Protecting the myocardium: A role for the β2 adrenergic receptor in the heart

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Objective: The sympathetic nervous system enhances cardiac muscle function by activating β adrenergic receptors (βARs). Recent studies suggest that chronic βAR stimulation is detrimental, however, and that it may play a role in the clinical deterioration of patients with congestive heart failure. To examine the impact of chronic β1AR and β2AR subtype stimulation individually, we studied the cardiovascular effects of catecholamine infusions in βAR subtype knockout mice (β1KO, β2KO).

Design: Prospective, randomized, experimental study.

Setting: Animal research laboratory.

Subjects: β1KO and β2KO mice and wild-type controls.

Interventions: The animals were subjected to 2 wk of continuous infusion of the βAR agonist isoproterenol. Analyses of cardiac function and structure were performed during and 3 days after completion of the infusions. Functional studies included graded exercise treadmill testing, in vivo assessments of left ventricular function using Mikro-Tip catheter transducers, right ventricular pressure measurements, and analyses of organ weight to body weight ratios. Structural studies included heart weight measurements, assessments of myocyte ultrastructure using electron microscopy, and in situ terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick-end labeling staining to quantitate myocyte apoptosis.

Measurements and Main Results: We found that isoproterenol-treated β2KO mice experienced greater mortality rates (p = .001, chi-square test using Fisher’s exact method) and increased myocyte apoptosis at 3- and 7-day time points (p = .04 and p = .0007, respectively, two-way analysis of variance).

Conclusion: The results of this study suggest that in vivo β2AR activation is antiapoptotic and contributes to myocardial protection.

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Key Words: adrenoceptor; knockout; myocardium; heart failure; apoptosis

The β1 adrenergic receptors (β1ARs) and β2ARs are closely related receptor subtypes that mediate the primary cardiovascular effects of catecholamines in the mammalian heart. Both receptors are G protein coupled and possess seven transmembrane helices. Both receptors have been shown to enhance heart contractility (1, 2) as well as relaxation (1). In addition, they share many common agonists and antagonists.

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Despite these similarities, β1AR and β2ARs differ in many ways. For instance, their gene loci are entirely different. The β1AR gene is located on chromosome 10 whereas the gene for the β2AR resides on chromosome 5. The receptor subtypes differ in size: The β2AR is 477 amino acids in length whereas the β1AR is 413 amino acids (3, 4). Also, recent investigations suggest that β1ARs and β2ARs have unique signaling properties. β2ARs in cultured rat and mouse ventricular myocytes couple to both stimulatory (Gs) and inhibitory (Gi) G proteins whereas β1ARs couple only to Gi. Furthermore, in rat neonatal cardiac myocytes, β2AR activation has been shown to deliver an antiapoptotic signal to cardiac myocytes through a Gi-dependent coupling to phosphatidylinositol 3’-kinase and Akt (protein kinase B). In contrast, β1AR activation in adult rat ventricular myocytes has been shown to increase apoptosis via a cyclic adenosine monophosphate-dependent mechanism (5, 6).

Previous studies using transgenic mice suggest that when β1ARs and β2ARs are expressed at levels many times greater than normal, their effects are also different. For example, five-fold transgenic overexpression of β1ARs in the murine heart causes cardiomyopathy (7), whereas overexpression of β2ARs, even at levels as high as 60-fold above normal, improves cardiac contractile force without any cardiomyopathic consequences (8).

Although these data suggest dissimilar roles for cardiac β1ARs and β2ARs, the degree to which they reveal differences between the two receptor subtypes during chronic activation is unclear. The effects of receptor overexpression may, in fact, differ from those of continuous stimulation. For example, the extent to which signaling elements are activated may be strongly influenced by variations in receptor expression levels. β2ARs, for instance, appear to operate within microdomains (9). Signal transduction elements within these domains may be finite, and it is conceivable that at some level overexpression exceeds the capacity of these microdomains and leads to atypical receptor signaling.

Our approach to the study of chronic βAR subtype activation has been to use knockout mice. Using this approach we
have investigated the effects of chronically stimulating individual βAR subtypes expressed at physiologic levels. Our pilot studies (mortality rate and exercise capacity studies using small numbers of animals) suggested that mice lacking β1ARs (β1KO and β1β2KO) might be resistant to isoproterenol-induced cardiac injury. They also suggested that β2KO mice might be more susceptible to the detrimental effects of isoproterenol infusion than wild-type animals. Based on these preliminary findings, we focused our investigation on β2KO mice. We examined the cardiovascular effects of chronic β1AR activation in the absence of β2AR stimulation in vitro.

