β-Agonist stimulation produces changes in cardiac AMPK and coronary lumen LPL only during increased workload

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An, Ding, Girsh Kewalramani, Dake Qi, Thomas Pulinilkunnil, Sanjoy Ghosh, Ashraf Abrahani, Rich Wambolt, Michael Allard, Sheila M. Innis, and Brian Rodrigues. β-Agonist stimulation produces changes in cardiac AMPK and coronary lumen LPL only during increased workload. Am J Physiol Endocrinol Metab 288: E1120–E1127, 2005. First published February 1, 2005; doi:10.1152/ajpendo.00588.2004.—Given the importance of lipoprotein lipase (LPL) in cardiac and vascular pathology, the objective of the present study was to investigate whether the β-agonist isoproterenol (Iso) influences cardiac LPL. Incubation of quiescent cardiomyocytes with Iso for 60 min had no effect on basal, intracellular, or heparin-releasable (HR)-LPL activity. Similarly, Iso did not change HR-LPL in Langendorff isolated hearts that do not beat against an afterload. In the intact animal, LPL activity at the vascular lumen increased significantly in the Iso-treated group, together with a substantial increase in rate-pressure product. This LPL increase was likely via mechanisms regulated by activation of AMP-activated protein kinase (AMPK) and inactivation of acetyl-CoA carboxylase (ACC280). In glucose-perfused hearts, simply switching from Langendorff to the isolated working heart (that beats against an afterload) induced increases in AMPK and ACC280 phosphorylation and enhanced HR-LPL activity. Provision of insulin and albumin-bound palmitic acid to the working heart was able to reverse these effects. In these hearts, introduction of Iso to the buffer perfusate duplicated the effects seen when this β-agonist was given in vivo. Our data suggest that Iso can influence HR-LPL only during conditions of increased workload, mechanical performance and excessive energy expenditure, and likely in an AMPK-dependent manner.

isoproterenol; acetyl-coenzyme A carboxylase; cardiomyocyte; Langendorff heart; working heart; adenosine 3’-5’-monophosphate-activated protein kinase; lipoprotein lipase

CARDIAC CONTRACTILITY REQUIREs an uninterrupted supply of ATP. Under normal physiological conditions, the heart utilizes two major substrates, glucose and fatty acids (FA; see Ref. 51). Compared with glucose, FA is the preferred substrate and accounts for ∼70% of ATP production. However, the heart has the ability to choose its substrates depending on their availability and the prevailing physiological (e.g., exercise) or pathophysiological (e.g., ischemia) conditions. AMP-activated protein kinase (AMPK) is likely a key player in modulating this substrate selection. Thus, during exercise (when ATP expenditure is augmented) or ischemia (when manufacture of ATP is hindered), changes in intracellular AMP/ATP levels promote threonine (Thr172) phosphorylation and activation of AMPK (22, 25). Upon stimulation, AMPK switches off energy-consuming processes like triglyceride (TG) and protein synthesis, whereas ATP-generating mechanisms are turned on (21, 26). In heart and skeletal muscle, phosphorylated AMPK stimulates glucose uptake (23, 55) and subsequent glycolysis through the activation of 6-phosphofructo-2-kinase (38). More importantly, through its control of acetyl-CoA carboxylase (ACC), AMPK facilitates FA oxidation (31, 32). AMPK has also been implicated in FA delivery to cardiomyocytes through its regulation of the FA transporter, CD36 (36). Finally, results from our laboratory have demonstrated a strong correlation between activation of cardiac AMPK and increases in coronary lumen lipoprotein lipase (LPL) activity (2).

LPL is a rate-limiting enzyme for hydrolysis of TG-rich lipoproteins, thus regulating the supply of FA to meet the metabolic demands of different tissues. It is synthesized in myocytes and subsequently transported onto heparan sulfate proteoglycan (HSPG) binding sites on the myocyte cell surface (13, 15). Through mechanisms that are not completely understood, LPL is then transported onto HSPG binding sites on the luminal surface of the capillary endothelium (43). At this location, the enzyme plays a crucial role in hydrolysis of TG-rich lipoproteins to FA, which are transported to the heart and used either for energy production or for resynthesis of TG. Recently, LPL-mediated hydrolysis of circulating TG was suggested to be the principal source of FA for cardiac utilization (4, 65). In addition to its role as a lipolytic enzyme, LPL also mediates a noncatalytic bridging function that allows it to bind simultaneously to both lipoproteins and specific cell surface proteins, facilitating cellular uptake of lipoproteins (40, 56, 63).

