Inhibition of p38 MAPK and AMPK restores adenosine-induced cardioprotection in hearts stressed by antecedent ischemia by altering glucose utilization

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Inhibition of p38 MAPK and AMPK restores adenosine-induced cardioprotection in hearts stressed by antecedent ischemia by altering glucose utilization. Am J Physiol Heart Circ Physiol 293: H1107–H1114, 2007. First published May 11, 2007; doi:10.1152/ajpheart.00455.2007—p38 mitogen-activated protein kinase (MAPK) and 5′-AMP-activated protein kinase (AMPK) are activated by metabolic stresses and are implicated in the regulation of glucose utilization and ischemia-reperfusion (IR) injury. This study tested the hypothesis that inhibition of p38 MAPK restores the cardioprotective effects of adenosine in stressed hearts by preventing activation of AMPK and the uncoupling of glycolysis from glucose oxidation. Working rat hearts were perfused with Krebs solution (1.2 mM palmitate, 11 mM [3H/14C]glucose, and 100 mU/l insulin). Hearts were stressed by transient antecedent IR (2 × 10 min I/5 min R) before severe IR (30 min I/30 min R). Hearts were treated with vehicle, p38 MAPK inhibitor (SB-202190, 10 μM), adenosine (500 μM), or their combination before severe IR. After severe IR, the phosphorylation (arbitrary density units) of p38 MAPK and AMPK, rates of glucose metabolism (μmol·g dry wt−1·min−1), and recovery of left ventricular (LV) work (Joules) were similar in vehicle-, SB-202190-, and adenosine-treated hearts. Treatment with SB-202190 + adenosine versus adenosine alone decreased p38 MAPK (0.03 ± 0.01, n = 3 vs. 0.48 ± 0.10, n = 3, P < 0.05) and AMPK (0.00 ± 0.00, n = 3 vs. 0.26 ± 0.08, n = 3, P < 0.05) phosphorylation. This was accompanied by attenuated rates of glycolysis (1.51 ± 0.40, n = 7 vs. 3.95 ± 0.65, n = 7, P < 0.05) and H+ production (2.12 ± 0.76, n = 7 vs. 6.96 ± 1.48, n = 7, P < 0.05), and increased glycogen synthesis (1.91 ± 0.25, n = 6 vs. 0.27 ± 0.28, n = 6, P < 0.05) and improved recovery of LV work (0.81 ± 0.08, n = 7 vs. 0.30 ± 0.15, n = 8, P < 0.05). These data indicate that inhibition of p38 MAPK abolishes subsequent phosphorylation of AMPK and improves the coupling of glucose metabolism, thereby restoring adenosine-induced cardioprotection.

p38 mitogen-activated protein kinase; 5′-adenosine monophosphate-activated protein kinase; glucose metabolism

ADENOSINE, an endogenous purine nucleoside, has been shown to be an effective pharmacological approach to limit cardiac injury during reperfusion following ischemia. Ischemia itself causes numerous disturbances, particularly an imbalance between energy substrate supply and energy demand. The ability of adenosine to improve the recovery of postischemic myocardial function may in part be related to its ability to restore the balance between energy substrate supply (coronary vasodilation) and energy demand (negative inotropism and chronotropicism) (26, 32) as well as its effects on myocardial carbohydrate metabolism (5). Previous reports demonstrate that adenosine-induced cardioprotection, manifest as an improved recovery of postischemic mechanical function, is accompanied by an inhibition of glycolysis and calculated proton (H+) production (5, 7, 8), which may serve to attenuate intracellular acidosis. This in turn reduces the potential for Na+ and Ca2+ overload that contributes to depressed mechanical function during reperfusion (9, 21).

Interestingly, if hearts are stressed by transient, antecedent ischemia (two cycles of 10-min ischemia and 5-min reperfusion) before the onset of severe global ischemia, the effects of adenosine on glucose metabolism are reversed such that glycolysis is stimulated. As the rate of glucose oxidation remains unchanged, coupling between glycolysis and glucose oxidation is reduced, and this leads to an acceleration of the rate of myocardial H+ production (6). As a consequence, the recovery of left ventricular (LV) work is impaired by adenosine in hearts stressed by transient ischemia (6). Thus stressing with transient ischemia may produce a model similar to the clinical scenario where adenosine mimetic compounds have limited cardioprotective effectiveness (34). Therefore, this model provides an ideal system to characterize the biochemical mediators responsible for the loss of adenosine-induced cardioprotection, as well as targets to potentially restore or enhance adenosine-induced cardioprotection.