METHODS

Mice. These experiments were reviewed and approved by our institution’s Subcommittee on Animal Studies and were in accordance with the provisions of the Animal Welfare Act, the Public Health Service Guide for the Care and Use of Laboratory Animals. The generation of β1KO, β2KO, and β1β2 adrenergic receptor double knockout (β1β2DKO) mice has been previously described (10–12). All mice used in these experiments were male mice between 25 and 35 g. The β2KO mice and their strain-specific wild-type control animals were on congenic FVB/N backgrounds.

We infused isoproterenol or saline into β2KO and strain-specific wild-type control animals for 2 hrs. The study groups were carefully matched in terms of age, weight, gender, and living environment. We studied 30 ± 3 g male mice between 12 and 14 wks of age. Within genotypes, littersmates were divided evenly and randomly assigned to isoproterenol or saline infusion, rather than assigning entire litters to one treatment or the other.

Reagents. (-)-Isoproterenol bitartrate (120 μg·g−1·day−1; Sigma Chemical, St. Louis, MO) was infused subcutaneously into each study animal via miniosmotic pumps (Alza Corporation, Palo Alto, CA). Control animals received infusions of sterile 0.9% sodium chloride (Abbott Laboratories, North Chicago, IL).

Surgery and Anesthesia. Miniosmotic pumps were placed subcutaneously in each mouse under general anesthesia. We used 3% isoflurane anesthesia for induction of anesthesia; 1.5% isoflurane was used for maintenance. Each animal was allowed to breathe spontaneously throughout the procedure. A small subcutaneous pouch was created on each animal’s flank using blunt-end forceps. The osmotic pumps were inserted, and the skin was closed using two 7.5 × 1.75 mm skin staples (Michel).

Mortality Investigation. The mortality investigation included mice that resided in individual cages for the duration of the study after undergoing infusion pump insertions. These animals were not included in any physiology experiments. Two separate mortality investigations were conducted. The data were pooled for analysis. In total, 36 isoproterenol-treated β2KO, 33 isoproterenol-treated wild-type, 22 saline-treated β2KO, and 30 saline-treated wild-type animals were studied.

Exercise Capacity. Several mice were challenged individually with a graded treadmill exercise protocol on a Simplex II rodent treadmill (Columbus Instruments, Columbus, OH). The animals were studied 1 day before and 17 days after osmotic pump insertion (3 days after the pump contents had been completely injected). Treadmill activity was initiated after each mouse had equilibrated in the exercise chamber for 10 mins. Stepwise increases in treadmill speed (2.5 m/min) and inclination (2°) were made every 3 mins until each mouse stopped running from exhaustion. Exercise capacity was calculated as the total distance run by the animal during the exercise protocol.

Left Ventricle Change in Pressure Over Time (dp/dt). Each animal studied was anesthetized with isoflurane as previously described. The animal’s neck was shaved and prepped in sterile fashion, and a midline neck incision was made. The right common carotid artery was isolated using a microscope. A suture was tied around the vessel approximately 0.25 cm below the skull base. Approximately 0.75 cm proximal to this point, blood flow was disrupted using a vascular clamp. This provided a site for insertion of a Mikro-tip catheter transducer (Millar Instruments, Houston Texas). The contralateral carotid artery was allowed to remain patent, maintaining adequate cerebral blood flow on the right side of the brain by perfusion through the circle of Willis. Proximal to the point of carotid artery ligation, a small arterotomy incision was made using a curved 25-gauge needle. The Mikro-tip catheter was introduced into the artery and advanced under waveform guidance into the left ventricle. A pressure waveform was generated using an amplifier (Gould Instrument Systems, Valley View, OH), a Transducer Control Unit TC-510 (Millar Instruments), a Dell Optiplex GX300 Computer (Dell Computer, Austin, TX), and WinDAQ Acquisition Software version 2.43 (DATAQ Instruments, Akron, OH). Ventricular pressure was measured before and after intraperitoneal injection of propranolol (30 mg/kg), which was used to block the effects of endogenous catecholamines that might have a greater impact on dp/dt in mice of one genotype relative to the other. To calculate dp/dt based on the left ventricle waveform, WinDAQ Waveform Browser software (DATAQ Instruments) was used.

