 4 for both, p ≤ 0.05), α1-AR density was not significantly affected by loss of both β1- and β2-ARs in comparison to wild types (50.3 ± 3.4 fmol/mg protein versus 54.1 ± 4.1 fmol/mg protein, respectively; n = 4 for both, p = not significant).

In Vitro Cardiac Responsiveness to G-protein-coupled Receptor Agonists—The effect of various G-protein-coupled receptor agonists was tested in either spontaneously beating atrial preparations (chronotropic assay) or paced right ventricular strips (inotropic assay). These experiments were performed to investigate the efficacy of these compounds relative to β-AR agonists, as well as to reveal any potential compensation for loss-of-β-AR signaling that could be more apparent as altered response relative to wild type preparations. An additional utility of these experiments was the potential to reveal any compensation with an indirect mechanism of action, since several potential compensatory signaling pathways could exert their effects through modulating the release of catecholamines and subsequent activation of β-ARs (21–23). The agonists tested included serotonin, angiotensin II, the β3-AR agonist CL316243, dopamine, histamine, the β-AR agonist isoproterenol (Iso), and the α-AR agonist phenylphrine. As can be seen in Fig. 5, there were no significant differences between wild type and β1/β2-AR double knockout preparations in response to any of these drugs, with the exception of isoproterenol, which has robust effects on both atrial rate and ventricular contractility in wild type preparations but no effect in β1/β2-AR double knockout preparations. The lack of effect of isoproterenol in the double knockout is virtually identical to that seen in β1-AR knockout preparations (13), supporting the predominant role of β1-ARs in the regulation of murine heart rate and contractility. The concentration of agonist used in each of these experiments (see Fig. 5 legend) was designed to elicit a maximal effect, based on prior experiments. It is notable that neither CL316243 nor isoproterenol has any appreciable effect on either atria or ventricles from β1–β2-AR double knockouts, given the purported negative coupling behavior of the β3-AR in human cardiac preparations (24).

In Vivo Left Ventricular Contractility in Anesthetized and Awakening Mice—Based on the observation that exercising β1/β2-AR double knockout mice can achieve similar workloads at reduced heart rates, we sought to test whether corresponding deficits were also present in the inotropic component of heart function in vivo, given the well known effects of β-AR agonists to regulate cardiac contractility. These studies were performed in both anesthetized and awakening mice in an attempt to reduce the cardiodepressant effects of anesthesia, using micromanometer-tipped left ventricular catheters (see “Materials and Methods”). Fig. 6 shows representative tracings from two mice in both anesthetized and waking states. Clearly both wild types and β1/β2-AR double knockouts show depressed cardiac contractility, both in terms of developed pressure and dP/dt (the first derivative of left ventricular press-
more, ventricular pressure tracings (self-righting became evident. Representative tracings from two mice were taken, and mice were allowed to recover until awakening mice. Micromanometer-tipped pressure-sensing catheters were advanced from the carotid to the left ventricle under anesthesia, measurements were taken, and mice were allowed to recover until self-righting became evident. Representative tracings from two mice are shown, in both the anesthetized and awakening state. Both the left ventricular pressure tracings (upper panels) and the first derivative of pressure, \(dP/dt\) (lower panels), are shown for a wild type and \(\beta_1/\beta_2\) AR knockout mouse. Time axis is in seconds.

**DISCUSSION**

The \(\beta\)-ARs are recognized as important components of the sympathetic nervous system, playing critical roles in the maintenance of cardiac, vascular, and metabolic homeostatic mechanisms. The purpose of these studies was to delineate the subtype-specific contributions of the \(\beta_1\)-AR and \(\beta_2\)-AR on these physiological processes by genetic knockout techniques, where \(\beta_1\)- and \(\beta_2\)-ARs were knocked out individually as well as in combination. By inference, we have also investigated cardiovascular functions specific to the remaining \(\beta_3\)-AR. Surprisingly, total elimination of both \(\beta_1\)- and \(\beta_2\)-ARs has little impact on resting cardiovascular tone or basal metabolism, although functional deficits are clearly revealed when mice are stimulated by \(\beta_3\)-AR agonists or maximal exercise. Such results underscore the notion that the \(\beta\)-ARs are modulators of these physiologic functions but not intrinsic to or required for the functions themselves.