Through its role in TG hydrolysis, LPL activity directly affects the level of circulating lipoprotein-TG. For example, in transgenic rabbits with global overexpression of LPL, attenuation of hypertriglyceridemia was observed, an effect suggested to contribute toward amelioration of insulin resistance and obesity (28). Contrary to systemic overexpression, tissuespecific overexpression of LPL in skeletal muscle and heart is associated with insulin resistance in these tissues as well as severe myopathy, characterized by both muscle fiber degeneration and extensive proliferation of mitochondria and peroxisomes (27, 34, 67). In a more recent study using genetically engineered mice that specifically overexpressed nontransferable cardiomyocyte surface-bound LPL, lipid oversupply and deposition were observed, along with excessive dilatation and...
impaired left ventricular systolic function (cardiomyopathy; see Ref. 69). In vascular smooth muscle, overexpression of LPL brings about FA loading and vascular dysfunction (16).

Heart failure, coupled with conditions such as coronary artery disease and hypertension, is often associated with activation of the sympathetic nervous system and higher levels of circulating catecholamines (14, 17). Although the β-agonist isoproterenol (Iso) had no influence on myocyte cell surface or intracellular LPL (60), or LPL measured in whole heart homogenates (12), its influence on LPL at cardiac coronary lumen is unknown. Given the importance of LPL in cardiac and vascular pathology, the objective of the present study was to investigate whether β-agonist stimulation influences LPL at its functionally relevant location, the coronary lumen. We demonstrate that Iso does indeed augment luminal LPL, likely secondary to an increase in workload and excessive energy expenditure, rather than a direct effect.

**MATERIALS AND METHODS**

**Experimental animals.** The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health and the University of British Columbia (UBC; Animal Care Certificate no. A04-1009). Adult male Wistar rats (220–240 g) were obtained from the UBC Animal Care Unit and supplied with a standard laboratory diet (PMI Feeds, Richmond, VA) and water ad libitum. All of the experiments were conducted between 0900 and 1300.

**In vivo hemodynamics.** Rats were anesthetized with 65 mg/kg pentobarbital sodium, and a cannula was inserted in the carotid artery to measure systemic blood pressure and heart rate. Anesthesia is known to cause cardio depression. After stabilization, animals were injected with heparin before death because it displaces LPL bound to HSPG on the capillary endothelium. Consequently, it was necessary to cannulate the heart quickly to avoid clotting of blood in the coronary arteries. After cannulation of the aorta, hearts were secured by tying below the innominate artery and perfused retrogradely with Krebs-Henseleit (KH) buffer containing 10 mM glucose (as the only substrate). The rate of coronary flow (7–8 ml/min) was controlled by a peristaltic pump. Where indicated, Iso (10 μM) was then added to the KH buffer.

**Isolated working heart perfusion.** Hearts were isolated and perfused as described previously (1). Briefly, rats were anesthetized using 2–3% halothane, and the hearts were carefully excised. Rats were not injected with heparin before death because it displaces LPL bound to HSPG on the capillary endothelium. Consequently, it was necessary to cannulate the heart quickly to avoid clotting of blood in the coronary arteries. After cannulation of the aorta, hearts were secured by tying below the innominate artery and perfused retrogradely with Krebs-Henseleit (KH) buffer containing 10 mM glucose (as the only substrate) and 2 mM calcium (pH 7.4, 37°C). Perfusion fluid was continuously gassed with 95% O₂-5% CO₂. After 10 min, hearts were then switched to a working mode (perfusion through the left atria) with a left atrial preload of 11.5 mmHg, and an aortic afterload of 80 mmHg, for 15 min. Heart function (rate-pressure product, RPP) was measured using a Direcwin physiograph (Raytech). Where indicated, Iso (10 μM) at 37°C for 1 h, and basal LPL activity in the medium and cell pellet (after centrifugation) was measured. To release surface-bound LPL activity, heparin (5 U/ml) was then added to the myocyte suspension. After incubation for 10 min, an aliquot of cell suspension was removed, and medium was separated by centrifugation in an Eppendorf microcentrifuge (1 min, 10,000 g) and assayed for LPL activity. The total cellular LPL activity was measured by sonication (Vibra Cell sonicator at a frequency of 40 Hz for 2 × 30 s) the cell pellets after resuspending them in 0.2 ml of 50 mM NaCl buffer (pH 8.0) containing 0.125% (vol/vol) Triton X-100. After sonication, the volume was adjusted to 1 ml using a sucrose buffer (0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol, and 10 mM HEPES, pH 7.4). Assay for cell sonicate LPL activity was done using 20 μl of the cell sonicate and heparin (2 U/ml).