Right Ventricle Mean Systolic Pressure. Each animal studied was anesthetized using isoflurane as previously described. The animal’s chest was shaved and prepped in sterile fashion. A 22-gauge needle was placed into the right ventricle using a transcutaneous subxiphoid approach. Using a Transpac IV Monitoring Kit (Abbott Critical Care Systems, North Chicago, IL), an SN-E8748 Interface Cable (Fogg System, Aurora, CO), a Gould 13-6615 Transducer (Gould Instrument Systems, Babylon, NY), a PowerLab 14SP (ADInstruments, Mountain View, CA), an Apple PowerMac G4 Computer running OS9.1 Operating System (Apple Computer, Cupertino, CA), and Chart version 3.6.3/s software (ADInstruments), the right ventricle pressure waveform was transduced and recorded for 5 secs. At a later time, the peak of each pressure waveform was identified manually and all the peak systolic pressures over the course of 5 secs were averaged to generate a right ventricle mean systolic pressure.

Noninvasive (Tail Cuff) Blood Pressure and Heart Rate. A BP 2000 Physiologic Research Instrument (Visitech Systems, Apex, NC) was used to determine systolic blood pressure at two time points before osmotic pump insertion and at nine time points after osmotic pump insertion for several animals. Before the study, each animal underwent 2 wks of blood pressure measurement training. At initial testing, each animal underwent 20 tail cuff blood pressure measurements every other day. At each time point during the study, each animal underwent ten practice measurements followed by ten actual measurements. A mean heart rate and systolic blood pressure were determined by the BP 2000 instrument for each animal at each time point.

Electrocardiography. Each animal studied was anesthetized using isoflurane as previously described. The animal’s chest was shaved and prepped in sterile fashion. Using transcutaneous needle electrodes, a PhysioTel TAJ01EA-P20 Transmitter (Data Sciences International, St. Paul, MN), a PhysioTel Receiver RPC-1 (Data Sciences International), a BCM100 Consolidation Matrix Device (Data Sciences International), a Powerlab 14SP (ADInstruments), an Apple PowerMac G4 Computer (Apple Computer), and Chart version 3.6.3/s software, an eight-lead electrocardiogram (ECG) was performed before and at several time points after initiation of saline or isoproterenol infusion. All ECGs were interpreted by a board eligible cardiologist.

Organ Weight to Body Weight Ratios. The mice that underwent exercise treadmill testing were anesthetized using 0.5 mg/g of body weight (about 0.5 mL) Avertin administered by injection into the peritoneum. Each animal was weighed and then killed using cervical dislocation. A clam-shell bilateral thoracotomy incision followed by a midline abdominal incision was immediately performed, and the heart, lungs, liver, spleen, and kidneys were removed. Each organ was rinsed in saline, blotted dry, and immediately weighed. Organ weight to body weight ratios were then calculated for each animal using these values.

Electron Microscopy and Analyses. Tissue was fixed for 24–48 hrs at room temperature in

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1.5% glutaraldehyde (cacodylate buffer, pH 7.4); sucrose was added to bring the milliosmolarity to 300. The tissue was stored at 4°C and dehydrated in ethanol, transferred to propylene oxide, and embedded in Epon 12 resin. Polymerized blocks were sectioned at 0.5–1 μm, and the sections were stained with toluidine blue for examination by light microscopy. Selected areas were sectioned at 0.05 μm, enhanced with uranyl acetate and lead citrate, and examined with a Philips 201 electron microscope. A quantitative analysis of myocyte injury was performed by a pathologist subspecializing in cardiac pathology. Blinded with regard to genotype and treatment group, the pathologist scored 113 midventricle images based on myofiber integrity, intracellular fluid collection, and mitochondrial degeneration. The grading scale ranged from 0 (no injury) to 4 (severe injury) (13).

