Differences in the Bioenergetic Response of the Isolated Perfused Rat Heart to Selective $\beta_1$- and $\beta_2$-Adrenergic Receptor Stimulation

Patrick McConville, PhD; Kenneth W. Fishbein, PhD; Edward G. Lakatta, MD; Richard G.S. Spencer, PhD, MD

Background—In the heart, striking functional differences exist after stimulation of the $\beta_1$- and $\beta_2$-adrenergic receptor (AR) subtypes. These may be linked to differences in metabolic response during $\beta_1$- and $\beta_2$-AR stimulation.

Methods and Results—The relation between work and metabolism was examined during selective $\beta_1$- and $\beta_2$-AR stimulation ($\beta_1$ and $\beta_2$ groups, respectively) in the isolated perfused rat heart. Measurements were made of rate-pressure product (RPP=LV developed pressure × heart rate), phosphorus-containing metabolites, and pH by $^{31}$P nuclear magnetic resonance spectroscopy and of O$_2$ consumption by fiber-optic oximetry. Experiments were performed under high constant flow (HCF) and under flow-limiting conditions (constant pressure, CP). Despite substantially greater RPP increases relative to baseline during $\beta_1$-AR (HCF, 475%; CP, 150%) than $\beta_2$-AR (HCF, 90%; CP, 72%) stimulation, the relative decrease in the intracellular energy charge relative to baseline was similar for the $\beta_1$ (HCF, 49%; CP, 64%) and $\beta_2$ (HCF, 59%; CP, 55%) groups. For each group, an increase in oxygen consumption ($\text{MVO}_2$) occurred commensurate with workload during HCF ($\beta_1$, 141%; $\beta_2$, 30%). During CP, however, the $\text{MVO}_2$ increase was similar ($\beta_1$, 39%; $\beta_2$, 34%), despite the large RPP difference between the groups. During both protocols, there was greater acidosis during $\beta_1$-AR than during $\beta_2$-AR stimulation. Thus, at a given workload, intracellular energy charge decreased, and $\text{MVO}_2$ (CP) increased to a greater extent during $\beta_2$ than $\beta_1$-AR stimulation.

Conclusions—The bioenergetic differences are consistent with access to an additional substrate pool during $\beta_1$-AR stimulation. This may occur via increased glycogenolysis during $\beta_1$-AR stimulation, facilitating increased energy production by oxidative phosphorylation, and under flow-limiting conditions, anaerobic glycolysis. (Circulation. 2003;107:2146-2152.)

Key Words: receptors, adrenergic, beta | metabolism | oxygen | imaging

Beta-adrenergic receptors (ARs) modulate the contractile response of the heart to stress. Although the importance of both the $\beta_1$- and $\beta_2$-AR subtypes has been recognized, there are substantial differences between $\beta_1$- and $\beta_2$-AR-mediated functional responses. 1–4

A recent study using canines found that cAMP signaling was coupled to PKA activation during $\beta_1$- but not during $\beta_2$-AR stimulation. 3 For $\beta_2$-AR–stimulated hearts, this led to PKA-dependent phosphorylation of the key regulatory proteins phospholamban, troponin I, and C protein and phosphorylation of the inactive b form of glycogen phosphorylase to the active a form. This suggested that the metabolic responses to $\beta_1$- and $\beta_2$-AR stimulation may differ. In particular, greater glycogenolysis may occur during $\beta_1$-AR than $\beta_2$-AR stimulation. 4

We hypothesized that as a result of these differences in cAMP signaling, the relationship between cardiac contractile function and bioenergetics differs between $\beta_1$- and $\beta_2$-AR stimulation. Studies in the intact heart permit an evaluation of the working organ with accurate regulation of substrate and oxygen delivery without the complexity of other neurohumoral factors present in the whole organism. $^{31}$P NMR spectroscopy provides real-time measurements of the intracellular concentrations of phosphocreatine (PCr), ATP, and inorganic phosphate (P$_i$), as well as pH. Previous applications of this technique have been made to $\beta$-adrenergic stimulation in isolated 6–8 and in situ 9,10 rat hearts, isolated guinea pig hearts, 11 in situ rabbit hearts 12 and human hearts, 13,14 each demonstrating decreased PCr concentration, but without specifically addressing differences between $\beta_1$- and $\beta_2$-AR–mediated effects.

