Effects of adenosine on myocardial glucose and palmitate metabolism after transient ischemia: role of 5′-AMP-activated protein kinase

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Adenosine is a major regulator of energy metabolism in the heart. It stimulates glycolysis in stressed hearts, an effect that may contribute to acidosis and reduced cardioprotection (11). Inasmuch as hearts stressed by transient ischemia may mimic some of the changes observed in the clinical setting, we utilized this experimental paradigm to determine signaling mechanisms that may account for changes in the metabolic responses to adenosine.

The stress-activated protein kinase 5′-AMP-activated protein kinase (AMPK) is an important regulator of energy metabolism and, when activated, inhibits ATP-consuming biosynthetic pathways while it activates ATP-generating catabolic processes (17). During ischemia-reperfusion, activation of AMPK may be beneficial, by increasing ATP production via stimulation of glucose transport, glycogenolysis, and glycolysis, as well as via stimulation of fatty acid oxidation through phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) (3, 30, 32, 36, 39). In contrast, activation of AMPK may be deleterious, inasmuch as stimulation of fatty acid oxidation leads to an inhibition of glucose oxidation (Randle cycle) (19, 40), which, in combination with elevated rates of glycolysis (27), promotes intracellular acidosis and Na+ and Ca2+ overload, which in turn contribute to LV mechanical dysfunction during reperfusion (28).

In this study, we investigated whether alterations in AMPK activity underlie changes in the effects of adenosine on energy substrate metabolism in hearts perfused aerobically after stress induced by transient ischemia. Our discovery that adenosine caused a marked activation of AMPK under these conditions allowed us to characterize further the effects of AMPK activation on carbohydrate and fatty acid metabolism under conditions of stable LV mechanical function. Specifically, this...
study tested the hypothesis that, in accordance with studies on ischemia-induced activation of AMPK, the activation of AMPK in response to adenosine in hearts stressed by transient ischemia also stimulates glycolysis, glucose uptake, and glycogenolysis. In addition, we tested the hypothesis that activation of AMPK in response to adenosine also accelerates myocardial fatty acid oxidation.

MATERIALS AND METHODS

Heart perfusions. All animals received humane care according to the guidelines of the Canadian Council on Animal Care and the University of Alberta Health Sciences Animal Welfare Committee. Hearts from pentobarbital sodium-anesthetized male Sprague-Dawley rats (300–350 g body wt) that had been fed ad libitum were excised, and their aortae were cannulated and perfused with Krebs-Henseleit solution (37°C, pH 7.4, gassed with 95% O2-5% CO2). The hearts were perfused in the Langendorff mode for 10 min and then switched to the working (ejecting) mode, as described previously (12). The perfusate (recirculating volume of 100 ml) consisted of a modified Krebs-Henseleit solution containing 2.5 mM Ca2+, 11 mM glucose, 1.2 mM palmitate prebound to 3% BSA (fraction V), and 100 mU/l insulin. Perfusions were performed at a constant workload (11.5 mmHg preload and 80 mmHg afterload) and heart rate (paced at 300 beats/min). Heart rate and systolic and diastolic aortic pressures (mmHg) were measured using a pressure transducer (model P21, Gould) attached to the aortic outflow line. Cardiac output (ml/min) and aortic flow (ml/min) were measured using ultrasonic flow probes (model T206, Transonic) placed in the left atrial inflow line and the aortic outflow line, respectively. LV work (joules) was calculated as cardiac output × LV developed pressure (systolic pressure − preload pressure)×1,000 × 0.133 and served as a continuous index of LV mechanical function.