**Cardiac LPL gene expression.** LPL gene expression was measured in the indicated groups using RT-PCR (2, 44). Briefly, total RNA from hearts (100 mg) was extracted using TRIzol (Invitrogen). After spectrophotometric quantification and resolving of RNA integrity using a formaldehyde agarose gel, reverse transcription was carried out using an oligo(dT) primer and Superscript II RT (Invitrogen). cDNA was amplified using LPL-specific primers (6), 5′-ATC-CAAGCTGACCTAATGTTT-3′ (left) and 5′-AATGGCTTCTCCAA-TGTTCG-3′ (right). The β-actin control was amplified as an internal control using 5′-TGTTGGATATGGCTCAGA AGG-3′ (left) and 5′-ATCCTGTCAAGCGATGCTTG G-3′ (right). The linear range was found to be between 15 and 30 cycles. The amplification parameters were set at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, for a total of 30 cycles. The PCR products were electrophoresed on a 1.7% agarose gel containing ethidium bromide. Expression levels were represented as the ratio of signal intensity for LPL mRNA relative to β-actin mRNA.

**Western Blotting for AMPK and ACC.** AMPK phosphorylation increases its activity ~50- to 100-fold (53, 54, 58). Activated AMPK phosphorylates and inactivates ACC (53, 54, 58). To determine total and phosphorylated AMPKαs and ACC, whole cell homogenates were isolated as described previously (3). Briefly, hearts were ground under liquid nitrogen, and 50 mg were homogenized. After centrifugation at 5,000 g for 20 min, the protein content of the supernatant was determined using a Bio-Rad Durosol protein assay. Protein extracts were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted using anti-AMPKα (1:1,000) and anti-ACC (1:1,000) antibodies. Blots were visualized using an enhanced chemiluminescence detection system.
quantified using a Bradford protein assay. Samples were diluted and boiled with sample loading dye, and 50 μg were used in SDS-PAGE. After transfer, membranes were blocked in 5% skim milk in Tris-buffered saline containing 0.1% Tween 20. Membranes were incubated either with rabbit AMPKα, phospho-AMPK (Thr172), or phospho-ACC (Ser79) antibodies and subsequently with secondary goat anti-rabbit horseradish peroxidase-conjugated antibodies and visualized using enhanced chemiluminescence (ECL) detection. Measuring the phospho form of AMPK is a surrogate for estimation of its activity.

Separation and characterization of cardiac lipids. Total cardiac lipids were extracted and solubilized in chloroform-methanol-ace-
tone-hexane (4:6:1:1 vol/vol/vol/vol). Separation of TG and FA was achieved using HPLC (Waters 2690 Alliance HPLC, Milford, MA) equipped with an auto-sampler and column heater. FA were quantified as their respective methyl esters using heptadecaenoic acid (17:0) as the internal standard with a Varian 3400 GLC equipped with a flame ionization detector, a Varian Star data system, and a SP-2330 capillary column (30 m × 0.25 mm; Supelco, Bellefonte, PA). Value of cardiac FA and TG were expressed as micrograms per milligram protein.

Serum measurements. Blood samples were removed from animals and centrifuged immediately to collect serum, which was then stored at −20°C until assay. Diagnostic kits were used to measure glucose, TG (Sigma), and nonesterified FA (Wako).

Statistical analysis. Wherever appropriate, one-way ANOVA followed by the Tukey or Bonferroni tests or the unpaired and paired Student’s t-test were used to determine differences between group mean values (as indicated in the specific legends for Figs. 1–6). The level of statistical significance was set at P < 0.05.

Materials. Iso was purchased from Sigma. Total AMPKα, phospho-AMPKα, and phospho-ACC antibodies were obtained from Cell Signaling (Beverly, MA). The ECL detection kit was obtained from Amersham.