In the present study, we used $^{31}$P NMR spectroscopy, oxygen consumption measurements, and measurements of contractile function to establish relationships between bioenergetics and work in the isolated perfused rat heart during $\beta_1$- and $\beta_2$-AR stimulation, under both limiting and nonlimiting flow conditions.
Methods

Isolated Heart Preparation

Male Wistar rats (Charles River, Wilmington, Mass), 3 to 4 months of age, were injected with 20 mg/kg 6-hydroxydopamine 24 hours before experimentation to attenuate the effect of endogenous catecholamines.15 Hearts were rapidly excised under anesthesia; perfused through the aorta with a filtered (0.45 μm), warmed (37°C), and gassed (95% O2/5% CO2) buffer in a nonrecirculating system; and allowed to beat spontaneously. The buffer consisted of (in mmol/L) 118 NaCl, 5 KCl, 0.5 Na2 EDTA, 1.2 MgSO4, 25 NaHCO3, 1.8 CaCl2, 11 glucose, and 1100 U/L heparin.

A water-filled polyethylene balloon connected to a pressure transducer was inserted into the left ventricle (LV) and used to measure the LV pressure. Balloon inflation was adjusted to achieve a preload of 10 to 15 mm Hg. RPP was used as an index of workload. To test the stability of the preparation, a control group of hearts (n=5) was perfused under constant perfusion pressure (CP) for 2 hours. During this time, LV developed pressure (LVDP), heart rate (HR), and PCr/Pi, ratio remained within 11.5±4% of baseline.

MVO2 Measurements

An O2-sensitive fiber-optic fluorometer (Presens GmbH) was used to measure the O2 saturation in the arterial line and in the venous effluent via a cannula sutured into the coronary sinus. MVO2 was derived from the arteriovenous O2 difference per unit coronary flow.

31P NMR Spectroscopy

Perfused hearts were placed in a glass tube, then inserted into a 9.4-T Magnex magnet (Magnex Scientific) interfaced to a Bruker DMX spectrometer (Bruker Analytik GmbH). Shimming was performed to achieve a water proton line width of ≤40 Hz. After a stabilization period, 31P spectra were acquired continuously over a period of 3 minutes using a pulse flip angle of 45°, repetition time of 1.5 seconds, spectral width of 12 kHz, 50-Hz line broadening, and zero filling to 8K points. A small tube containing 400 mmol/L methylenediphosphonate (MDP) was placed outside the heart and used as a chemical shift and signal intensity standard. Quantification was performed with Lorentzian deconvolution. The ratio of the PCr and chemical shift and signal intensity standard. Quantification was performed with Lorentzian deconvolution. The ratio of the PCr and Pi resonances was used as an index of the intracellular energy charge.

Experimental Protocols

Experiments were performed both under conditions of high constant flow (HCF) and CP. In the HCF experiments, the fixed flow rate (28.4±0.1 mL/min) was equal to the highest flow rates observed under CP (at the start of the perfusion) and is at the upper end of the range typically used in isolated rat heart perfusion experiments.

Under the CP protocol (CP=120 mm Hg), the coronary flow rate decreased over the duration of the experiment for both groups (β1, 24%; β2, 28%), with the respective coronary flow time courses not significantly different (P=0.3). Therefore, the CP protocol provided a convenient method for examining flow-limiting and hence oxygen-limiting conditions.

Under each protocol, hearts underwent either selective β1-AR stimulation (β1 group) or selective β2-AR stimulation (β2 group). The doses used were based on previous studies2-3 and are listed in the Table. Three 12-minute doses of increasing concentrations of the β1 agonist norepinephrine or the β2 agonist zinterol (Bristol-Myers Squibb) were administered to the β1 and β2 groups, respectively. To increase selectivity of the receptor stimulation, an α-AR antagonist, prazosin, or a β2-AR antagonist, bisoprolol (Merck), was used for β1 and β2-stimulated hearts, respectively, in combination with the agonists.2-4 Figure 1 shows typical responses of RPP and MVO2 for a β1- and a β2-AR-stimulated heart during HCF at baseline and during each of the 3 doses. Typical 31P NMR spectra obtained during baseline and β-AR stimulation are shown in Figure 2.