Perfusion protocol. Hearts were perfused under aerobic conditions in working mode for 45 min [normal group, no transient ischemia (−TI)] or under aerobic conditions for 15 min before stress by transient ischemia [stressed group, two 10-min periods of global no-flow ischemia, each followed by 5 min of reperfusion (+TI)]. The hearts were paced at 300 beats/min in all experimental groups, except during the periods of global no-flow ischemia. Normal and stressed hearts were then frozen for biochemical analyses (before treatment) or perfused aerobically for a further 35 min with vehicle (saline) or 500 μM adenosine. Thereafter, untreated and adenosine-treated hearts were frozen for biochemical analyses (end treatment; Fig. 1A). Two additional groups of stressed hearts were perfused aerobically during the 35-min treatment period with 1 μM phenolamine or 1 μM phenolamine + 500 μM adenosine.

Immunoblot analysis of AMPK and ACC. Heart homogenates were obtained by homogenization of frozen LV tissue in a solution containing 20 mM Tris·HCl (pH 7.4 at 4°C), 50 mM NaCl, 50 mM NaF, 5 mM sodium pyrophosphate, 0.25 mM sucrose, protease inhibitor cocktail (Sigma), phosphatase inhibitor cocktail (Sigma), and 1 mM dithiothreitol. After homogenization for 30 s, protein contents of the homogenates were determined using the Bradford protein assay. The samples were diluted and boiled in protein sample buffer, subjected to SDAS-PAGE, and transferred to nitrocellulose, as previously described (42). The membranes were blocked in 5% (wt/vol) skim milk powder in Tris-buffered saline (TBS) containing 0.1% (vol/vol) Tween and then immunoblotted with rabbit anti-phospho-AMPK (Thr172) and rabbit anti-AMPK-total (1:1,000 dilution) in 5% BSA (wt/vol) in TBS or rabbit anti-phospho-ACC (Ser79; 1:1,000 dilution) in 5% BSA (wt/vol) in TBS and peroxidase-conjugated streptavidin (1:500 dilution) in 5% BSA (wt/vol) in TBS. After the membranes were extensively washed, they were incubated with a peroxidase-conjugated goat anti-rabbit secondary antibody in 5% skim milk powder (wt/vol) in TBS when appropriate. The membranes were washed further, and the antibodies were visualized using the Pharmacia enhanced chemiluminescence Western blotting and detection system. Densitometric analyses of immunoblots (n = 3 per experimental group) were performed using Quantity One (version 4.4.0) software (Bio-Rad Laboratories). Densitometric values of the phosphorylated proteins were normalized to the total amount of the protein detected and expressed as arbitrary density units.

Measurement of AMPK activity. The activity of AMPK (nmol·mg protein−1·min−1) was measured in 6% polyethylene glycol fractions extracted from 200 μg of frozen LV tissue, as described previously (25, 26). Activity of AMPK in the presence of S'-AMP (200 μM) was assayed in the 6% polyethylene glycol fraction by following the

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Fig. 1. Experimental protocol for heart perfusions. A: hearts were perfused under aerobic conditions in working mode for 45 min [normal group, no transient ischemia (−TI)] or perfused aerobically for 15 min before stress induced by transient ischemia (+TI), two 10-min periods of global no-flow ischemia (gray bars), each followed by 5 min of reperfusion (stressed group)]. Normal and stressed hearts were frozen for biochemical analyses (end of treatment, arrows) or assigned randomly to a vehicle (saline)-treated group (n = 9 for −TI, n = 10 for +TI) or a group that was treated with 500 μM adenosine (n = 9 for −TI, n = 7 for +TI) and perfused aerobically for another 35 min. Hearts were frozen for biochemical measurements (end of treatment, arrows). B: mechanical function [left ventricular (LV) work] of normal [vehicle (○)] and stressed [vehicle (●) and adenosine (■)] hearts. Values are means ± SE.
incorporation of \(\gamma^32-P\) from \([\gamma^32-P]\)ATP into a Ser79 phosphorylation site-specific SAMS peptide (HRMSAMSGLHVKRR), as previously described (5, 26).