RESULTS

Coronary luminal LPL activity increases after a single dose of Iso given in vivo. After Iso administration, RPP increased significantly compared with control (Table 1). Changes in plasma parameters after 1 h of Iso included a significant increase in serum free FA, with no effect on either glucose or TG (Table 1). To determine whether Iso controls LPL (at its functionally relevant location, the coronary lumen), hearts isolated from Iso-treated animals were perfused retrograderly with heparin. Compared with saline-injected rats, there was a substantial increase in LPL activity (∼300%) at the vascular lumen after 1 h of Iso (Fig. 1, top). This increase in LPL activity was maintained for an additional 3 h (Fig. 1, top).

Table 1. General characteristics of the animals

<table>
<thead>
<tr>
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<th>Control</th>
<th>Iso</th>
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<tr>
<td>Blood glucose, mM</td>
<td>5.7±0.2</td>
<td>5.4±0.2</td>
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<tr>
<td>Serum free fatty acid, mM</td>
<td>0.34±0.04</td>
<td>0.49±0.05*</td>
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<tr>
<td>Serum triglyceride, mM</td>
<td>1.4±0.2</td>
<td>1.3±0.1</td>
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<tr>
<td>Systolic pressure, mmHg</td>
<td>116±4</td>
<td>131±10</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>421±16</td>
<td>474±18*</td>
</tr>
<tr>
<td>Rate-pressure product</td>
<td>49,192±3,121</td>
<td>62,039±5,378*</td>
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Values are means ± SE for 6 animals in each group. Rats were anesthetized with 25 mg/kg pentobarbital sodium, and a cannula was inserted in the carotid artery to measure systemic blood pressure and heart rate. After stabilization, animals were either injected ip with saline or 10 μg/kg of the β-agonist isoproterenol (Iso). Blood pressure and heart rate were recorded every 20 min for 1 h, and averages are presented. For measurement of serum parameters, blood was collected at termination (1 h after Iso). *Significantly different from control, P < 0.05.

Notably, alterations in luminal LPL activity were independent of shifts in mRNA levels (Fig. 1, bottom).

Iso injected intraperitoneally augments cardiac AMPK and ACC phosphorylation. Because changes in luminal LPL activity were independent of shifts in LPL mRNA, the LPL increase at the coronary lumen is likely via posttranscriptional mechanisms. Interestingly, upon injection of Iso, the increase in LPL was also associated with phosphorylation of AMPK, both at 1 and 4 h after administration (Fig 2, top). Once activated, AMPK phosphorylates and inactivates ACC. As ACC catalyzes the conversion of acetyl-CoA to malonyl-CoA, AMPK, by phosphorylating ACC, is able to decrease malonyl-CoA and minimize its inhibition of FA oxidation (53, 54, 58). In the heart, ACC280 (280-kDa) is the predominant isoform. After 1 h of Iso, phosphorylation of ACC280 increased; although ACC280 phosphorylation appeared higher at 4 h, no statistical difference could be identified (P = 0.086; Fig. 2, bottom). Interestingly, Iso induced a time-dependent drop in cardiac TG, without affecting cardiac free fatty acid (FFA; Table 2).

Iso does not influence LPL activity and AMPK phosphorylation in myocytes or Langendorff-perfused hearts. Previous studies have shown that incubation of cardiomyocytes with Iso for 30 min had no effect on either basal or HR-LPL activity (60). In the present study, incubation with this β-agonist was...
extended to 1 h, and no effect was observed on basal (unpublished observation), HR (control, 847 ± 0.02; Iso, 814 ± 0.04 units) or retrograde perfused hearts (Fig. 3; as measured by densitometry; control, 1.0 ± 0.1; Iso, 1.03 ± 0.14 units).