We have recently described that the adenosine-induced acceleration of glycolysis and H+ production during aerobic perfusion of stressed hearts is accompanied by a suppression of the rate of glycogen synthesis and an activation of the 5′-AMP-activated protein kinase (AMPK), independently of differences in LV work (13). Furthermore, we have demonstrated that inhibition of p38 mitogen-activated protein kinase (MAPK) inhibits the activation of AMPK, attenuates the rates of glycolysis and H+ production, as well as relieving the suppression of glycogen synthesis (14). Whether this mechanism persists in stressed hearts during reperfusion following severe ischemia and translates into cardioprotection has yet to be determined.

p38 MAPK and AMPK are stress-responsive protein kinases involved in ischemia-reperfusion (IR) injury and in the regulation of glucose utilization. In addition, these two kinases form a functional signaling cascade (19, 37). The roles of both p38 MAPK and AMPK in myocardial IR injury are complex. With regard to p38 MAPK and the recovery of postischemic...
myocardial function, there is controversy. Some studies suggest that p38 MAPK activation is beneficial (24, 25), whereas other studies suggest that its activation is detrimental, and that its inhibition during reperfusion is cardioprotective (2, 16, 35, 38). Similarly, the role of AMPK in the evolution of IR injury is not clearly defined. Previous studies suggest that the activation of AMPK is cardioprotective via inhibition of apoptosis and via increased glucose uptake and glycolysis (28, 30). However, increased rates of glycolysis during reperfusion coupled with the rapid recovery of fatty acid oxidation at the expense of glucose oxidation accelerates myocardial H⁺ production and so contributes to depressed contractile function and cardiac efficiency during reperfusion (20, 21).

In this study we investigated whether the inhibition of p38 MAPK can inhibit AMPK as well as glycolysis and H⁺ production during reperfusion following severe ischemia in hearts stressed by transient antecedent ischemia. In accordance with our findings in stressed hearts during aerobic perfusion, we hypothesized that inhibition of p38 MAPK will inhibit AMPK and attenuate glycolysis and H⁺ production. Furthermore, we hypothesized that these alterations in glucose metabolism restore the cardioprotective effectiveness of adenosine during reperfusion following severe ischemia in hearts stressed by antecedent ischemia.

MATERIALS AND METHODS

Animals. 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.

Heart perfusions. 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 (37°C, pH 7.4, gassed with a 95% O₂-5% CO₂ mixture) was initiated. Hearts were perfused in the Langendorff mode for 10 min and thereafter switched to the working (ectopic) mode as described previously (13). 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 pre-bound to 3% bovine serum albumin and 100 mU/l insulin. Perusions were performed at a constant workload (preload, 11.5 mmHg; afterload, 80 mmHg) and heart rate (paced at 300 beats/min). Heart rate and systolic and diastolic arterial pressures (mmHg) were measured using a Gould P21 pressure transducer attached to the aortic outflow line. Cardiac output (ml/min) and aortic flow (ml/min) were measured by using ultrasonic flow probes (Transonic T206) placed in the left atrial inflow line and the aortic outflow line, respectively. LV work (in 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 15 min before stressing by transient ischemia [two 10-min periods of global no-flow ischemia (shaded bars) each followed by 5 min reperfusion] and were then treated with either vehicle (saline) (n = 6), SB-202190 (10 μM) (n = 6), adenosine (Ado) (500 μM) (n = 6), or SB-202190 (10 μM) + Ado (500 μM) (n = 6) before 30 min severe global no-flow ischemia. Stressed hearts were then frozen for biochemical analyses at the end of severe ischemia (↓). Hearts were perfused in a similar manner as in A; however, after treatment with vehicle (n = 10), SB-202190 (10 μM) (n = 8), Ado (500 μM) (n = 8), or SB-202190 (10 μM) + Ado (500 μM) (n = 7), they were subjected to 30 min severe ischemia and 30 min reperfusion. Hearts were then frozen for biochemical analyses at the end of ischemia or reperfusion (↓).