Measurement of steady-state rates of palmitate oxidation. Steady-state rates of palmitate oxidation were measured from the release of \(^{3}H_2O\) from the metabolism of \([9,10-^{3}H]palmitate\). For separation of \(^{3}H_2O\) from \([9,10-^{3}H]palmitate\), 0.5 ml of perfusate sampled at predetermined times (5, 14, 29, 44, 50, 60, 70, and 80 min) was mixed with 1.88 ml of 1:2 (vol/vol) chloroform-methanol and 1.0 ml of chloroform and then 1.0 ml of 1.1 mol/l KCl-0.9 mol/l HCl was added. The samples were allowed to separate into polar and nonpolar phases. A 1.0-ml sample of the polar layer was removed and mixed with 1.0 ml of chloroform, 1.0 ml of methanol, and 0.9 ml of the KCl-HCl solution. Again samples were allowed to separate into polar and nonpolar phases, and 0.5-ml aliquots of the polar phase were subjected to scintillation counting for determination of \(^{3}H_2O\) content. 

Measurement of adenine nucleotide content. Adenine nucleotides were extracted from \(\sim100\) mg of frozen ventricular tissue into 1 ml of 6% ice-cold perchloric acid by homogenization with a pestle in a cold mortar. The tissue-perchloric acid mixture was centrifuged at 4°C, and the supernatant was neutralized with K\(_2\)CO\(_3\). High-performance liquid chromatography was used to measure nucleotide and nucleoside content in the neutralized extracts (10).

Measurement of steady-state rates of glycolysis and glucose oxidation. Glycolysis and glucose oxidation rates were measured directly from the simultaneous production of \(^{3}H_2O\) (liberated at the enolase step of glycolysis) and \(^{14}CO_2\) (liberated at the level of pyruvate dehydrogenase complex and in the citric acid cycle), respectively, from \([5-{^3}H]glucose\) and \([U-{^{14}C}]glucose\), as described previously (13,15). Per fusate was sampled at predetermined times (5, 14, 29, 44, 50, 60, 70, and 80 min), and steady-state rates (\(\mu\)mol \([5-{^3}H]glucose\) or \([U-{^{14}C}]glucose\) metabolized/g dry wt \(-1\) min \(-1\) ) were calculated for baseline and treatment periods.

Calculation of rate of proton production arising from exogenous glucose metabolism. When glucose (from endogenous or exogenous sources) is metabolized by glycolysis and subsequently oxidized 1:1, with the associated synthesis and hydrolysis of ATP, the net production of protons is zero. However, if the rate of glycolysis exceeds that of glucose oxidation, there is a net production of two protons per molecule of exogenous glucose that passes through glycolysis, which is not subsequently metabolized. Therefore, the rate of proton production attributable to the hydrolysis of ATP arising from exogenous glucose metabolism can be calculated as follows: \(2 \times (rate\ of\ glycolysis - rate\ of\ glucose\ oxidation)\).

Assay of glycogen content and glucose uptake. Frozen LV tissue was powdered using a mortar and pestle maintained at the temperature of liquid N\(_2\). Glycogen, in 200 mg of powdered tissue, was converted to glucose by reaction with 4 M H\(_2\)SO\(_4\). The amount of glucose (\(\mu\)mol glucose units/g dry wt) thus obtained was determined using a Sigma glucose analysis kit. The net rate of glycogen synthesis (\(\mu\)mol glucose·min\(^{-1}\)·g dry wt\(^{-1}\) ) in hearts during the 35-min aerobic treatment period was calculated from the increase in \([5-{^3}H]\)- and \([U-{^{14}C}]\)glucosyl units in total myocardial glycogen in hearts frozen at the end of treatment relative to hearts frozen before treatment. The rate of glucose uptake (\(\mu\)mol·min\(^{-1}\)·g dry wt\(^{-1}\) ) during the treatment period was calculated as the sum of the net rate of glycogen synthesis and the rate of glycolysis in individual hearts. The net rate of glycogen degradation was calculated as the difference between the unlabeled myocardial glycogen content in the before-treatment and end-treatment groups.

