p38 mitogen-activated protein kinase mediates adenosine-induced alterations in myocardial glucose utilization via 5′-AMP-activated protein kinase

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Jaswal JS, Gandhi M, Finegan BA, Dyck JR, Clanachan AS. p38 mitogen-activated protein kinase mediates adenosine-induced alterations in myocardial glucose utilization via 5′-AMP-activated protein kinase. Am J Physiol Heart Circ Physiol 292: H1978–H1985, 2007. First published December 15, 2006; doi:10.1152/ajpheart.01121.2006.—Adenosine-induced acceleration of glycolysis in hearts stressed by transient ischemia is accompanied by suppression of glycogen synthesis and by increases in activity of adenosine 5′-monophosphate-activated protein kinase (AMPK). Because p38 mitogen-activated protein kinase (MAPK) may regulate glucose metabolism and may be activated downstream of AMPK, this study determined the effects of the p38 MAPK inhibitors SB202190 and SB203580 on adenosine-induced alterations in glucose utilization and AMPK activity. Studies were performed in working rat hearts perfused aerobically following stressing by transient ischemia (2 × 10-min ischemia followed by 5-min reperfusion). Phosphorylation of AMPK and p38 MAPK each were increased fourfold by adenosine, and these effects were inhibited by either SB202190 or SB203580. Neither of these inhibitors directly affected AMPK activity. Attenuation of the adenosine-induced increase in AMPK and p38 MAPK phosphorylation by SB202190 and SB203580 occurred independently of any change in tissue ATP-to-AMP ratio and did not alter glucose uptake, but it was accompanied by an increase in glycogen synthesis and glycogen content and by inhibition of glycolysis and proton production. There was a significant inverse correlation between the rate of glycogen synthesis and AMPK activity and between AMPK activity and glycogen content. These data demonstrate that AMPK is likely downstream of p38 MAPK in mediating the effects of adenosine on glucose utilization in hearts stressed by transient ischemia. The ability of p38 MAPK inhibitors to relieve the inhibition of glycogen synthesis and to inhibit glycolysis and proton production suggests that these agents may restore adenosine-induced cardioprotection in stressed hearts.

adenosine; adenosine 5′-monophosphate-activated protein kinase; glycogen metabolism

CARDIOPROTECTION INDUCED BY adenosine and the adenosine A1 receptor agonist N6-cyclohexyladenosine may be related to their ability to stimulate glycogen synthesis and inhibit glycolysis and proton production during reperfusion following ischemia (7, 9). However, our laboratory has shown that in hearts stressed by transient ischemia, there is a loss of adenosine-induced cardioprotection (8), an effect consistent with the lower cardioprotective effectiveness of adenosine and adenosine mimetic agents in studies involving patients with coronary artery disease (24). Because this model of transient ischemia differs from ischemic preconditioning, in that the transient ischemia does not enhance the recovery of left ventricular (LV) function following severe ischemia (8), stressing by transient ischemia may provide a unique system in which to study the mechanisms underlying the loss of adenosine-induced cardioprotection observed in the clinical setting.

Alterations in glycogen metabolism and glucose utilization may contribute to the loss of adenosine-induced cardioprotection in stressed hearts. Our group has reported previously that poor recovery of postischemic function is associated with an acceleration of glycolysis and proton production (8). Adenosine-induced acceleration of glycolysis also occurs in stressed hearts during aerobic perfusion, suggesting that the increase in glycolysis, proton production, and the potential for Na+ and Ca2+ overload is a cause, rather than a consequence, of depressed postischemic mechanical function. Whereas the effects of adenosine on glycogen synthesis have been extensively characterized, less well characterized are its effects on glycogen metabolism and the signaling pathways involved.