Results

Dose Responses

Preliminary experiments using both perfusion protocols showed that the maximum agonist doses chosen elicited a

<table>
<thead>
<tr>
<th>Dose Protocol Used After the 30-Minute Equilibration Period</th>
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<tbody>
<tr>
<td>Dose Period</td>
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<tr>
<td>(12 minutes)</td>
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<tr>
<td>β1 Group</td>
</tr>
<tr>
<td>β2 Group</td>
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<tr>
<td>Baseline</td>
</tr>
<tr>
<td>10^6 praz</td>
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<tr>
<td>1.5×10^6 bis</td>
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<tr>
<td>Dose 1</td>
</tr>
<tr>
<td>10^6 NE=10^6 praz</td>
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<tr>
<td>10^6 zint+1.5×10^6 bis</td>
</tr>
<tr>
<td>Dose 2</td>
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<tr>
<td>10^7 NE=10^6 praz</td>
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<tr>
<td>10^6 zint+1.5×10^6 bis</td>
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<tr>
<td>Dose 3</td>
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<tr>
<td>10^6 NE=10^6 praz</td>
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<td>10^6 zint+1.5×10^6 bis</td>
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Values are in mol/L. Praz indicates prazosin; NE, norepinephrine; bis, bisoprolol; and zint, zinterol.
near-maximal response in RPP. In particular, the larger range of RPPs achieved in response to \( \beta_1 \)-AR stimulation could not be achieved during \( \beta_2 \)-AR stimulation. The mean baseline values of all measured parameters were generally consistent between the \( \beta_1 \) and \( \beta_2 \) groups.

**LV Developed Pressure**

LVDP increased significantly in a dose-dependent manner in both groups (Figure 3A). Under HCF, the maximum response for the \( \beta_1 \) group (415% of baseline) was significantly greater than that of the \( \beta_2 \) group (158% of baseline). Under the flow-limiting CP protocol, the maximum \( \beta_1 \)-AR–mediated LVDP response was only half that under HCF (187% of baseline), whereas the response of the \( \beta_2 \)-AR–stimulated groups was similar to that under HCF (132% of baseline).

**Heart Rate**

Dose-dependent increases in HR were observed for each group (Figure 3B). The range of this response was somewhat greater during \( \beta_1 \)-AR (141% of baseline) than during \( \beta_2 \)-AR (124% of baseline) stimulation. No such difference was found under the CP protocol (134% and 132% of baseline for the \( \beta_1 \) and \( \beta_2 \) groups, respectively).

**Rate-Pressure Product**

The dose dependence of cardiac workload as measured by the RPP is shown in Figure 3C. For the \( \beta_1 \)-AR–stimulated hearts, the workload was influenced predominantly by the LVDP response, leading to much greater RPPs at maximum dose (HCF, 575%; CP, 250%), compared with the \( \beta_2 \) group (HCF, 190%; CP, 172%).

**Oxygen Consumption**

\( \text{MVO}_2 \) increased relative to baseline at all doses (Figure 3D) in both groups. During the HCF protocol, the \( \beta_1 \) group showed substantially greater maximum \( \text{MVO}_2 \) response (241% of baseline) than the \( \beta_2 \) group (130% of baseline). Under the flow-limiting CP protocol, significantly lower maximal \( \text{MVO}_2 \) was observed in the \( \beta_1 \) group only (\( \beta_1 \), 139%; \( \beta_2 \), 134%).

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**Figure 2.** Typical examples of \( ^{31}\text{P} \) NMR spectra during (A) baseline and (B) \( \beta_1 \)-AR stimulation (\( 10^{-7} \) mol/L norepinephrine), showing MDP, Pi, and PCR resonances and \( \gamma \), \( \alpha \), and \( \beta \)-phosphate resonances of ATP. A decrease in PCR concentration and corresponding increase in Pi concentration occurs as predicted by creatine kinase and ATP hydrolysis equilibria. Note that ATP concentration remained relatively constant.