Materials. \(\Delta-[5-{^3}H]\)glucose, \(\Delta-[U-{^{14}C}]\)glucose, and \([9,10-^{3}H]palmitate\) were purchased from Dupont Canada; adenosine from Research Biochemicals International (Natick MA); and anti-phospho-AMPK (Thr172), anti-AMPK (total), and anti-phospho-ACC antibodies and peroxidase-conjugated streptavidin from Cell Signaling Technology (Beverly, MA). All other chemicals were reagent grade.

Statistical analysis. Values are means ± SE of n observations. The significance of differences between untreated and adenosine-treated groups was estimated by Student’s t-test. When required, multiple comparisons were made using one-way ANOVA. When ANOVA revealed significant differences, individual groups were compared using Bonferroni’s multiple comparisons test. Differences were considered significant when \(P < 0.05\).

RESULTS

LV work in normal and stressed hearts. LV work was stable and similar in normal and stressed hearts before they were assigned to the vehicle- or adenosine-treated groups. Although all measurable LV work ceased during the periods of transient ischemia, it recovered quickly and remained stable throughout subsequent perfusion in the absence (vehicle-treated) or presence of adenosine. Adenosine had no effect on LV work in normal or stressed hearts (Fig. 1B). Coronary flow and coronary vascular conductance were increased by adenosine (\(P < 0.05\)) in normal, but not in stressed, hearts (Table 1).

Phosphorylation of AMPK and ACC in normal and stressed hearts. To determine the consequences of transient ischemia per se, phosphorylation of AMPK and its downstream target ACC was assessed in normal and stressed hearts before they were subjected to scintillation counting for determination of \(^{3}H_2O\) content. The net rate of glycogen synthesis (\(\mu\)mol glucose·min\(^{-1}\)·g dry wt\(^{-1}\) ) in hearts during the 35-min aerobic treatment period was calculated from the increase in \([5-{^3}H]\)- and \([U-{^{14}C}]\)glucosyl units in total myocardial glycogen in hearts frozen at the end of treatment relative to hearts frozen before treatment. The rate of glucose uptake (\(\mu\)mol·min\(^{-1}\)·g dry wt\(^{-1}\) ) during the treatment period was calculated as the sum of the net rate of glycogen synthesis and the rate of glycolysis in individual hearts. The net rate of glycogen degradation was calculated as the difference between the unlabeled myocardial glycogen content in the before-treatment and end-treatment groups.

Effects of adenosine on phosphorylation of AMPK and ACC. Adenosine decreased AMPK phosphorylation by 70% and activity by 50% in normal hearts (\(P < 0.05\), by unpaired t-test), consistent with our previous study in which we used \(N^e\).

Table 1. Cardiac function in normal and stressed hearts

<table>
<thead>
<tr>
<th>Cardiac Parameter</th>
<th>Vehicle</th>
<th>+TI (n = 10)</th>
<th>Adenosine</th>
<th>+TI (n = 7)</th>
</tr>
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<tbody>
<tr>
<td>HR, beats/min</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>RPP (HR × PSP × 10(^{-3}))</td>
<td>38.3±0.7</td>
<td>35.2±1.8</td>
<td>35.7±0.3</td>
<td>36.1±1.6</td>
</tr>
<tr>
<td>AF, ml/min</td>
<td>46.5±2.2</td>
<td>38.9±5.1</td>
<td>38.9±2.2</td>
<td>36.7±6.8</td>
</tr>
<tr>
<td>CF, ml/min</td>
<td>31.3±2.7</td>
<td>26.1±2.6</td>
<td>40.4±2.0†</td>
<td>27.1±1.9</td>
</tr>
<tr>
<td>CVC, mL·min(^{-1})·mmHg(^{-1})</td>
<td>0.332±0.028</td>
<td>0.300±0.025</td>
<td>0.449±0.022†</td>
<td>0.300±0.020</td>
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</table>

Values are means ± SE; n, number of hearts. TI, transient ischemia; HR, heart rate; RPP, rate-pressure product; PSP, peak systolic pressure; AF, aortic flow; CF, coronary flow; CVC, coronary vascular conductance. *Significantly different from vehicle. †Significantly different from adenosine + TI.
cyclohexyladenosine, an adenosine A1 receptor agonist (15).