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is a multisubstrate enzyme involved in the control of cellular energy metabolism (6). Upon activation in response to metabolic stresses, including hypoxia and ischemia, AMPK phosphorylates enzymes involved in the regulation of both fatty acid and glucose metabolism, thereby increasing ATP production (6). Despite the importance of AMPK in regulating energy substrate metabolism, its role in regulating myocardial glycolysis is not well understood. However, in skeletal muscle, AMPK regulates glycogen content via mechanisms related to alterations in glucose uptake and the activity of glycogen synthase (2, 11). Furthermore, AMPK activity and glycogen content are inversely correlated in skeletal muscle (38), but whether such a reciprocal relationship exists in the heart has not been determined. Although adenosine does not stimulate glucose uptake in stressed hearts, it shifts the balance between the relative rates of glycogen synthesis and glycolysis such that glucose taken up by the stressed myocardium is preferentially metabolized by glycolysis and so contributes to an increase in proton production (17). We have recently demonstrated that adenosine-induced acceleration of glycolysis and inhibition of glycogen synthesis in stressed hearts are accompanied by the activation of AMPK (17), thereby suggesting a relationship among glycogen synthesis, glycogen content, and AMPK activity.

Recent reports suggest that p38 mitogen-activated protein kinase (MAPK), another stress-responsive protein kinase, is activated downstream of AMPK and that together, these kinases may regulate glucose uptake and subsequent myocardial...
glucose utilization (22, 26, 36, 39). Despite this established role of AMPK/p38 MAPK in glucose uptake, it is not known whether AMPK and p38 MAPK form a functional signaling cascade in the regulation of glycogen metabolism and glycolysis. Given that the effects of adenosine on carbohydrate metabolism are intimately linked to its cardioprotective properties, elucidation of involved biochemical mediators may provide novel protective strategies to enhance recovery of posts ischemic mechanical function.

This study investigated the involvement of AMPK and p38 MAPK in adenosine-induced alterations in glycogen and glucose metabolism in hearts stressed by transient ischemia using the selective p38 MAPK inhibitors SB202190 and SB203580. Alterations in AMPK and p38 MAPK phosphorylation, as well as rates of glycogen and glucose metabolism, were measured during aerobic perfusion of hearts previously stressed by transient ischemia, where LV work is stable and cellular signaling events are not influenced by confounding factors such as energy substrate supply and energy demand.

MATERIALS AND METHODS

Heart perfusions. All animals received humane care according to the Canadian Council on Animal Care, and the study protocol was approved by the University of Alberta Health Sciences Animal Welfare Committee. Hearts from pentobarbital sodium-anesthetized male Sprague-Dawley rats (300–350 g) that had been fed ad libitum were excised, their aortas were cannulated, and a perfusion using Krebs-Henseleit solution containing 2.5 mM Ca²⁺, 11 mM glucose, 1.2 mM palmitate prebound to 3% bovine serum albumin (BSA; fraction V), and 100 mM/L insulin. Perfusions were performed at a constant workload (preload, 11.5 mmHg; afterload, 80 mmHg) and heart rate (paced at 300 beats/min). Heart rate, systolic and diastolic aortic pressures (mmHg), cardiac output, and coronary flow were measured as described previously (17). 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% bovine serum albumin (BSA; fraction V), and 100 mL/L insulin. Perfusions were performed at a constant workload (preload, 11.5 mmHg; afterload, 80 mmHg) and heart rate (paced at 300 beats/min).

Perfusion protocol. Hearts were perfused under aerobic conditions for 15 min and then stressed by transient ischemia: two 10-min periods of global no-flow ischemia (I; shaded bars) each followed by 5 min of reperfusion. Stressed hearts were then either frozen for biochemical analyses before treatment or assigned randomly to groups treated with vehicle (saline, n = 8), SB202190 (10 μM, n = 6), adenosine (Ado; 500 μM, n = 7), SB202190 (10 μM)/Ado (500 μM, n = 8), or SB203580 (10 μM)/Ado (500 μM, n = 10) and perfused aerobically for a further 35-min aerobic treatment period. Hearts were then frozen for biochemical measurements (end treatment, ↓). B: left ventricular (LV) work of hearts perfused in the absence or presence of Ado, SB202190, and SB203580. The absence or presence of Ado, SB202190, and SB203580 is indicated by − and +, respectively. Values represent means ± SE.