**Basal Cardiovascular Function**—Loss of both \(\beta_1\)- and \(\beta_2\)-ARs has minimal impact on basal heart rate and blood pressure. Based on the phenotype of both the \(\beta_1\)-AR knockout (15, 18) and the \(\beta_2\)-AR knockout (17), these results are not surprising. Such results would be unexpected, however, when considered in the context of numerous pharmacological studies using either non-selective or selective \(\beta\)-AR antagonists that are commonly used to lower heart rate and blood pressure. Why do mice lacking both adrenergic receptors fail to exhibit abnormalities at rest? First, there may be fundamental differences between animals that lack a given receptor from conception onwards and animals treated with antagonists at a discrete point in time. Furthermore, the bulk of evidence from knockouts of the \(\alpha_1\)-, \(\alpha_2\)-, \(\alpha_2\)-, \(\beta_2\)-, and \(\beta_2\)-ARs also reveals that basal physiological functions are not significantly perturbed, again failing to reproduce the phenotypes observed by acute subtype-specific blockade in normal animals (25). Our data suggest that there are alternative control points for such critical physiological functions such as cardiac rate and contractility, vascular tone, and metabolic state, which can be altered to compensate for the lack of \(\beta_2\)-AR signaling. The parasympathetic nervous system, which acts in functional opposition to signals generated by the sympathetic nervous system (26, 27), has the potential to compensate for absent \(\beta_2\)-AR signaling, as do a variety of other hormone or neurotransmitter systems. Our demonstration that cardiac muscarinic receptor density is reduced in the \(\beta_1/\beta_2\) double knockout may reflect a counterbalancing reduction in a receptor that is known to functionally antagonize stimulatory \(\beta_3\)-ARs.

**\(\beta_3\)-AR Function**—Another example of a compensatory change in G-protein-coupled receptor signaling resulting from \(\beta_1\)- and \(\beta_2\)-AR deficiency is the supranormal response of \(\beta_1\)- \(\beta_2\)-AR knockouts to the \(\beta_3\)-AR agonist CL316243. Stimulation of \(\beta_3\)-ARs by this agonist (and 37344 from Life Technologies, Inc.) in rats and dogs elicits sustained increases in both blood pressure and total peripheral resistance (20). In wild type mice, we have demonstrated that \(\beta_3\)-AR stimulation also results in a robust and sustained hypotensive response. Mice deficient in both \(\beta_1\)- and \(\beta_2\)-ARs show an exaggerated response to CL316243. There are several possible explanations for such altered responses. First, vascular \(\beta_3\)-ARs may be up-regulated in the \(\beta_1/\beta_2\)-AR double knockout. The demonstration that \(\beta_1\)-ARs are up-regulated in adipose tissue of \(\beta_3\)-AR knockout mice (12) supports the contention that deficiencies in \(\beta_3\)-AR signaling can be counteracted by increases in the density of and/or signaling efficiency of other \(\beta\)-AR subtypes. Another possibility is that there is an up-regulation of the signaling machinery distal to \(\beta\)-ARs in the vascular beds of \(\beta_1/\beta_2\)-AR double knockouts, secondary to disuse. The phenomenon of \(\beta_3\)-AR supersensitization following prolonged \(\beta_3\)-AR antagonist therapy is well known (28–30) and may be analogous to the situation in mice when both \(\beta_1\)- and \(\beta_2\)-ARs are absent.

Experiments with the \(\beta_3\)-AR agonist CL316243 demonstrate that residual responses (defined as the additional isoproterenol-induced vasodilatory response during full \(\beta_3\)-AR stimulation) of \(\beta_1\)-ARs differ from that of \(\beta_2\)-ARs in mice which lack one or the other subtype. In these experiments, \(\beta_1\)-AR knockouts possess identical residual isoproterenol responses in comparison to wild types, whereas \(\beta_2\)-AR knockouts showed attenuated residual responses. This could be due to either increased efficacy of the \(\beta_2\)-AR in mediating vasodilatation, different mechanism(s) of receptor activation, or to differences in the distribution among the three \(\beta\)-ARs within vascular beds. As an example, preferential distribution of \(\beta_2\)-ARs in small resistance arterioles and \(\beta_1\)-ARs in large conductance vessels would tend to favor \(\beta_2\)-ARs in the primary control of peripheral vascular resistance. Additionally, the attenuated response of \(\beta_2\)-AR knockouts to isoproterenol in these experiments could indicate an overlap of \(\beta_1\)- and \(\beta_3\)-AR expression in the same vascular beds, such that \(\beta_3\)-AR stimulation with CL316243 precludes a maximal response through \(\beta_1\)-ARs co-expressed in the same resistance vessels.

**Metabolic and Physiologic Response to Exercise**—The ability of \(\beta_1\)-AR antagonists to alter the metabolic response to exercise is well known (31–33). \(\beta_1\)-AR activation normally stimulates glycogenolysis as well as lipolysis, reflected in the rise of plasma glucose and free fatty acids during exercise. Whereas free fatty acid mobilization is impaired in \(\beta_1\)-AR-blocked exer-
The primary G-protein-coupled receptors regulating cardiac function are not surprising that metabolic demands are reduced in the present study, the chronotropic effect of agents such as histamine or serotonin, despite their ability to stimulate cardiac adenylate cyclase in vitro (39, 40), suggests that β-ARs are the primary G-protein-coupled receptors regulating cardiac function in vivo.