Adenosine increased AMPK phosphorylation (9-fold) and activity (2.3-fold) in stressed hearts ($P < 0.05$; Fig. 3). AMPK phosphorylation and activity were 30- and 6-fold higher, respectively, in adenosine-treated stressed hearts than in adenosine-treated normal hearts (Fig. 3).

The short-term regulation of fatty acid oxidation is, in part, achieved via the reversible phosphorylation and inactivation of ACC. Adenosine inhibited the phosphorylation of ACC$\beta$- and ACC$\alpha$-isoform in normal hearts (Fig. 4A); however, as we reported previously (8), it did not affect rates of palmitate oxidation (Fig. 4B). Interestingly, despite the marked adenosine-induced activation of AMPK in stressed hearts, adenosine did not increase the phosphorylation of ACC$\beta$- or ACC$\alpha$-isoform, nor did it accelerate rates of palmitate oxidation.

Effects of adenosine on adenine nucleotide content. Inasmuch as an increase in AMP concentration and/or a decrease in the ATP-to-AMP ratio regulates AMPK activity, the contents of these adenine nucleotides were assessed in vehicle- and adenosine-treated hearts. Although adenosine did not affect ATP content in stressed hearts (Fig. 5A), it increased AMP content 1.6-fold ($P < 0.05$; Fig. 5B) and, consequently, decreased the ATP-to-AMP ratio by 40% ($P < 0.05$; Fig. 5C), thereby mimicking changes expected to occur with a decrease in cellular energy state. Although adenosine increased AMP content of normal hearts, it also increased ATP content and, therefore, did not change the ATP-to-AMP ratio (data not shown).

Effects of AMPK activation on glucose metabolism. Steady-state rates of glucose metabolism (glycolysis and glucose oxidation) and calculated rates of proton production were assessed to characterize the consequences of the adenosine-induced activation of AMPK in stressed hearts. Because the rates of glycolysis (1.8-fold, $P < 0.05$) and glucose oxidation (1.8-fold, $P < 0.05$) were accelerated in hearts with elevated AMPK activity (Fig. 6, A and B), the calculated rate of proton production from exogenous glucose was significantly greater (1.7-fold, $P < 0.05$; Fig. 6C).

Effects of AMPK activation on glucose uptake and glycogen metabolism. Rates of glucose uptake, as well as glycogen synthesis and degradation, were also calculated in hearts perfused in the absence and presence of adenosine. Despite the marked activation of AMPK in adenosine-treated hearts, the rate of glucose uptake was not different from that calculated in vehicle-treated hearts (Fig. 7A). Inasmuch as an increase in glucose uptake was not responsible for the elevated rate of
glycolysis observed in hearts with high AMPK activity, glycogen metabolism (glycogen synthesis and glycogen degradation) was assessed. In vehicle-treated hearts, the net rate of glycogen synthesis was \( \frac{1}{22} \) of the rate of glucose uptake. In hearts with high AMPK activity, glycogen synthesis was completely suppressed \((P < 0.05; \text{Fig. } 7B)\); consequently, the rates of glucose uptake (Fig. 7A) and glycolysis (Fig. 6A) were equivalent. The net rate of glycogenolysis was not different between hearts with low and high AMPK activity (Fig. 7C). Inhibition of glycogen synthesis and unaltered glycogen degradation in hearts with high AMPK activity resulted in a slight, but not statistically significant, decrease in glycogen content at the end of the treatment period in adenosine-treated hearts (Fig. 7D).