Measurement of AMPK Activity. The activity of AMPK (nmol·mg protein⁻¹·min⁻¹) was measured in 6% polyethylene glycol (PEG) fractions extracted from 200 mg of frozen LV tissue by following the incorporation of γ-³²P from [γ-³²P]ATP into a Ser79 phosphorylation site-specific SAND peptide (HMRSAMGSLHVKRR), as previously described (4, 19, 20).

Assay of glycogen content and glucose uptake. Glycogen, in 200 mg of powdered tissue, was converted to glucose by reacting with 4 M H₂SO₄. The amount of glucose (expressed as μmol glucose units/g dry wt) thus obtained was determined using a Sigma glucose analysis kit. The net rate of glycogen synthesis (μmol glucose·g dry wt⁻¹·min⁻¹) during the 35-min aerobic treatment period was calculated from the increase in [⁵⁺H₃]glucosyl and [¹⁴C]glucose units in total glycogen in hearts frozen at end treatment relative to hearts frozen immediately following transient ischemia (“before treatment”). The rate of glucose uptake (μmol glucose·g dry wt⁻¹·min⁻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 (7, 17). 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.

Measurement of steady-state rates of glycolysis and glucose oxidation. Glycolysis and glucose oxidation rates were measured directly from the simultaneous production of ³H₂O and ¹⁴CO₂, respectively.
from [5-^3^H]glucose and [U-^1^4^C]glucose, as described previously (7, 9, 17). Perfusate was sampled at predetermined time points (5, 14, 29, 44, 50, 60, 70, 80 min), and steady-state rates (expressed as μmol [5-^3^H]glucose or [U-^1^4^C]glucose metabolized g dry wt^-1^-min^-1^) were calculated for the aerobic treatment period.

**Calculation of the rate of proton production arising from exogenous glucose metabolism.** When glucose is metabolized by glycolysis and completely oxidized, the associated synthesis and hydrolysis of ATP results in a net proton production of 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 that is not subsequently oxidized. Therefore, the rate of proton production attributable to the hydrolysis of ATP arising from exogenous glucose metabolism can be calculated as 2 x (rate of glycolysis - rate of glucose oxidation).

**Materials.** D-[5-^3^H]glucose and D-[U-^1^4^C]glucose were purchased form Dupont Canada (Ontario, Canada). SB202190 and SB203580 were purchased from Calbiochem (San Diego, CA). Adenosine was purchased from Research Biochemicals International (Natick, MA). Anti-phospho-p38 MAPK (Thy180/Tyr182), anti-p38 MAPK (total), anti-phospho-AMPK (Thr172), and anti-AMPK (total) antibodies were obtained from Cell Signaling Technology (Beverly, MA). All other chemicals were reagent grade.

**Statistical analysis.** All values are presented as means ± SE (n observations). The significance of the differences for multiple comparisons between treated and untreated groups was estimated using one-way analysis of variance. If significant, selected data sets were compared using Bonferroni’s multiple comparison test. Differences were considered significant when P < 0.05.

**RESULTS**

**Effects of p38 MAPK inhibitors and adenosine on LV work and coronary flow.** As reported previously for stressed hearts (8, 17), LV work during the aerobic treatment period was not altered by adenosine (500 μM). LV work also was unaffected by the p38 MAPK inhibitor SB202190 (10 μM) or by administration of SB202190 (10 μM) plus adenosine (500 μM) or SB203580 (10 μM) plus adenosine (500 μM) (Fig. 1B). Furthermore, coronary flow was not affected by any of these treatments (data not shown).