Increasing workload promotes phosphorylation of AMPK and ACC and enlarges the coronary lumen LPL pool. Given that the Iso-induced increase in RPP was associated with phosphorylation of AMPK and ACC, and coronary luminal LPL activity in vivo, and that myocytes or retrograde perfused hearts do not represent a physiological contractile circumstance, we considered whether simply increasing workload by switching from Langendorff to the isolated perfused working heart would induce similar changes. Compared with retrograde perfusion, the working heart perfused only with glucose (but in the absence of insulin) demonstrated higher phospho-AMPK (Fig. 4; as measured by densitometry; Langendorff heart, 0.91 ± 0.15; working heart, 2.15 ± 0.6 units, P < 0.05), and phospho-ACC280 (Fig. 4; as measured by densitometry; Langendorff heart, 0.9 ± 0.08; working heart, 1.4 ± 0.04 units, P < 0.05), and HR-LPL activity (Fig. 4). These results suggest that, when increasing workload augments cardiac mechanical performance and energy expenditure, LPL is recruited to the coronary lumen.

determine whether Iso influences LPL in the intact heart, we perfused hearts retrogradely with this β-agonist. Retrograde perfused hearts allow for adequate coronary perfusion with limited expenditure of energy (these hearts do not beat against an afterload). Similar to myocytes, Iso did not change HR-LPL in Langendorff isolated hearts (Fig. 3). Overall, our data suggest that, specifically in these preparations in vitro, Iso does not have a direct effect in regulating LPL activity. In a recent report, we described that recruitment of LPL to the coronary lumen can be regulated by AMPK (2). The relationship between AMPK phosphorylation and LPL activity was evaluated in isolated myocytes and Langendorff-perfused hearts. We demonstrate that Iso did not affect AMPK phosphorylation in either myocytes (as measured by densitometry; control, 0.97 ± 0.02; Iso, 0.88 ± 0.04 units) or retrograde perfused hearts (Fig. 3; as measured by densitometry; control, 1.0 ± 0.1; Iso, 1.03 ± 0.14 units).

Fig. 2. AMP-activated protein kinase (AMPK) and 280-kDa acetyl-CoA carboxylase (ACC280) phosphorylation in hearts isolated from animals injected with Iso for 1 or 4 h. After Iso injection for 1 or 4 h, hearts were removed and snap-frozen in liquid nitrogen. AMPKα (total and phosphorylated; top) and phospho-ACC280 (bottom) were measured using Western blotting and rabbit AMPKα, phospho-AMPK (Thr172), or phospho-ACC280 (Ser79) antibodies. Data are means ± SE of 5 different hearts in each group. *Significantly different from control, P < 0.05.

Table 2. Cardiac lipids in control and Iso-treated groups

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<th>Triglyceride, μg/mg protein</th>
<th>Free Fatty Acid, μg/mg protein</th>
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<tr>
<td>Control</td>
<td>1.7 ± 0.2</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>Iso 1 h</td>
<td>1.1 ± 0.1*</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Iso 4 h</td>
<td>0.7 ± 0.1*</td>
<td>3.4 ± 0.1</td>
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Values are means ± SE for 4 animals in each group. After in vivo injection of Iso (1 or 4 h) hearts were collected, and cardiac triglyceride and free fatty acid were measured using HPLC. *Significantly different from control, P < 0.05.

Fig. 3. HR-LPL activity and AMPK phosphorylation in Langendorff hearts perfused with Iso. To determine whether Iso influences LPL in the intact heart, we perfused hearts retrogradely with this β-agonist (10 μM) for 1 h. Subsequently, LPL from control or Iso-perfused hearts was displaced by heparin, and activity was determined. Data are means ± SE of 4 different isolated hearts in each group. Subsequently to displacement of LPL, hearts were removed and snap-frozen in liquid nitrogen. AMPKα (total and phosphorylated; top) was measured using Western blotting. Data are means ± SE of 4 different isolated hearts in each group.
Augmentation of substrate supply reduces AMPK and ACC phosphorylation and HR-LPL activity in the isolated perfused working heart. Glucose entry in the heart is predominantly insulin dependent (7). Compared with glucose, FA is the preferred substrate and, when supplied at physiological levels, contributes \( \frac{70\%}{11011} \) of the ATP necessary for normal heart function (47, 48). Interestingly, provision of insulin and albumin-bound palmitic acid to the working heart reduced phosphorylation of AMPK (Fig. 5, top), ACC\(_{280}\) (Fig. 5, bottom), and HR-LPL activity (Fig. 6) without changing the RPP (glucose only, 25,400 \( \pm \) 2,000; insulin + palmitate, 24,700 \( \pm \) 300). In these hearts, introduction of Iso to the buffer perfusate increased RPP (control, 24,700 \( \pm \) 300; Iso, 33,600 \( \pm \) 900, \( P < 0.05 \)) and reversed the effects on AMPK and ACC\(_{280}\) phosphorylation and LPL activity (Figs. 5 and 6). Taken together, our results suggest that the Iso-induced increase in AMPK and ACC phosphorylation occurs through indirect mechanisms.