Overall, these data suggest that an alteration in glucose utilization, i.e., the partitioning of glucose between glycogen synthesis and glycolysis after uptake, rather than an increase in glucose uptake, accounts for the acceleration of glycolysis in hearts with high AMPK activity.

**Effects of AMPK activation on steady-state rates of ATP production.** The activation of AMPK by adenosine in stressed hearts significantly increased calculated rates \((\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1})\) of ATP production from glycolysis (from \(6.1 \pm 1.1\) to \(10.9 \pm 0.9\), \(P < 0.05\)) and glucose oxidation (from \(21.1 \pm 2.6\) to \(35.5 \pm 4.9\), \(P < 0.05\)) but had no significant effect on the calculated rate of ATP production from palmitate oxidation (from \(115.5 \pm 19.4\) to \(133.8 \pm 21\)). Inasmuch as palmitate oxidation is the major energy source, overall ATP production was not increased in hearts with high AMPK activity.

**Effects of phentolamine on AMPK phosphorylation.** We previously demonstrated that phentolamine (1 \(\mu\text{M}\), an \(\alpha\)-ad-
renoreceptor antagonist, prevents the adenosine-induced acceleration of glycolysis and the calculated rate of proton production in hearts stressed by transient ischemia (9). Examination of the effects of phentolamine on the phosphorylation of AMPK showed that although it has no effect on AMPK phosphorylation per se, it prevented the adenosine-induced increase in AMPK phosphorylation in stressed hearts (Fig. 8).

**DISCUSSION**

This study investigated the role of AMPK in mediation of the effects of adenosine on myocardial energy metabolism. During aerobic perfusion of hearts stressed by transient ischemia and under conditions of stable mechanical function, adenosine stimulated AMPK activity. Despite the marked increase in AMPK activity, ACC phosphorylation and palmitate oxidation were not affected. However, increases in glycolysis and glucose oxidation were observed. Inasmuch as glucose uptake in hearts with high AMPK activity was unchanged, the observed increase in glucose metabolism arose from an altered partitioning of glucose after its uptake away from glycogen synthesis to glycolysis and glucose oxidation. This indicates that, in contrast to the ischemia-induced activation of AMPK,
Increased AMPK activity in hearts stressed by transient ischemia 

evidence that, relative to vehicle-treated hearts, adenosine activity) and direct assays of AMPK activity provide clear under aerobic conditions of stable energy supply and demand.

adenosine-induced activation of AMPK under conditions of stable LV work is not sufficient to stimulate fatty acid oxidation or glucose uptake. Thus previous associations between high AMPK activity and elevated rates of fatty acid oxidation and glucose uptake in ischemic tissue may have arisen as a consequence of other ischemia-induced changes, rather than a direct activation of AMPK.

These studies were performed using isolated working rat hearts, a well-established model for the simultaneous and direct measurement of cardiac mechanical and metabolic function. The inclusion of glucose and palmitate as energy substrates in the perfusate ensured that myocardial glycogen content was not depleted, thereby preserving normal values of glycogen turnover (11, 14). The presence of insulin ensured adequate glucose transport, so that glucose availability was not rate limiting for glycolysis and glucose oxidation. Furthermore, the concentrations of energy substrates provided in the perfusate mimic some of the metabolic conditions that occur after myocardial ischemia. The periods of transient ischemia that caused alterations in the responses to adenosine do not induce ischemic preconditioning, inasmuch as recovery of LV mechanical function after a period of sustained severe ischemia is unaltered (11). Also, inasmuch as transient ischemia is not of sufficient duration to cause LV mechanical dysfunction during the treatment periods, this experimental model provided the opportunity to characterize the role of AMPK in mediating adenosine-induced alterations in energy substrate metabolism under aerobic conditions of stable energy supply and demand.