**Effects of p38 MAPK inhibitors on phosphorylation of AMPK and p38 MAPK.** As described previously, transient ischemia per se does not alter the phosphorylation of AMPK compared with hearts perfused aerobically in the absence of transient ischemia (17). Thus the possible involvement of p38 MAPK on AMPK activation was assessed during the aerobic treatment period in the presence or absence of adenosine. Perfusion of hearts with SB202190 alone did not affect the phosphorylation of AMPK compared with hearts treated with vehicle. Consistent with our previous findings, adenosine caused a marked increase in the phosphorylation of AMPK. The adenosine-induced increase in AMPK phosphorylation was abrogated by cotreatment with either SB202190 or SB203580 (Fig. 2A). The changes in AMPK phosphorylation were consistent with changes in AMPK activity (Fig. 2B). A similar pattern of changes in phosphorylation was observed for p38 MAPK. SB202190 alone did not affect p38 MAPK phosphorylation compared with hearts treated with vehicle, whereas adenosine increased p38 MAPK phosphorylation. The adenosine-induced increase in p38 MAPK phosphorylation was eliminated by either SB202190 or SB203580 (Fig. 2B).

**Effects of p38 MAPK inhibitors and adenosine on nucleoside and nucleotide content.** The content of adenosine and adenine nucleotides in LV tissue was determined to characterize potential mechanisms responsible for alterations in p38 MAPK and AMPK phosphorylation. SB202190 alone did not alter adenosine content compared with vehicle-treated hearts. As expected, exogenous adenosine significantly increased adenosine content, which was not altered by either SB202190 or SB203580. None of the treatments significantly
altered either ATP or AMP content or the ATP-to-AMP ratio, suggesting that the mechanisms underlying alterations AMPK and p38 MAPK phosphorylation occurred independently of changes in nucleotide content (Table 1).

**Effects of p38 MAPK inhibitors on AMPK activity.** To assess any direct effects of SB202190 and SB203580 on AMPK activity, we included graded concentrations (0 to 100 μM) of each p38 MAPK inhibitor in PEG fractions isolated from hearts with an elevated AMPK activity. Neither of the compounds had any direct effect on AMPK activity (Fig. 3).

**Effects of p38 MAPK inhibitors on glucose uptake and glycogen metabolism.** A previous study described the effects of adenosine on the rates of glucose uptake, glycogen synthesis, glycogen degradation, and glycogen content in hearts stressed by transient ischemia (17). A portion of those data (vehicle-treated and adenosine-treated hearts) are restated presently to facilitate comparison with effects of the p38 MAPK inhibitors. The rate of glucose uptake during the aerobic treatment period was similar to that reported previously for stressed hearts and was not affected by adenosine, SB202190, or adenosine in combination with the p38 MAPK inhibitors (Fig. 4A). Whereas SB202190 did not affect the rate of glycogen synthesis compared with vehicle-treated hearts, SB202910 and SB203580 each prevented the adenosine-induced suppression of glycogen synthesis (Fig. 4B). The rate of glycogen degradation was not altered by any of the treatments (Fig. 4C). Consequently, glycogen content was significantly greater in hearts treated with a combination of either SB202190 and adenosine or SB203580 and adenosine compared with hearts treated with adenosine alone (Fig. 4D). As a result of the changes in glycogen metabolism, there was a significant inverse correlation between the rate of glycogen synthesis and AMPK activity ($r^2 = 0.94, P < 0.05$) (Fig. 5A) and AMPK activity and glycogen content ($r^2 = 0.93, P < 0.05$) (Fig. 5B).

**Effects of p38 MAPK inhibitors on glucose metabolism.** Whereas SB20190 alone did not affect the rate of glycolysis, the marked adenosine-induced acceleration of glycolysis, which was described previously (17), was abolished by either SB202190 or SB203580 (Fig. 6A). The rate of glucose oxidation was not altered by any of the treatment combinations (Fig. 6B). The rate of proton production, which is an index of uncoupling of glycolysis and glucose oxidation, was increased by adenosine as reported previously (8, 17) and was not affected by SB20190 alone. However, SB202190 and SB203580 each prevented the adenosine-induced stimulation of the rate of proton production (Fig. 6C).