**DISCUSSION**

FA delivery and utilization by the heart involves 1) release from adipose tissue and transport to the heart after complexing with albumin (35), 2) provision through the breakdown of endogenous cardiac TG stores (42, 57), 3) internalization of whole lipoproteins (20, 61, 64), and 4) hydrolysis of circulating TG-rich lipoproteins to FA by LPL positioned at the endothelial surface of the coronary lumen (5). It should be noted that the molar concentration of FA bound to albumin is \( \sim 10\)-fold less than that of FA in lipoprotein-TG (39), and, recently, LPL-mediated hydrolysis of lipoproteins to FA was suggested to be the principal source of FA for cardiac utilization (4). LPL is synthesized in cardiomyocytes and subsequently secreted onto HSPG binding sites on endothelial cells in the coronary lumen (13, 15, 39, 41). Thus, even though the majority of enzyme is located in myocytes, vascular endothelial-bound LPL likely determines the rate of plasma lipoprotein-TG clearance (41). Previously, incubation of cardiomyocytes with Iso for 30 min did not change either HR-LPL or cellular LPL activity (60). We have confirmed these findings even after incubation with Iso was prolonged for 1 h. Because cardiomyocytes are quiescent, we extended these experiments to an isolated retrograde perfused heart and demonstrated a similar absence of effect of this \( \beta \)-agonist on LPL. The models above are not truly representative of a heart beating in vivo. In the only study that examined the influence of Iso in the intact animal, LPL activity was measured in whole heart homogenates (12), which does not distinguish between the HR (localized on capillary endothelial cells) and cellular (that represents a storage form of the enzyme) pools of LPL. Our results, for

**Fig. 4.** Comparison of HR-LPL activity, AMPK, and ACC\(_{280}\) phosphorylation in perfused Langendorff or working hearts. Langendorff (no pressure-volume work) or working (beating against an afterload) hearts were perfused for 15 min with glucose as the sole substrate. Subsequently, LPL was displaced by heparin, and activity was determined. Data are means \( \pm \) SE of 3 different hearts in each group. *Significantly different from control, \( P < 0.05 \). Subsequently, AMPK\(_{\alpha}\) (total and phosphorylated) and phospho-ACC\(_{280}\) (top) were measured using Western Blotting. Data are means \( \pm \) SE of 3 different hearts in each group.

**Fig. 5.** Consequence of additional substrate provision in regulating AMPK and ACC\(_{280}\) phosphorylation in isolated working hearts in the absence or presence of Iso. Working hearts were perfused either with glucose or with buffer containing glucose, insulin, and palmitic acid. In an additional group, Iso was added to buffer containing all of the above components. Subsequently, AMPK\(_{\alpha}\) (total and phosphorylated; blots on top) and phospho-ACC\(_{280}\) (bottom) were measured using Western Blotting. Data are means \( \pm \) SE of 3 different hearts in each group. *Significantly different from control, \( P < 0.05 \).
the first time, suggest that a single injection of Iso, given in vivo, can increase cardiac HR-LPL.

Through its interaction with cell surface adrenergic receptors, β-agonists increase intracellular messengers such as cAMP, which leads to increased adipose and cardiac tissue lipolysis, heart rate, and contractility (49, 68). Indeed, plasma FFA were augmented, as well as heart function, after a single injection of Iso. It is interesting that, despite an increase in HR-LPL and plasma FFA, measurement of intracellular lipids revealed a drop in TG with no change in FA. These data imply that circulating FFA are either inefficiently transported in the myocyte or excessively utilized. Increasing evidence has demonstrated that β-agonists increase glucose, glycogen, and FA utilization (10, 19, 59). Additionally, Iso is known to augment TG lipolysis in quiescent myocytes, an effect that was independent of its inotropic response (30). Given that HR-LPL only increased after in vivo administration of Iso, it is likely that, under conditions of excessive cardiac workload and energy expenditure, LPL is recruited to the coronary lumen. Interestingly, increasing cardiac workload after exercise is also associated with enhanced HR-LPL (18, 66). Overall, these data suggest that the influence of Iso on LPL is not via a direct mechanism but is likely through its effects on regulating cardiac workload and energy expenditure.