Given that both β1-AR knockouts (18) and the β1/β2-AR double knockout show normal exercise capacities at submaximal heart rates, we wanted to investigate whether there were any significant differences in cardiac inotropic state that could represent an adaptation to the loss of β-ARs. Based on our previous findings with β1-AR knockouts (13, 41), the inotropic state is largely determined by the presence or absence of the β1-AR and any underlying sympathetic tone. Our results here suggest that there may be some residual sympathetic tone in anesthetized mice, which in wild type mice manifests itself as an increased dP/dt and decreased −dP/dt in comparison to the β1/β2-AR double knockout; this is even further accentuated in the awakening state. Alternatively, the difference in heart rates between wild types and double knockout mice during anesthesia and upon awakening could be responsible for this difference in dP/dt. Interestingly, inotropic state is greatly enhanced even in β1/β2-AR double knockouts upon transition from anesthetized to awakening states. Depression of myocardial contractility while under isoflurane anesthesia can be due to indirect effects to reduce sympathetic outflow, as well as direct inhibitory effects on cardiac muscle (42–44). Despite the reduced positive and negative dP/dt values in β1/β2-AR double knockouts, these mice develop equivalent peak left ventricular pressures in comparison to their wild type counterparts. Taken together with the exercise studies, our results would suggest that the β1-AR-mediated increases in heart rate and contractility in the mouse are not necessary for maximal performance during a stress such as exercise. In fact, at the extremely high heart rates typical for a mouse at maximal exercise, reduced diastolic filling time may serve to limit any further increases in cardiac output. The demonstration that humans under β-AR blockade can increase stroke volume via the Frank-Starling mechanism (while heart rate remains depressed) supports the idea that chronotropic and inotropic stimulation through β-ARs are not required for maximal exercise performance (45). Other studies have shown that during exercise, intrinsic mechanisms such as increased venous return enhances diastolic filling and hence cardiac output (46, 47) and can be preferentially utilized over heart rate changes in some pathological states to maintain cardiac output (48). Together with our data in genetically altered mice, such results underscore the importance of intrinsic preload and afterload mechanisms and tend to mitigate the requirement for β-AR signaling in the adaptive responses to exercise.

In summary, the present study has further characterized the physiological role of β1- and β2-ARs in mice by means of genetic knockout techniques. The impact of β1-, β2-, or β1/β2-AR loss on basal physiological functions such as heart rate, blood pressure, or metabolic rate is remarkably minor; however, striking differences between these knockouts and their wild type counterparts can be seen following β-agonist administration or during the stresses of exercise. Given what is known

### Table II

### Summary of left ventricular contractility measurements

| Anesthetized | | | | | | Waking | | | |
|--------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| HR          | Max LVP       | Min LVP        | Max dP/dt      | Min dP/dt      | | HR          | Max LVP       | Min LVP        | Max dP/dt      | Min dP/dt      |
| Beats/min   | mm Hg         | mm Hg          | mm Hg/ms       | mm Hg/ms       | | Beats/min   | mm Hg         | mm Hg          | mm Hg/ms       | mm Hg/ms       |
| Wild type (5) | 384.6        | 100.0          | −0.3          | 5.00           | −3.90          | 471.6        | 122.2          | −8.9          | 8.28           | ±0.6          | −6.46         |
|             | ±22.9         | ±3.6           | ±2.4          | ±0.19          | ±0.36a         | ±27.0         | ±4.2           | ±1.1          | ±1.1           | ±0.48         |
| β1/β2 knockout (6) | 316.0        | 94.8           | 2.4           | 4.03           | −3.4           | 331.0        | 116.9          | 1.8           | 5.55           | −4.05         |
|             | ±12.4a        | ±4.9           | ±3.2          | ±0.29a,b       | ±0.11a,b       | ±8.7b        | ±5.4           | ±3.3b         | ±0.72b         | ±0.25b        |

* p < 0.05, anesthetized versus waking, within a genotype.

* p < 0.05 wild type versus β1/β2 knockout.
about the important role that β1- and β2-ARs play in both physiological and pathophysiological processes, one can speculate as to whether the gene knockout technique has revealed the “true” role of these receptor subtypes. More thorough studies involving different types of stresses (such as induced heart failure) and longitudinal studies will help to clarify this issue. Despite the limitations of the model systems studied to date, certain β-AR-modulated functions such as heart rate and contractility are well defined and can be attributed to single β-AR members (in this case the β1-AR). Other functions, such as β-AR-mediated vasodilatation, are additive and integrated, with all three β-AR subtypes contributing at some level. For other functions, such as metabolic rate, β-AR actions appear to be redundant, and deficiencies are not apparent until both β-AR subtypes are knocked out. Mice lacking β1- and/or β2-ARs represent useful model systems for the study of β-AR-modulated function in vivo, as well as the role that β-ARs play in pathophysiology.

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