### Table 1. Adenosine and adenine nucleotide content in stressed hearts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adenosine</th>
<th>ATP</th>
<th>AMP</th>
<th>ATP/AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.18 ± 0.04</td>
<td>13.69 ± 2.1</td>
<td>4.61 ± 0.3</td>
<td>3.03 ± 0.5</td>
</tr>
<tr>
<td>SB202190</td>
<td>0.15 ± 0.02</td>
<td>18.55 ± 2.1</td>
<td>4.00 ± 0.4</td>
<td>4.94 ± 0.7</td>
</tr>
<tr>
<td>Ado</td>
<td>2.97 ± 0.4</td>
<td>19.47 ± 2.2</td>
<td>8.57 ± 2.5</td>
<td>3.07 ± 0.7</td>
</tr>
<tr>
<td>SB202190/Ado</td>
<td>2.24 ± 0.1</td>
<td>16.46 ± 0.9</td>
<td>4.35 ± 1.3</td>
<td>3.19 ± 0.5</td>
</tr>
<tr>
<td>SB203580/Ado</td>
<td>3.09 ± 0.3</td>
<td>19.52 ± 2.5</td>
<td>6.45 ± 1.3</td>
<td>3.44 ± 0.4</td>
</tr>
</tbody>
</table>

*Values represent means ± SE. Adenosine (Ado) and adenine nucleotide contents (μmol/g dry wt) in perchloric acid extracts from ventricular tissue from stressed hearts frozen at the end of the aerobic treatment period.

*Significantly different from vehicle-treated hearts.

**DISCUSSION**

LV work is stable during aerobic perfusion of hearts stressed by transient ischemia, but there is a loss of adenosine-induced cardioprotection following a subsequent period of severe ischemia, possibly due to changes in adenosine-mediated alterations in myocardial glucose metabolism. This study investigated the roles of AMPK and p38 MAPK in the regulation of glycogen metabolism and glucose utilization in stressed hearts, a system where adenosine activates AMPK and accelerates glycolysis. Whereas the p38 MAPK inhibitor SB202190 did not affect the phosphorylation of AMPK or p38 MAPK in the absence of adenosine, SB202190 and SB203580 each abolished the adenosine-induced increase in both AMPK and p38 MAPK phosphorylation. The p38 MAPK inhibitors also inhibited the adenosine-induced acceleration of glycolysis and the adenosine-induced suppression of glycogen synthesis. In contrast to previous reports that describe p38 MAPK to be downstream of AMPK in the regulation of glucose metabolism (22, 26, 39), we provide evidence using selective p38 MAPK inhibitors that p38 MAPK is upstream of AMPK and that its activation stimulates AMPK phosphorylation and activity. The ability of the p38 MAPK inhibitors to prevent AMPK activation by adenosine, as well as its consequences on glycolysis and glycogen synthesis, indicates that p38 MAPK is upstream of AMPK in the pathway that alters myocardial glucose utilization in response to adenosine (Fig. 7).
function and coronary flow allowed the characterization of AMPK and p38 MAPK signaling events involved in their regulation independent of confounding factors such as differences in energy supply, O2 availability, or energy demand. Inhibitors of p38 MAPK prevented adenosine-induced increase in both AMPK and p38 MAPK phosphorylation, but their mechanism has not been clearly defined. Although a previous report has demonstrated that both SB202190 and SB203580 inhibit adenosine uptake in cultured human erythroleukemia (K562) cells (14) and thus may prevent adenosine-induced alterations in the ATP-to-AMP ratio, they had no significant effect on either adenosine uptake or adenine nucleotide content in this study. The reported values for LV adenosine content represent total tissue adenosine and so take into account both intracellular and extracellular adenosine content. Given that adenosine undergoes rapid intracellular metabolism, the reported adenosine content likely represents adenosine in the extracellular space. Interestingly, α-adrenoceptors are involved in activating both AMPK and p38 MAPK (15, 26), and the α-adrenoceptor antagonist phentolamine prevents the adenosine-induced increase in AMPK phosphorylation in stressed hearts (17). Thus α-adrenoceptors may participate in the alterations in both AMPK and p38 MAPK phosphorylation in response to adenosine and the p38 MAPK inhibitors and in the subsequent regulation of glucose utilization (Fig. 7).