**Apoptosis.** Mice were killed for cardiac apoptosis studies 3, 7, or 10 days after initiation of isoproterenol or saline infusion. Each animal was anesthetized by an intraperitoneal injection of Avertin. Each animal was then killed using cervical dislocation. The heart was immediately excised using a bilateral clamshell incision. A 20-gauge needle was inserted into the beating right ventricle to serve as a vent. Six milliliters of chilled (4°C) saline was injected into the beating left ventricle via a 20-gauge needle. Immediately after saline injection, 30 mL of chilled (4°C) 4% paraformaldehyde fixative was injected into the left ventricle. Each animal’s heart was then removed and stored in 4% paraformaldehyde at 4°C until it was sliced into three sections perpendicular to the long axis of the ventricle. Sections were then stored in 10% neutral buffered formalin for 24 hrs and then embedded in paraffin. Slide sections (5 μm) from the midventricular level were stained in situ terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) using an in situ apoptosis detection kit (TACS 2 TD, Trevigen, Gaithersburg MD) (14). TUNEL-positive nuclei stained blue in this assay. To count the number of TUNEL-positive nuclei, the entire section was scanned at ×100 by light microscopy. When TUNEL-positive cells were detected, magnification was changed to ×400 to assess whether staining occurred in myocytes. Only TUNEL-positive myocytes were counted in each slice. Any nuclei that were ambiguous were not counted. Round homogeneous dark dots (considered to be artifact) and stained cell debris were carefully eliminated from counting. Brownish nuclei-like deposits, considered to be Formalin crystals, were not counted. The number of apoptotic nuclei was normalized to apoptotic nuclei per 10,000 cardiac myocyte nuclei. This was done using the morphometric method. From the positive control of each section, 12 light microscopic fields (400×) were chosen to count the number of myocyte nuclei. Endocardial, midmyocardial, and epicardial areas were chosen from the septum, lateral, anterior, and posterior walls of the left ventricle. The number of cardiac myocyte nuclei in each microscopic field was counted under a grid covering a total area of 0.0625 mm². Only nuclei that were completely contained within the myocyte contours were counted. The number of nuclei within 12 fields was averaged. The number of TUNEL-positive cells per 10,000 cardiomyocyte nuclei in each slide was calculated as the total number of TUNEL-positive cells in each slide divided by the estimated total of myocyte nuclei in each slide multiplied by 10,000.

**Statistics.** All data were expressed as mean ± SEM. Most of the statistical analyses involved two-way analysis of variance (ANOVA), with predictors for drug treatment and genotype as well as an interaction term. An exception was the comparison of left ventricular contractility (dp/dt max). In this experiment, isoproterenol-treated β2KO mice were compared with isoproterenol-treated wild-type animals, and a Student’s t-test was used for the statistical analysis. The saline-treated β2KO group was studied simply for comparison with published normal values to validate our experimental technique.

Several of the experiments described were affected by mortality rate among the isoproterenol-treated animals. We investigated whether mortality rate affected the statistical significance of the results by censoring the cohorts where fewer mice died. We repeated the statistical analyses withholding the highest values and then withholding the lowest values from these cohorts. In only one case was the effect of mortality rate significant: liver weight to body weight ratio. When high values were removed, we observed a statistically significant interaction between genotype and treatment (p = .006; two-way analysis of variance).

**RESULTS**

**Mortality.** Mortality was defined as death within 17 days of drug infusion onset. On day 17, all animals were killed for analysis. Statistical analysis revealed significantly greater mortality rate among isoproterenol-treated β2KO mice compared with isoproterenol-treated wild-type mice (p = .001, chi-squared test using Fisher’s exact method). We found that 14 of 36 isoproterenol-treated β2KO mice died during two mortality studies, whereas two of 33 isoproterenol-treated wild-type animals died (no saline-treated β2KO or wild-type mice died).

**Exercise Capacity.** To assess whether isoproterenol infusion affected overall cardiovascular function of β2KO or wild-type animals, we performed graded exercise treadmill studies. Our data revealed a decrease in exercise capacity after isoproterenol infusion for both wild-type and β2KO animals. Although the drug effect was found to be significant, the genotype effect was not significant (Fig. 1).

**Cardiac Function.** An increase in lung weight reflects left ventricular dysfunction due to pulmonary congestion as the left ventricle fails. We used lung weight to body weight ratios as an indirect measure of left ventricular cardiac function in β2KO and wild-type mice. Analysis of data from this study revealed a significant drug effect (p < .0001, two-way ANOVA) but no genotype effect (p = .7843, two-way ANOVA).

Using Mikro-Tip catheter transducers, we performed direct measurements of left ventricular function in isoproterenol-treated β2KO and wild-type animals. We also studied saline-treated β2KO mice and compared their left ventricle dp/dt measurements to previously reported values from wild-type mice to validate our experimental technique. The dp/dt values measured in our saline-treated β2KO animals were comparable to values previously reported for wild-type mice (15, 16). Our data did not reveal significant differences in dp/dt max or –dp/dt when isoproterenol-treated β2KO and isoproterenol-treated wild-type mice were compared (p = .377 and p = .306, respectively, Student’s t-test) (Fig. 2).