Measurement of AMPK activity. The activity of AMPK (pmol·mg protein⁻¹·min⁻¹) was measured in 6% polyethylene glycol (PEG) fractions extracted from 200 mg of frozen LV tissue as described...
previously (17, 18). Activity of AMPK in the presence of 5'-AMP (200 μM) was assayed in the 6% PEG fraction by following the incorporation of [γ-32P]ATP into a serine-79 phosphorylation site-specific HMRASMSGLVHVK (SAMS) peptide as previously described (3, 18).

**Assay of glycogen content and glucose uptake.** Frozen LV tissue was powdered using a mortar and pestle maintained at the temperature of liquid N₂. 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 by using a Sigma glucose analysis kit. The net rate of glycogen synthesis (μmol·g dry wt⁻¹·min⁻¹) in hearts during the 30-min reperfusion period was calculated from the increase in [5-3H]- and [14C]glycosyl units in total myocardial glycogen in hearts frozen at the end of reperfusion relative to hearts frozen at the end of severe ischemia. The rate of glucose uptake (μmol·g dry wt⁻¹·min⁻¹) during the reperfusion period was calculated as the sum of the net rate of glycogen synthesis and the rate of glycolysis in individual hearts.

Measurement of steady-state rates of glycolysis and glucose oxidation. Glycolysis and glucose oxidation rates were measured directly from the simultaneous production of H₂O (liberated at the enolase step of glycolysis) and 14CO₂ (liberated at the level of pyruvate dehydrogenase complex and in the citric acid cycle), respectively, from [5-3H]glucose and [U-14C]glucose, as described previously (8). Perfusate was sampled at predetermined time points (5, 14, 29, 44, 75, 85, 95, and 105 min), and steady-state rates (expressed as μmol [5-3H]glucose or [U-14C]glucose metabolized·g dry wt⁻¹·min⁻¹) were calculated.

Calculation of the 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 2×(rate of glycolysis – rate of glucose oxidation).

**Materials.** d-[5-3H]glucose and d-[U-14C]glucose were purchased from DuPont Canada (Ontario, Canada). SB-202190 was purchased from Calbiochem (San Diego, CA). Adenosine was purchased from Research Biochemicals International (Natick, MA). Anti-phospho-p38 MAPK (Thy180/Yyr182), 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.** Values are means ± SE of n observations. The significance of the differences for multiple comparisons between treated and untreated groups was estimated by ANOVA. If significant, selected data sets were compared by Bonferroni’s multiple comparison test. Differences were considered significant when P < 0.05.

**RESULTS**

**LV work in stressed hearts before and after severe ischemia.** LV work (in joules) was stable and similar during the baseline period of perfusion and in hearts before being assigned to vehicle, SB-202190, adenosine, or SB-202190 + adenosine-treated groups as described in MATERIALS AND METHODS (data not shown). All measurable LV work ceased during the periods of transient and antecedent ischemia and recovered to similar levels in all groups immediately before the onset of severe ischemia (Fig. 2A). All measurable LV work ceased during the 30-min period of severe ischemia. During reperfusion, LV work recovered to similar extents in vehicle (0.39 ± 0.12, n = 10), SB-202190 (0.25 ± 0.12, n = 8), and adenosine-treated (0.30 ± 0.15, n = 8) hearts, indicating a loss of the cardioprotective effects of adenosine in hearts stressed by antecedent ischemia (Fig. 2B). However, there was a significant improvement in the recovery of LV work in hearts treated with SB-202190 + adenosine (0.81 ± 0.08, n = 7, P < 0.05), indicating that the p38 MAPK inhibitor restores adenosine-induced cardioprotection.