Similar to previous reports, our results indicate that AMPK and p38 MAPK form a common signaling cascade and participate in the regulation of glucose metabolism (22, 26, 39). Interestingly, previous reports have implicated transforming growth factor-β-activated protein kinase 1-binding protein 1

![Fig. 4. Glucose uptake and glycogen metabolism in stressed hearts. Glucose uptake (A), glycogen synthesis (B), glycogen degradation (C), and glycogen content (D) were assessed as described in MATERIALS AND METHODS for hearts treated with vehicle (n = 6), SB202190 (10 μM, n = 6), Ado (500 μM, n = 6), SB202190 (10 μM)/Ado (500 μM, n = 6), or SB203580 (10 μM)/Ado (500 μM, n = 5). Values represent means ± SE. *Significantly different from vehicle-treated hearts. †Significantly different from Ado-treated hearts.

![Fig. 5. AMPK activity and indexes of glycogen metabolism in stressed hearts. Correlation between AMPK activity in ventricular homogenates of hearts frozen at end treatment and the rate of glycogen synthesis calculated during the treatment period (A, n = 3–6 per group) and glycogen content measured at the end of the treatment period (B, n = 3–6 per group). Hearts were treated with vehicle (○), SB202190 (●), Ado (■), SB202190/Ado (▲), or SB203580/Ado (▲). Values represent means ± SE.
those found in the ATP-binding pocket of AMPK (10, 37). Moreover, the selectivity of SB202190 and SB203580 versus a variety of other protein kinases, including AMPK, has been validated previously (5). We have further confirmed that these p38 MAPK inhibitors have no direct effect on AMPK activity in our preparations, given that AMPK retains 94–96% of its activity in vitro in the presence of SB202190 (10 μM) or SB203580 (10 μM). Although this study did not assess the activity of LKB1, a recently identified AMPK kinase (AMPKK), which appears not to be responsive to metabolic stresses including hypoxia or ischemia (1, 32), or the activity of other alternate, yet to be identified AMPKKs, our results strongly support the notion that p38 MAPK is upstream of AMPK in the signaling cascade linking adenosine with changes in myocardial glycogen metabolism and glycolysis (Fig. 7).

Myocardial glucose uptake is stimulated by metabolic stresses, including hypoxia and ischemia, by insulin-independent mechanisms (36). AMPK and p38 MAPK also regulate glucose uptake in response to metabolic stresses (12, 28, 36), possibly by distinct mechanisms. Whereas AMPK stimulates glucose uptake by increasing the translocation of GLUT4 transporters to the cell surface (12, 16, 25, 28), p38 MAPK may stimulate glucose uptake by increasing the intrinsic activity of GLUT4 transporters already at the cell surface (18, 21, 33). Despite these potential synergistic effects of AMPK (GLUT4 translocation) and p38 MAPK (GLUT4 activation), differences in glucose uptake were not observed in stressed hearts despite marked differences in the extent of AMPK and p38 MAPK phosphorylation. The lack of effect of SB202190 and SB203580 on glucose uptake despite their ability to abrogate the adenosine-induced increase in both AMPK and p38 MAPK suggests that these two kinases do not significantly regulate glucose uptake in the fatty acid-perfused rat heart during aerobic conditions.