The “fuel gauge” AMPK regulates cellular metabolism (53, 54, 58). During ischemia or exercise, changes in intracellular AMP/ATP levels promote activation of AMPK, an important regulator of both lipid and carbohydrate metabolism (22, 25). Thus, in heart and skeletal muscle, phosphorylated AMPK stimulates glycolysis through activation of 6-phosphofructo-2-kinase and facilitates FA utilization via control of ACC (31, 32). AMPK also regulates substrate uptake. For example, AMPK induces glucose transport by recruiting GLUT4 to the plasma membrane (33, 55). In addition, AMPK has also been implicated in FA delivery to cardiomyocytes through its regulation of CD36 (36). More recently, we have also reported that AMPK can regulate cardiac LPL by facilitating movement of this enzyme to the coronary lumen (2). Because AMPK phosphorylation was only observed after the administration of Iso in vivo, and not in the isolated nonbeating myocytes or retrograde nonworking hearts, it appears that activation of AMPK is secondary to augmented workload and energy expenditure. Although intracellular ATP remains unchanged in skeletal muscle during exercise, increased energy expenditure augments AMP, leading to a change in the AMP-to-ATP ratio and subsequent activation of AMPK (8, 62). Similar effects on AMPK activation in the heart were also observed after increased heart rate and contractility during exercise (11). Because AMPK activation phosphorylates and inhibits ACC280, and likely controls the increases in LPL at the coronary lumen, our data imply that, after Iso, with its associated increase in energy expenditure, FA delivery and oxidation are increased to maintain cellular ATP.

In Langendorff hearts, retrograde perfusion through the aorta allows for adequate coronary perfusion that maintains normal beating, but does not generate pressure-volume work as observed in vivo. In contrast, in the working heart, buffer enters through the left atrium, fills the left ventricle, and is expelled against an afterload. Under these conditions, energy expenditure in the Langendorff heart is likely lower compared with the working heart and probably explains why AMPK and HR-LPL remains unchanged even after 1 h of perfusion with glucose as the sole substrate. In contrast, in the working heart perfused in this manner, ATP generation is likely not matched to ATP expenditure, and AMPK would be expected to increase. Indeed, in the working heart, AMPK and ACC280 phosphorylation and HR-LPL activity were higher compared with the Langendorff heart. Whether this augmentation of AMPK phosphorylation is the result of insufficient ATP production or increased AMP accumulation is unknown. In an attempt to decrease AMPK and ACC phosphorylation and increase ATP generation, we provided the working heart with insulin (known to decrease AMPK phosphorylation in an Akt-dependent manner; see Ref. 29) and albumin-bound FA, in addition to glucose. Interestingly, although we were able to lower both AMPK and ACC280 phosphorylation, and HR-LPL activity, it is unclear whether these effects are a consequence of changes in energy status or the direct effects of insulin. Under these in vitro conditions, provision of Iso was able to duplicate the effects seen when this β-agonist was given in vivo. Overall, these experiments further validate the idea that, after increased workload and high energy expenditure, AMPK activation not only promotes FA oxidation but likely, through its effect on HR-LPL, also provides the hearts with FA. Interestingly, in a different study that perfused isolated mouse hearts, basal (constitutive) LPL release was higher from working compared with Langendorff hearts (37).

In summary, our data suggest that Iso can influence HR-LPL only during conditions of increased workload and excessive energy expenditure and is highly associated with phosphorylation of AMPK. Whether this increase in LPL after Iso can induce cardiovascular damage is currently unknown. However, LPL-mediated lipoprotein hydrolysis can provide excessive FA that may affect the biology of the vessel wall (9, 16). Indeed, when endothelial cell monolayers are incubated with increasing concentrations of FA, the permeability of the monolayers is enhanced, allowing albumin to traverse faster across the vessel wall (24). Additionally, tissue-specific overexpression of LPL is associated with severe myopathy (27, 34, 67) and promotion...
of atherosclerosis (16). Thus a novel mechanism by which excessive β-agonists could induce cardiovascular complications is through their control of HR-LPL.

GRANTS

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REFERENCES