**Phosphorylation of p38 MAPK and AMPK in stressed hearts.** The phosphorylation (densitometric analysis) of p38 MAPK (Fig. 3A) and AMPK (Fig. 3B), as well as AMPK activity (Fig. 3C), was measured in hearts frozen at the end of the 30-min period of severe ischemia (i.e., before reperfusion). Values were similar in vehicle, SB-202190-, adenosine-, and SB-202190 + adenosine-treated hearts at the end of severe ischemia. In hearts frozen at the end of reperfusion, the phosphorylation of p38 MAPK was similar in vehicle, SB-202190-, and adenosine-treated hearts but was significantly decreased in hearts treated with SB-202190 + adenosine (Fig. 4A). In parallel, the phosphorylation of AMPK (Fig. 4B) and AMPK activity (Fig. 4C) were similar in vehicle, SB-202190, and adenosine-treated hearts frozen at the end of reperfusion but were significantly decreased (phosphorylation to nondetectable levels) in hearts treated with SB-202190 + adenosine.

**Glucose metabolism in stressed hearts during reperfusion following severe ischemia.** Steady-state rates of glucose metabolism (glycolysis and glucose oxidation) and calculated rates of H⁺ production were assessed to characterize the
metabolic consequences of the alterations in p38 MAPK and AMPK phosphorylation during reperfusion in hearts stressed by antecedent ischemia. The rates of glycolysis were similar during reperfusion in vehicle-, SB-202190-, and adenosine-treated hearts where the levels of p38 MAPK and AMPK phosphorylation were also similar. However, in hearts treated with SB-202190 + adenosine, the rate of glycolysis was decreased by 62% \((P < 0.05)\) where the phosphorylation of both p38 MAPK and AMPK was attenuated when compared with hearts treated with adenosine alone (Fig. 5A). The rates of glucose oxidation were similar among the experimental groups during the reperfusion period (Fig. 5B). As glycolysis was decreased and glucose oxidation was unaltered in hearts with attenuated levels of p38 MAPK and AMPK phosphorylation, the calculated rate of \(\text{H}^+\) production from exogenous glucose was concomitantly decreased by 70% \((P < 0.05)\) compared with hearts treated with adenosine alone (Fig. 5C).

Glucose uptake and glycogen metabolism in stressed hearts during reperfusion. The rates of glucose uptake and glycogen metabolism were also calculated during reperfusion in hearts stressed by antecedent ischemia. Despite the marked attenua-
tion of p38 MAPK phosphorylation, as well as AMPK phosphorylation and activity in hearts treated with SB-202190 + adenosine, the rate of glucose uptake remained unaltered (Fig. 6A). In hearts treated with vehicle, SB-202190, or adenosine, the rates of glycogen synthesis ranged between 6 and 19% of the rates of glucose uptake. However, in hearts treated with SB-202190 + adenosine, where the phosphorylation of p38 MAPK and AMPK is attenuated, the rate of glycogen synthesis was ~56% of the rate of glucose uptake. Consequently, myocardial glycogen accumulated to a significantly greater extent in hearts with low levels of p38 MAPK and AMPK phosphorylation (Fig. 6C). Furthermore, whereas the proportions of radiolabeled glycogen (expressed as % total glycogen) in hearts treated with vehicle, SB-202190, or adenosine were 67.1 ± 3.7%, 68.2 ± 2.8%, and 63.0 ± 4.1%, respectively, it was significantly greater in hearts treated with SB-202190 + adenosine (84.4 ± 2.6%), indicative of greater glycogen turnover during reperfusion (Fig. 6D). Overall, these data suggest that it is alterations in glucose utilization (i.e., the coordinated balance between glycogen synthesis and glycolysis) that account for the decreased rate of calculated H⁺ production and the restoration of the cardioprotective effects of

Fig. 5. Rates of glucose metabolism and calculated proton production in stressed hearts during reperfusion following severe ischemia. Glycolysis (A), glucose oxidation (B), and the calculated rate of proton production from glucose metabolism (C) were assessed as described in MATERIALS AND METHODS for hearts treated with vehicle (n = 7), SB-202190 (n = 8), Ado (n = 7), or SB-202190 + adenosine (n = 7). Values (μmol·g dry wt⁻¹·min⁻¹) represent means ± SE. The absence or presence of adenosine or SB-202190 is indicated by − and + respectively. *Significant difference from adenosine-treated hearts.