Therefore, our data contrast with reports suggesting a requirement for AMPK activation in ischemia-induced stimulation of myocardial glucose uptake and for p38 MAPK phosphorylation in the acceleration of glucose uptake in adipocytes, myotubes, isolated adult cardiomyocytes, and the isolated perfused mouse heart (26, 34, 40). Although the low statistical power for the comparison of glucose uptake may not have detected a significant difference, the difference in the mean values of glucose uptake under the conditions of low and high AMPK activity is still very minor relative to changes in glucose uptake reported previously using this method, where the removal of fatty acid from the perfusate nearly doubled the rate of glucose uptake (31). It should be noted that in the current study, AMPK and p38 MAPK activation was achieved during nonischemic conditions, where the supply of glucose is not rate limiting. Moreover, isolated cardiomyocytes have minimal energy requirements; rates of oxidative metabolism are 50 to 100 times less than in isolated working rat hearts, and therefore do not represent a normal energy demand (3). Under these more physiological conditions of appropriate energy substrate supply and energy demand, glucose uptake (determined from the rates of glycolysis and glycogen synthesis) appears insensitive to changes in p38 MAPK or AMPK activity. This observation is further supported by...
a previous study demonstrating that the activation of cardiac AMPK in vivo is not sufficient to increase glucose clearance (30).

Although glucose uptake was similar among experimental groups, there were important differences in glucose utilization with regard to glycogen synthesis and total glycogen content, as well as glycolysis and proton production. Although the p38 MAPK inhibitor SB202190 alone did not affect the rate of glycogen synthesis, SB202190 or SB203580 each prevented the adenosine-induced inhibition of glycogen synthesis. The restoration of normal rates of glycogen synthesis by the p38 MAPK inhibitors allowed glycogen to accumulate normally during the aerobic treatment period. Thus, under these conditions, we have shown that AMPK activation inhibits glycogen synthesis and that inhibition of p38 MAPK prevents AMPK activation, restores glycogen synthesis, and allows glycogen to accumulate to normal levels. To our knowledge, this is the first report to demonstrate a significant inverse correlation between myocardial glycogen content and AMPK and p38 MAPK activities and supports a study by Wojtaszewski et al. (38) that demonstrated an inverse relationship between the activities of glycogen synthase and AMPK, as well as glycogen content and AMPK activity, in rat skeletal muscle. Thus glycogen content becomes an important consideration in investigations of cardiac AMPK activity.

The prevention of adenosine-induced inhibition of glycogen synthesis by SB202190 or SB203580 resembles the profile of glucose utilization observed in normal hearts with low AMPK activity, where the balance in the fate of glucose between glycogen synthesis and glycolysis favors glycolysis synthesis. Glycogen accumulation and the preferential oxidation of glucose liberated from glycogen may provide an energetic advantage, and it also lessens the potential for glycolysis and proton production (13, 29). Glycogen synthesis is an important target in cyclohexyladenosine-induced cardioprotection (7), but whether the ability of p38 MAPK inhibitors to relieve the adenosine-induced suppression of glycogen synthesis in stressed hearts translates into enhanced cardioprotection following severe ischemia remains to be determined.

In conclusion, in hearts stressed by transient ischemia, adenosine activates both AMPK and p38 MAPK. These effects are inhibited by either of the p38 MAPK inhibitors, SB202190 or SB203580, and are unrelated to changes in nucleotide content. Because the p38 MAPK inhibitors lack any direct effect on AMPK activity, the results suggest that p38 MAPK is upstream of AMPK in this system. In contrast to previous reports, alterations in both AMPK and p38 MAPK phosphorylation do not affect glucose uptake; rather, the consequences of their activation are manifest as an alteration in the partitioning of glucose between glycogen synthesis and glycolysis. The inverse correlation between AMPK activity and myocardial glycogen synthesis and glycogen content was associated with an attenuation of the rates of glycolysis and proton production. These results suggest that the cardioprotective effectiveness of adenosine that is lost in hearts stressed by transient ischemia may be due to activation of AMPK (8). Because there is currently a lack of selective AMPK inhibitors, inhibitors of p38 MAPK may be useful to restore adenosine-induced cardioprotection following further ischemic challenge via the downstream inhibition of AMPK, an effect that elicits salutary alterations in glucose metabolism.

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GRANTS

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