**Figure 3.** Responses under CP and HCF plotted as percent of baseline for each perfusion period: (A) LVDP, (B) HR, (C) RPP, (D) \( \text{MVO}_2 \), (E) PCR/Pi ratio, and (F) pH. Data are shown for \( \beta_1 \) (solid lines) and \( \beta_2 \)-AR–stimulated hearts (dotted lines) and CP (circles) and HCF (squares). Where error bars are not visible, they fall within symbol area. \( \beta_1 \) group: CP, \( n=10 \); HCF, \( n=5 \), and \( \beta_2 \) group: CP, \( n=8 \); HCF, \( n=6 \). In all cases, a significant main effect of dose was found at \( P<0.05 \) level. *\( P<0.05 \) for response/flow protocol interaction (for each protocol); †\( P<0.05 \) for response/\( \beta \)-stimulation group interaction (for each protocol).
Intracellular Energy Charge
In the HCF protocol, the IEC (Figure 3E) decreased in a dose-dependent manner in both groups to similar final values (β₁, 36%; β₂, 45%). In the CP protocol, the dose dependence of this decrease was not significantly different for either the β₁-AR (P = 0.2) or β₂-AR (P = 0.8) group (IEC = 51% and 41% at maximum dose, respectively).

pH
During HCF, significant acidosis occurred in the β₁-AR group only (Figure 3F). During CP, acidosis developed in both groups as a function of dose, although the pH reduction at maximum dose was greater in the β₁-AR group (HCF, 0.06; CP, 0.09).

Comparison of Bioenergetic Responses to β₁- and β₂-AR Stimulation
Substantially greater RPP dose responses were achieved during β₁-AR than during β₂-AR stimulation (HCF and CP), despite similar decreases in IEC (HCF and CP) and MVO₂ (CP). We sought to determine the metabolic cost associated with a given workload during β₁- and β₂-AR stimulation by plotting IEC and MVO₂ as functions of RPP. A significant difference in the IEC versus RPP relationship was observed under both HCF and CP (Figure 4), with β₂-AR-stimulated hearts showing a significantly greater decrease (HCF, P = 0.05; CP, P = 0.001) in IEC than β₁-AR-stimulated hearts.

Figure 4. PCr/Pi vs rate-pressure product for β₁- and β₂-AR groups under (A) HCF and (B) CP. Each data point corresponds to average responses measured during baseline or 1 of 3 doses. This plot demonstrates response in PCr/Pi ratio (IEC) for a given RPP increase, removing dose dependence of specific agonists and concentrations that were used. Under conditions of both HCF and CP, for a given increase in RPP, β₂-AR-stimulated hearts show a significantly greater decrease (HCF, P = 0.05; CP, P = 0.001) in IEC than β₁-AR-stimulated hearts.

Discussion
The major aim of this study was to compare the correlations between bioenergetics and LV workload during stimulation...
Figure 6. Schematic showing major factors expected to influence energy supply and workload demand and hence intracellular energy charge during β-adrenergic stimulation. Arrows and their directions show potential influences. Combination of our measurements under HCF and CP (flow-limiting) suggests that influences of certain factors shown in this figure are flow dependent, as indicated. Coronary flow can potentially be mediated via β-AR stimulation (arrow 1), although our data showed no difference in flow between β₁ and β₂ groups. However, flow was clearly influenced by choice of perfusion protocol. β₁-AR-mediated activation of phosphorylase kinase can influence provision of endogenous glycogen as substrate (arrow 2). Total available substrate may then be utilized via aerobic (arrow 3) or anaerobic (arrow 4) pathways, providing energy supply. β-AR workload response is mediated via increased Ca⁺ release and reuptake by sarcoplasmic reticulum (arrow 5). However, calcium ions act as dual messengers by also activating mitochondrial dehydrogenases, NAD-isocitrate, 2-oxoglutarate, and pyruvate dehydrogenase, thereby regulating generation of ATP by oxidative phosphorylation (arrow 6). Feedback is also regulated between workload and oxidative phosphorylation via phosphorylation potential [ATP/(ADP×P)] and mitochondrial NADH/NAD ratio (arrow 7).