Examination of AMPK phosphorylation status (indicative of activity) and direct assays of AMPK activity provide clear evidence that, relative to vehicle-treated hearts, adenosine increased AMPK activity in hearts stressed by transient ischemia. Activation of AMPK was not due to transient ischemia per se, inasmuch as AMPK phosphorylation after transient ischemia, but before adenosine treatment, was identical to that in normal hearts. The mechanism underlying the activation of AMPK by adenosine is unlikely to be mediated by adenosine A₁ receptors, inasmuch as the A₁ receptor agonist N⁶-cyclohexyladenosine inhibits AMPK activation in isolated working rat hearts during low-flow ischemia as well as after transient ischemia (13, 15). Adenosine A₂ receptor stimulation is also not likely involved, inasmuch as the selective adenosine A₂ receptor agonist CGS-21680 inhibits AMPK activation (4).

Table 2. End-treatment values of PCr and Cr content in ventricular tissue

<table>
<thead>
<tr>
<th>High-Energy Phosphate</th>
<th>Vehicle (n = 8)</th>
<th>Adenosine (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr, μmol/g dry wt</td>
<td>62.2±5.1</td>
<td>59.7±4.7</td>
</tr>
<tr>
<td>Cr, μmol/g dry wt</td>
<td>194.7±5.1</td>
<td>194.0±10.6</td>
</tr>
<tr>
<td>PCr/Cr</td>
<td>0.32±0.03</td>
<td>0.31±0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE measured in stressed hearts frozen at the end of treatment. PCr, phosphocreatine; Cr, creatine. There were no significant differences between groups.
the ATP-to-AMP ratio may rule out a role for Ca\(^{2+}\)/calmodulin-dependent protein kinase kinases as AMPK-activating kinases under these conditions, inasmuch as Ca\(^{2+}\)/calmodulin-dependent protein kinase kinases appear to activate AMPK independent of changes in the ATP-to-AMP ratio and are not influenced by AICAR (21).

Numerous reports have linked AMPK activation to the stimulation of fatty acid oxidation. AMPK activation has been associated with enhanced clearance of long-chain fatty acid in cardiac muscle preparations in vivo (41) and with increased rates of myocardial fatty acid oxidation during posts ischemic reperfusion (25). However, it was not clearly defined whether enhanced fatty acid utilization was due solely to AMPK activation, rather than other changes that may have occurred as a consequence of ischemia and altered LV mechanical function. The lack of association between AMPK activation and a stimulation of the rate of palmitate oxidation observed in this study indicates that AMPK activation is not sufficient to stimulate fatty acid oxidation in the aerobically perfused working rat heart. Moreover, although the underlying mechanism for the stimulation of fatty acid oxidation by AMPK has been considered to result from AMPK-induced phosphorylation and inactivation of ACC, the extent of ACC (\(\alpha\)- and \(\beta\)-isoforms) phosphorylation was unaltered in the face of markedly elevated AMPK activity. Furthermore, although the phosphorylation of ACC is attenuated in response to adenosine in normal hearts (absence of transient ischemia), there is no associated change in palmitate oxidation. These observations differ from previous reports that suggested a negative correlation between AMPK and ACC activity, coupled to increased fatty acid oxidation (25, 32, 33, 36, 46). However, these differences may arise because of differences in experimental models (skeletal vs. cardiac muscle and severity of myocardial ischemia). Moreover, the dissociation between AMPK and ACC phosphorylation in this study is consistent with a recent report indicating a lack of ACC phosphorylation, despite AMPK activation in rat heart after mild ischemia (1).

After entry into the cardiac myocyte, \(~80\%\) of glucose enters the glycolytic pathway, which generates a limited amount of ATP (2 mol/mol glucose) and pyruvate for subsequent oxidation. The observation that glycolysis was accelerated in hearts with high AMPK activity is compatible with previous observations that indicate AMPK stimulation of the phosphorylation and activation of 6-phosphofructo-2-kinase, an enzyme that generates fructose 2,6-bisphosphate, a potent stimulator of 6-phosphofructo-1-kinase, the rate-limiting step of glycolysis (6, 30). Glucose oxidation, the final stage of glucose metabolism, at which pyruvate undergoes oxidative decarboxylation by pyruvate dehydrogenase to generate acetyl-CoA for the tricarboxylic acid cycle, was also accelerated by adenosine. Although previous studies suggest that increasing glycolysis during ischemia and reperfusion plays a protective role in limiting myocardial damage (39), the associated increase in the calculated rate of proton production and potential for Na\(^+\) and Ca\(^{2+}\) overload (28) may explain the loss of cardioprotective effectiveness of adenosine in stressed hearts, as reported previously (11).