Fig. 6. Glucose uptake and glycogen metabolism in stressed hearts during reperfusion following severe ischemia. Glucose uptake (A), glycogen synthesis (B), total glycogen content (C), and [3H/14C]glycogen content (D) were assessed as described in MATERIALS AND METHODS for hearts treated with vehicle, SB-202190, Ado, or SB-202190 + adenosine (n = 6 per group). Values for A and B (μmol·g dry wt⁻¹·min⁻¹) and C and D (μmol/g dry wt) represent means ± SE. *Significant difference from adenosine-treated hearts.
adenosine in hearts where the phosphorylation of p38 MAPK and AMPK is attenuated.

DISCUSSION

This study investigated the roles of p38 MAPK and AMPK in adenosine-induced cardioprotection and in the regulation of glucose and glycogen metabolism during posts ischemic reperfusion in hearts stressed by antecedent ischemia. Relative to the marked beneficial actions of adenosine in normal hearts, there was a loss of adenosine-induced cardioprotection following severe ischemia in hearts stressed by antecedent ischemia. However, the p38 MAPK inhibitor SB-202190 restored adenosine-induced cardioprotection as evinced by the marked improvement in the recovery of posts ischemic LV work. This was accompanied by an inhibition of p38 MAPK and AMPK phosphorylation as well as an inhibition of AMPK activity during reperfusion. The inhibition of p38 MAPK and AMPK was associated with an inhibition of the rates of glycolysis and calculated H⁺ production. The inhibition of glycolysis occurred independently of changes in glucose uptake, but was accompanied by an elevated rate of glycogen synthesis, and thus an accumulation of myocardial glycogen content. These data indicate that the inhibition of both p38 MAPK and AMPK during reperfusion following severe ischemia is cardioprotective via alterations in glucose metabolism. Rather than being due to changes in glucose uptake, cardioprotection due to inhibition of p38 MAPK and AMPK was related to an alteration in the partitioning of glucose between glycolysis and glycogen synthesis.

The role of p38 MAPK in mediating myocardial IR injury is complex, with studies describing its activation as being both protective and deleterious. Previous reports implicate increased p38 MAPK phosphorylation as an important component of the cardioprotective effects of ischemic preconditioning (25, 36). However, the model utilized in this study is not one of ischemic preconditioning, because the antecedent ischemia protocol fails to enhance the recovery of LV work during reperfusion following severe ischemia. Rather, the antecedent ischemia protocol produces a model where there is a loss of the cardioprotective effects of adenosine. The mechanisms underlying the loss of adenosine-induced cardioprotection in hearts stressed by antecedent ischemia are not yet well defined. However, changes in p38 MAPK phosphorylation in signaling events linking adenosine to alterations in glucose metabolism are strongly implicated as inhibition of p38 MAPK prevents adenosine-induced uncoupling of glucose metabolism in hearts stressed by antecedent ischemia (14), and this then allows adenosine to partially inhibit glycolysis and H⁺ production in a manner similar to that described for normal hearts (13). Interestingly, in this study the level of p38 MAPK phosphorylation is similar between experimental groups at the end of the period of severe ischemia. This may be accounted for by the transient nature of ischemia-induced p38 MAPK phosphorylation (27). In contrast to the above, previous reports also suggest that the activation of p38 MAPK is detrimental during the posts ischemic period, as inhibitors of p38 MAPK reduce apoptosis and infarct size and improve the recovery of cardiac function (2, 11, 22). Although the p38 MAPK inhibitor SB-202190 alone did not affect the phosphorylation of p38 MAPK or recovery of LV work during reperfusion, the combination of SB-202190 + adenosine induced robust cardioprotection and reduced the extent of p38 MAPK phosphorylation at the end of reperfusion, indicating that inhibition of p38 MAPK preserves the cardioprotective effects of adenosine in hearts stressed by antecedent ischemia.