When the right ventricle becomes dysfunctional, central venous pressures increase. An increase in liver weight may reflect right ventricular dysfunction because this organ can become more congested when venous return to the heart is impeded by rising central pressures. An increase in liver weight may also occur for reasons unrelated to cardiac function. We determined liver to body weight ratios and found a significant genotype effect (p = .028, two-way ANOVA; Fig. 3). When the data were censored to correct for mortality by removing the highest liver weight values from both cohorts, the interaction between isoproterenol treatment and genotype was significant (p = .006, two-way ANOVA).

Elevated pulmonary artery pressure can lead to right ventricle dysfunction by forcing the right ventricle to eject against a greater resistance. To determine whether the absence of β2ARs in the pulmonary vasculature might lead to higher pulmonary artery pressure in β2KO mice, we evaluated right ventricle mean systolic pressure. Right ventricular mean systolic pressure reflects the pulmonary artery pressure because the pulmonary valve is open during this phase of the
cardiac cycle. Mice without infusion pumps as well as animals that had received saline or isoproterenol infusions were studied. We failed to detect a significant genotype effect on right ventricle mean systolic pressure ($p = .488$, two-way ANOVA).

**Cardiac Structure.** We employed three techniques for a study of cardiac structure: heart weight to body weight ratio analysis, assessment of myocyte ultrastructure using electron microscopy, and quantitative measurement of myocyte apoptosis using *in situ* TUNEL staining.

Heart weight to body weight ratio analysis is a simple technique for identi-
roni posttests), suggesting that a baseline difference might exist between β2KO and wild-type mice in terms of myocyte apoptosis.

**Electrophysiology.** We evaluated five isoproterenol-treated β2KO mice and five saline-treated β2KO animals using ECG. No evidence of myocardial injury was detected in isoproterenol-treated β2KO animals by ECG. No abnormalities in terms of rate, rhythm, intervals, or axis were observed either at baseline or after isoproterenol or saline infusions (23).

**Effects of Isoproterenol Infusion on Blood Pressure and Heart Rate.** Afterload reduction is an indirect means by which peripheral β2AR activation might afford protection to the hearts of wild-type animals relative to β2KO mice. Activation of β2ARs in the peripheral vasculature has been shown to mediate vasodilation (24, 25). Using a noninvasive blood pressure measurement device, we performed a series of experiments to determine whether differences in blood pressure existed between β2KO and wild-type mice. We found no significant differences in systolic blood pressure between isoproterenol-treated β2KO and wild-type mice (p = .09, Bonferroni/Dunn post hoc analysis, repeated-measures ANOVA; Fig. 8).

Tachycardia-induced cardiac injury has been well documented (26, 27). To determine whether heart rate differences existed between isoproterenol-treated β2KO and wild-type mice, we compared heart rates in the mice during the infusions. Results of this experiment suggested no significant differences between the heart rates of isoproterenol-treated β2KO and isoproterenol-treated wild-type animals (p = .99, Bonferroni/Dunn post hoc analysis, repeated-measures ANOVA).

**DISCUSSION**

Our results provide evidence that β2ARs mitigate some of the deleterious effects of chronic β1AR stimulation. In mice subjected to a chronic infusion of the nonselective βAR agonist isoproterenol, we observed higher mortality rate in β2KO mice than in wild-type animals. Moreover, we observed more apoptosis in the hearts of β2KO mice.

The results of our study indicate that β2KO and wild-type mice are more dissimilar than we initially believed. Previous investigations suggested that β2KO animals were normal in size and demonstrated heart rates and blood pressures similar to wild-type control animals. Only during significant stress were differences between β2KO and wild-type mice apparent. β2KO mice were found to become hypertensive in response to exercise. However, they also demonstrated enhanced exercise capacity and more efficient oxygen utilization during exercise (lower respiratory exchange ratio at a given workload) (12). Our data suggest that even modest stress (such as saline infusion) can elicit differences in myocyte apoptosis between β2KO and wild-type mice, indicating that the absence of β2AR signaling is important even when physiologic differences are not apparent.
A growing body of evidence suggests that β2AR stimulation is protective (6, 9, 28). However, the mechanisms by which β2AR-mediated protection is conferred remain unclear. β2AR-mediated reduction in myocyte apoptosis by a Gi-dependent signaling pathway is likely one factor. The capacity of β2ARs but not β1ARs to couple to Gi represents an important point of divergence between β1AR and β2AR signaling, and other Gi-dependent signaling pathways may also contribute to the protective effect of β2AR stimulation.