Greater Energy Availability and Contractile Response During β₁-AR Stimulation
During the CP protocol, we observed a far greater RPP response during β₁- than during β₂-AR stimulation. Despite this difference, the decrease in IEC was similar in the β₁- and β₂-AR-stimulated hearts (Figure 4B). This indicates a similar energy supply:demand ratio and therefore an increase in energy production and therefore oxygen delivery) under the HCF protocol resulted in an almost 2-fold greater contractile response compared with the response under the flow-limiting CP protocol. For the β₂-AR-stimulated hearts, however, the responses under the 2 flow protocols were similar. This suggests that under the CP protocol, the maximal β₂-AR-mediated increases in workload were flow limited. Despite the very large difference in the maximum workload achieved during β₁- and β₂-AR stimulation with HCF, the net decrease in IEC was again similar (Figure 4A), implying that energy production was augmented during β₁-AR stimulation to meet the greater demand.

Increased Anaerobic Metabolism During β₁-AR Stimulation Under Oxygen-Limited Conditions
Under CP, the greater workload demand and energy production during β₁- compared with β₂-AR stimulation
occurred without a large increase in oxygen requirements (Figure 5B). This suggests that during $\beta_1$-AR stimulation, anaerobic metabolism was used for this additional energy production. This is supported by the intracellular pH measurements (Figure 3F), which demonstrated significantly greater acidosis during the maximum response to $\beta_1$-AR stimulation, consistent with increased lactate production from glycolysis. In contrast, during HCF, the increased work achieved by $\beta_1$-AR stimulation was associated with a commensurate increase in $\text{MV}_0$ (Figure 5A) and a smaller degree of acidosis.

**$\beta_1$-AR Stimulation Shows Increased IEC at a Given Workload**

Our measurements allow us to examine a range of workloads common to both $\beta_1$- and $\beta_2$-AR–stimulated hearts and specifically the metabolic responses at a given workload during $\beta_1$- or $\beta_2$-AR stimulation. Under both flow protocols, at a fixed RPP, the IEC was preserved to a greater extent during $\beta_1$-AR stimulation (Figure 4), indicating that during $\beta_1$-AR stimulation there was greater energy reserve, consistent with increased energy production. Under CP, at a given RPP, a clear difference in the $\text{MV}_0$ was found between the $\beta_1$- and $\beta_2$-AR–stimulated hearts (Figure 5), a difference not present in the HCF protocol. This suggests that during $\beta_1$-AR stimulation under $O_2$-limiting conditions, the increase in workload was facilitated by energy production through anaerobic pathways.

**Regulation of Energy Charge and the Workload Response**

Intracellular calcium ([Ca$^{2+}$]) levels are known to be greater during $\beta_1$-AR stimulation$^{1-4}$ and may be a more significant regulator of oxidative phosphorylation (Figure 6, arrow 5) during $\beta_1$-AR stimulation than during $\beta_2$-AR stimulation. This regulation would be expected to be more effective when $O_2$ is not limiting and is consistent with the greater augmentation of energy supply and workload demand during $\beta_1$-AR stimulation under HCF compared with that under CP. This could lead to more effective augmentation of the other regulators of oxidative phosphorylation, such as phosphorylation potential and mitochondrial NADH/NAD ratio (Figure 6, arrow 7), and ultimately to increased feedback between these regulators and cardiac workload. During $\beta_2$-AR stimulation, energy supply and demand regulation by [Ca$^{2+}$] potential, and mitochondrial NADH/NAD ratio may be reduced, with a similar effect occurring under reduced flow during $\beta_2$-AR stimulation.

The $\text{MV}_0$ data and pH data under CP suggest that when oxygen limits the aerobic energy supply, anaerobic metabolism can be used during $\beta_1$-AR stimulation in conjunction with additional substrate delivery. When oxygen delivery is increased (HCF), [Ca$^{2+}$] regulation of oxidative phosphorylation could act in concert with the additional substrate to preserve the IEC, leading to the even larger workload responses.