The ability to activate AMPK in the absence of confounding factors that occur 1) during severe ischemia, 2) with alterations in mechanical function and energy demand, or 3) in isolated cell systems (low energy demand) provided a unique opportunity to characterize the relationships between AMPK activity and the pathways of glucose uptake and glycogen metabolism under conditions of stable LV work. Acceleration of myocardial glucose uptake by various stresses is independent of insulin signaling and may be due to the activation of AMPK (18, 38, 54). Indeed, activation of AMPK by metabolic stresses in isolated skeletal muscle, or pharmacologically in isolated heart muscle by AICAR, stimulates glucose uptake, which may result from an AMPK-mediated translocation of GLUT4 transporters from an intracellular membrane pool to the cell surface (23, 35, 38). Whether activation of AMPK alone is sufficient to increase glucose uptake in the absence of ischemia or changes in energy demand has not been clearly defined. However, this study has shown that, despite marked increases in AMPK activity, glucose uptake was not markedly affected. The low statistical power of this comparison may have concealed a significant increase, but the observed 15% difference was small relative to the more than twofold increase in glucose uptake that can be achieved in the absence of palmitate (42). That result also indicates that the lack of effect of AMPK activation on glucose uptake was not due to maximal rates of uptake preexisting in the working heart. Thus it appears that AMPK activation is not sufficient to stimulate glucose uptake in working cardiac muscle in the absence of other stimuli that may occur concomitantly with ischemia. This finding is in agreement with a recent study that demonstrated that AMPK activation does not affect cardiac glucose clearance in vivo (41). Furthermore, in contrast to previous reports linking increased glucose uptake and elevated rates of glycolysis (6, 20, 30), the present study indicates that increased glucose uptake does not underlie the acceleration of glycolysis in response to the adenosine-induced activation of AMPK, suggesting that these two processes are differentially regulated under conditions of stable LV work.

Glycogen is an important source of endogenous glucose, and its turnover (simultaneous synthesis and degradation) is a key factor in the control of glucose utilization (14, 15). Glycogen accumulates to physiological levels (150–160 \(\mu\)mol/g dry wt) in aerobic working rat hearts perfused with glucose, insulin, and palmitate, and although there is continuous glycogen turnover, the net rate of glycogenolysis is negligible (14). The demonstration that net glycogen synthesis was depressed in hearts with high AMPK activity, whereas glycogenolysis was unaffected, reflects an altered partitioning of glucose utilization after uptake in hearts with elevated AMPK activity. This may arise because of suppression of glycogen synthase activity (2, 45), thereby directing glucose away from energy storage (glycogen synthesis), or by an activation of glycogenolysis, thereby diverting glucose toward energy production (glycolysis and glucose oxidation).

In conclusion, this study has shown that the activation of AMPK by adenosine during aerobic perfusion and under conditions of stable energy supply and demand does not alter overall rates of energy production. Rather, activation of AMPK stimulates glycolysis and glucose oxidation and accelerates the calculated rate of proton production but has no effect on rates of glucose uptake and palmitate oxidation. This indicates that AMPK regulates myocardial glucose utilization in a coordinated manner by influencing the relative rates of glycolysis and glycogen synthesis. Inasmuch as AMPK activation increases the calculated rate of proton production and the potential for...
acidsis, inhibition of AMPK may be a useful approach to restore adenosine-induced cardioprotection in stressed hearts.

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