Evidence now suggests that in cardiac myocytes, receptor targeting and trafficking mediated by interactions with cellular scaffolding proteins dictate subtype-specific signaling. The PDZ domain binding motifs at the carboxyl termini of βARs differ significantly between β1ARs and β2ARs and mediate interactions with different cellular scaffolding proteins (29–32). For instance, the β1AR PDZ motif interacts with postsynaptic density protein-95 and related proteins. This interaction prevents agonist-induced internalization (33) and appears to prevent β1AR coupling to Gi. Disruption of this motif leads to efficient β1AR-Gi coupling.
The results of this study suggest that in vivo β2 adrenergic receptor activation is antiapoptotic and contributes to myocardial protection.

in neonatal cardiac myocytes (29). In contrast, the β2AR PDZ motif interacts with different PDZ domain-containing proteins, such as ezrin-binding-50/sodium-hydrogen exchange regulatory factor family proteins, which are responsible for receptor sorting between recycling and degradative endocytic pathways (32). In vitro studies demonstrate that mutation of the β2AR PDZ motif disrupts β2AR-C coupling possibly by interfering with receptor recycling after endocytosis (30).

Our data suggest that β2AR activation reduces myocyte apoptosis in vivo. Whether myocyte apoptosis contributed directly to the higher mortality rate among isoproterenol-treated β2KO animals is unclear. We did not observe greater impairment of cardiac function in isoproterenol-treated β2KO mice relative to isoproterenol-treated wild-type mice. However, structural injury leading to events such as lethal arrhythmias remains a possible cause of the higher mortality. β2KO mice may be more susceptible to arrhythmias due to altered signaling in their myocytes. Other investigators have reported that in a canine model, arrhythmogenicity increases during β1AR (but not β2AR) activation by virtue of a cyclic adenosine monophosphate-dependent pathway (34). Other investigators have also reported that β2AR activation attenuates β1AR-mediated arrhythmias (35). We did not observe arrhythmias during our ECG analyses. However, this approach cannot be considered an adequate assessment method.

Noncardiac events may also have played a role in the higher mortality rate among our isoproterenol-treated β2KO mice. For example, changes in the hepatic, renal, and/or central nervous systems may have adversely affected the β2KO mice. In addition, isoproterenol infusion may have affected metabolic and neuroendocrine pathways differently in β2KO mice compared with wild-type animals.

Our data also revealed a significant genotype effect on liver weight to body weight ratios. However, we have not determined why this phenomenon occurred. One possibility is that the livers of β2KO mice are more susceptible to direct effects of isoproterenol infusion. Another possibility is that venous congestion occurs in the livers of β2KO mice due to impaired right heart function, although we did not obtain direct evidence of right ventricular dysfunction.

In summary, the results of this study suggest that in vivo β2AR activation is antiapoptotic and contributes to myocardial protection.

REFERENCES


Figure 7. Myocyte apoptosis after 7 days of saline or isoproterenol infusion. We detected a genotype effect (#) (p < .0001, two-way analysis of variance) as well as saline and isoproterenol treatment effects (×, *) (p = .03 and p < .0001, respectively, two-way analysis of variance) in terms of myocyte apoptosis when β2 adrenergic receptor knockout (β2KO) and wild-type mice were compared at 7 days of infusion. However, further analysis suggested that the genotype-treatment interaction terms were not significant (F = 3.87 and F = 1.92, respectively, Bonferroni posttests). The numbers of animals in each study group were eight isoproterenol-treated β2KO mice, five isoproterenol-treated wild-type mice, eight saline-treated β2KO mice, eight saline-treated wild-type mice, five β2KO mice without infusion pumps, and nine wild-type mice without infusion pumps. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

Figure 8. Noninvasive blood pressure measurements during isoproterenol infusion: wild-type vs. β2 adrenergic receptor knockout (β2KO) mice. No significant difference in systolic blood pressure was detected when isoproterenol-treated β2KO mice were compared with isoproterenol-treated wild-type animals (p = .99; Bonferroni/Dunn post hoc analysis, repeated-measures analysis of variance).
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