At the maximum workloads achieved, there was no statistical difference in the IEC between the $\beta_1$ and $\beta_2$ groups during CP or HCF. This suggests that the energy supply:demand ratio was similar under these conditions of near maximal stimulation. This could indicate a lower limit for IEC, which might then limit the workload response. Alternatively, however, IEC can be thought of as an energy “reserve” that can be rapidly utilized after sudden increases in demand. In this context, this reserve may be “depleted” by workload to some limiting extent during maximal stimulation. The maintenance of this increased workload would then be regulated by energy supply through the usual aerobic and anaerobic pathways, which would in turn depend on both $O_2$ and substrate supply.

**Mechanisms for Increased Energy Charge**

Although we cannot draw absolute conclusions about the origin of the additional energy available under $\beta_1$-AR stimulation, an attractive possibility is increased substrate supply from the breakdown of endogenous glycogen, followed by anaerobic and/or aerobic glucose metabolism. This is consistent with previous results demonstrating that glycogen phosphorylase kinase is activated during $\beta_1$- but not during $\beta_2$-AR stimulation.$^{4}$ Glycogen availability under $\beta_1$-AR stimulation may be a result of specific signaling that leads to conversion of glycogen phosphorylase from the inactive $b$ form to the active $a$ form.

Significant reliance on glycogenolysis and subsequent glucose metabolism during epinephrine-induced mixed $\beta$-adrenergic stimulation in the isolated heart has been observed with radiolabeling.$^{18,19}$ However, epinephrine is a potent agonist for both the $\beta_1$- and $\beta_2$-AR subtypes. Our results suggest, for the first time, that reliance on an alternative energy source to meet requirements under $\beta$-adrenergic stimulation occurs specifically under $\beta_1$-AR stimulation.

Although these results are consistent with glycogen being the origin of additional substrate available during $\beta_1$-AR stimulation, we cannot distinguish between this and the possibility of enhanced delivery of exogenous glucose, for example, via an unspecified $\beta_1$-AR–mediated activation of glucose transporters.

The $\text{MV}_0$ and pH differences between the $\beta_1$- and $\beta_2$-AR–stimulated hearts suggest that during the flow-limiting conditions of the CP experiments, a significant proportion of any potential additional substrate is metabolized anaerobically. Under HCF, the IEC data are still consistent with increased use of glycogen in the $\beta_1$ hearts, but the $\text{MV}_0$ and pH data suggest that given sufficient $O_2$, this substrate is metabolized primarily aerobically.

Furthermore, it should be noted that the $\beta_1$/$\beta_2$-AR differences in $\text{MV}_0$ and IEC are also consistent with increased oxidative efficiency during $\beta_1$-AR stimulation. This may result from decreased utilization of $O_2$ for noncontractile cellular processes or futile cycles. This could result in greater preservation of IEC because of an effectively greater energy supply for contraction. However, other evidence suggests that the opposite may be true, namely, that $\beta_1$-AR stimulation is less energy effi-
cient than β₂-AR stimulation. One possible mechanism that has been proposed for this is spontaneous [Ca²⁺]ᵢ oscillations during β₁-AR stimulation.³

**Effect of Competing Substrates**

In the intact organism, free fatty acids (FFAs) are the preferred substrate of the heart in the fasted state. However, it has been shown that under β-AR–induced stress in the perfused rat heart, carbohydrates become the preferred substrate, with glucose and glycogen showing large increases in oxidation compared with relatively minor increases in oxidation of exogenous FFAs and endogenous triglycerides.²⁰,²¹ Furthermore, the endogenous lipolytic capacity of the heart is considerably smaller than the capacity for glycolysis and subsequent carbohydrate oxidation, which is directly regulated by mitochondrial feedback. Therefore, we expect that metabolism of exogenous FFAs and endogenous triglycerides would make only a minor contribution to the observed differences between β₁- and β₂-AR stimulation.

**Summary**

We have demonstrated for the first time, in a working heart preparation, a difference in metabolic response to β₁- and β₂-AR stimulation. These differences are consistent with differences in energy supply, possibly mediated by β-AR subtype–dependent endogenous substrate utilization. These metabolic differences may underlie the large differences in β₁- and β₂-AR–mediated functional responses.

**References**

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