AMPK interacts with p38 MAPK to regulate glucose metabolism (19, 37). However, in contrast to the former reports, which suggest p38 MAPK functions downstream of AMPK in a rat liver cell line (37) and in the isolated, perfused mouse heart (19), our results are consistent with our previous findings in aerobically perfused hearts that suggest that AMPK is downstream of p38 MAPK in the control of myocardial glucose metabolism (14). Whether AMPK functions to limit myocardial IR injury is uncertain. Previous reports suggest that AMPK plays a protective role in limiting IR injury via its involvement in ischemic preconditioning (33), as well as by promoting glucose uptake, glycolysis, and preventing apoptosis associated with IR injury (28). In the isolated, fatty acid-perfused rat heart, the activation of AMPK does indeed accelerate glycolysis; however, it also accelerates the rate of H⁺ production (13), which has been demonstrated to impair LV functional recovery during reperfusion (6). As the uncoupling between glycolysis and glucose oxidation is a significant source of H⁺, it can delay the recovery of intracellular pH and so contribute to impaired cardiac work and efficiency during reperfusion (21). The lack of available selective AMPK inhibitors precluded assessing the direct effects of AMPK inhibition per se. Nevertheless, the results obtained in this study suggest that the inhibition of AMPK activation during reperfusion (subsequent to inhibition of p38 MAPK) is responsible for the marked cardioprotection that was associated with partial inhibition of glycolysis and H⁺ production from uncoupled glucose metabolism. These effects have previously been demonstrated to enhance the recovery of LV function during reperfusion following ischemia (8). Previous reports indicate that AMPK increases the production of fructose 2,6-bisphosphate, a potent stimulator of 6-phosphofructo-1-kinase, the rate-limiting enzyme of glycolysis (4, 23). The observation that glycolysis is partially inhibited in hearts with low p38 MAPK phosphorylation, as well as low AMPK phosphorylation and activity, is consistent with this recognized role of AMPK in regulating the glycolytic pathway. The inhibition of glycolysis occurred independently of alterations in glucose uptake but was accompanied by changes in glycogen metabolism, such that glycogen synthesis was increased.

The role of AMPK in regulating glycogen metabolism is most well studied in skeletal muscle where there is an inverse relationship between AMPK activity and glycogen content (1, 15). Less well characterized is the role of AMPK in regulating myocardial glycogen metabolism. However, our previous findings in stressed hearts perfused under aerobic conditions have demonstrated that increased AMPK activity is accompanied by a suppression of the rate of glycogen synthesis and an acceleration of the rate of glycolysis (13). Furthermore, these studies demonstrated that decreased AMPK activity is accompanied by an increase in the rate of glycogen synthesis and an inhibition of the rate of glycolysis (14). In this study, we have extended these findings to a model where stressed hearts are subjected to severe ischemia, where the inhibition of AMPK activity is also accompanied by an increase in the rate of glycogen synthesis and an inhibition of the rate of glycolysis.
The inhibition of AMPK and the associated alterations in glucose metabolism are accompanied by marked cardioprotection during reperfusion following severe ischemia. A greater rate of glycogen synthesis during reperfusion in hearts with low AMPK activity allowed for the accumulation of myocardial glycogen content. Previous reports suggest that glucose liberated from the myocardial glycogen pool is preferentially oxidized (10, 12). This may contribute to the observed cardioprotection in hearts with low AMPK activity by an improved coupling of the rates of glucose oxidation to glycolysis and thereby decreasing the rate of H⁺ production, which has been shown to enhance myocardial functional recovery during reperfusion (8). As the changes in p38 MAPK and AMPK phosphorylation, as well as the alterations in glucose metabolism during reperfusion following severe ischemia, also occur in stressed hearts during aerobic perfusion and in the absence of severe ischemia and differences in LV work (14), our results indicate that these changes during reperfusion cannot simply be a consequence of changes in LV mechanical function. Rather, the current and previous results suggest that the changes in p38 MAPK, AMPK, and glucose metabolism are the cause, and not the consequence, of the extent of adenosine-induced cardioprotection.

In conclusion, this study has shown that the inhibition of p38 MAPK and the subsequent inhibition of AMPK restores adenosine-induced cardioprotection during reperfusion following severe ischemia in hearts stressed by antecedent ischemia. Cardioprotection was associated with an inhibition of the rates of glycogen synthesis and H⁺ production that occurred independently of alterations in glucose uptake. The reduction of glycogen and H⁺ production was associated with increased glycogen synthesis and an accumulation of myocardial glycogen content. As the inhibition of p38 MAPK and AMPK reduces the calculated rate of H⁺ production and the subsequent potential for acidosis, the inhibition of either p38 MAPK or AMPK is a novel strategy to restore the protective effects of adenosine in hearts stressed by antecedent ischemia.

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