AMP deaminase in piglet cardiac myocytes: effect on nucleotide metabolism during ischemia

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Hohl, Charlene M. AMP deaminase in piglet cardiac myocytes: effect on nucleotide metabolism during ischemia. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1502–H1510, 1999.—The purpose of this study was to examine in situ regulation of AMP deaminase in newborn piglet cardiac myocytes and to determine its role in nucleotide metabolism during ischemia. When a rapid deenergization paradigm was used to assay AMP deaminase, enzyme activity depended on the hormonal and metabolic status of cells just before deenergization. Inosine 5′-monophosphate (IMP) formation was increased 150% in deenergized myocytes pretreated with phorbol 12-myristate 13-acetate (PMA; EC50 = 4.7 × 10⁻⁸ M). This effect was 90% blocked with the protein kinase C (PKC) inhibitor staurosporine. In addition, the β-adrenergic agonist isoproterenol stimulated AMP deaminase activity (EC50 = 1.5 × 10⁻⁸ M), and IMP formation was directly correlated to intracellular cAMP levels (r² = 0.9). Furthermore, adenosine increased IMP formation, whereas nonrespiring, glycolyzing piglet myocytes had reduced AMP deaminase activity. Pretreatment of perfused piglet hearts with adenosine, but not PMA, before exposure to global ischemia resulted in enhanced conversion of AMP to IMP during the ischemic period. Similar results were obtained in piglet myocytes preincubated with adenosine or PMA before exposure to simulated ischemia. These results may be relevant to the preconditioning phenomenon.

WITH A SUDDEN INCREASE in ATP demand, a reduction in coronary flow, or inadequate substrate availability, the myocardium degrades ATP stores to AMP, nucleosides, and bases. The predominant pathway of AMP catabolism in heart muscle is dephosphorylation to adenosine via 5′-nucleotidase and subsequent deamination of adenosine to inosine. Adenosine is a potent vasodilator and, as well, antagonizes cAMP-dependent inotropic effects through adenosine receptor-mediated action on adenylate cyclase. Both adenosine and inosine are freely permeable and are washed from the interstitium on reperfusion. AMP can also be deaminated directly to form inorganic adenosine 5′-monophosphate (IMP) in the initial reaction of the purine nucleotide cycle. A complete turn of this cycle allows salvage of AMP and generates substrates for the citric acid cycle at the expense of aspartate and GTP through the actions of adenylosuccinate synthetase and lyase. Moreover, AMP deaminase is the key enzyme that integrates adenine and guanine metabolism, permitting the synthesis of guanine nucleotides from the larger adenylate pool.

Under aerobic conditions, cellular cytosolic AMP concentrations are much lower than the Michaelis-Menten constant (Km) for nonstimulated cardiac AMP deaminase, and IMP levels are low. However, during severe ATP depletion, AMP increases and is subsequently either deaminated to IMP or dephosphorylated to adenosine via 5′-nucleotidase in a competing reaction. Whether IMP or adenosine formation predominates appears to be dependent on both the species and the hormonal and metabolic state of the heart just before deenergization. Using a rapid ATP-depletion paradigm to measure AMP deaminase activity in situ in isolated intact cardiac myocytes, we have previously determined (12) that enzyme activity is quite high in aerobic rat heart myocytes that are subsequently rapidly deenergized, whereas nonrespiring, glycolyzing cells form little IMP. Furthermore, β-adrenergic agonists stimulate rat cardiac AMP deaminase through a protein kinase C (PKC)-dependent pathway (12, 13). Adenosine as well as agents that raise intracellular cAMP also increase AMP deaminase activity; however, their mechanism of action has not been established (13, 14).

Interestingly, many of the same agents that activate AMP deaminase are also involved in the preconditioning response of ischemic myocardium. Preconditioning is the phenomenon whereby subjecting myocardium to transient cycles of ischemia protects it from the damaging effects of prolonged ischemia (28). Although the exact mechanism of preconditioning is still controversial, adenosine and PKC mediation have been implicated (7, 18, 40). Furthermore, in many species, treatment with adenosine or adenosine deaminase inhibitors mimics preconditioning. Although preconditioning has been shown to have a sparing effect on high-energy phosphates during the subsequent longer ischemic period in adult canine (29) and porcine hearts (17, 27), it is not known whether adenosine nucleotide degradation pathways are altered. Moreover, no information is available on how these agents affect neonatal hearts from a larger mammalian species such as human and swine. The purpose of this study was to examine in situ regulation of AMP deaminase in cardiac myocytes isolated from newborn piglet ventricles and to determine its role in nucleotide metabolism during ischemia. Furthermore, the effect of adenosine and the PKC activator phorbol 12-myristate 13-acetate (PMA) on altering ATP-degradation and -resynthesis pathways in ischemic neonatal swine myocardium was investigated. The results demonstrate that AMP deaminase activity is stimulated in neonatal piglet cardiac myocytes by phorbol esters, adenosine, and β-adrenergic agonists. Moreover, pretreatment of intact hearts with
ADENOSINE ACTIVATES AMP DEAMINASE DURING ISCHEMIA

H1503

METHODS

Isolation of myocytes. Ventricular myocytes were isolated from newborn (<24 h) mixed-breed swine hearts using a collagenase procedure, as described previously (21). Isolated piglet myocytes were suspended in HEPES-buffered medium containing (in mM) 110 NaCl, 4.8 KCl, 1.2 MgSO_4, 1.2 KH_2PO_4, 1 CaCl_2, 5 NaHCO_3, 25 HEPES (pH 7.3), 11 glucose, 5 pyruvate, 20 taurine, 10 creatine, 0.68 glutamine, and 0.001 insulin as well as amino acids, vitamins, and penicillin-streptomycin. Myocytes were incubated in this medium for experiments examining regulatory mechanisms of AMP deaminase. In studies simulating in vivo ischemia, myocytes were suspended in medium containing (in mM) 110 NaCl, 4.8 KCl, 1.2 MgSO_4, 1.2 KH_2PO_4, 1 CaCl_2, 5 NaHCO_3, and 25 HEPES (pH 7.3) as well as amino acids and penicillin-streptomycin.

Newborn piglet ventricular myocytes used in this study were 89 ± 1% viable as estimated by trypan blue exclusion criteria. Cellular metabolite values and other characteristics of these preparations are described elsewhere (11, 21).

Induction of ischemia in intact myocardial chunks and isolated myocytes. Newborn piglet hearts were cannulated via the aorta and perfused (~5 ml·min·g wet wt) on a Langendorff apparatus at 37°C for a 15- to 20-min stabilization period followed by an additional 8-min perfusion with either buffer (control), 100 nM PMA, or 10 µM adenosine. Piglet hearts were then rapidly removed from the apparatus and sliced into small chunks (~200 mg) that were subsequently incubated at 37°C in sealed zipper-lock storage bags. Samples were freeze-clamped at regular intervals, extracted with perchloric acid, and analyzed by HPLC for nucleotide content.

Freshly isolated piglet myocytes were suspended at 1.5 mg protein/ml in substrate-free HEPES-buffered medium and incubated for 10 min at 37°C in the presence of 300 nM PMA or either 20 µM or 1 mM adenosine plus 3 µM coformycin (to inhibit endogenous adenosine deaminase). Myocytes were removed from the water bath, treated with 5 mM 5-ethyl-5-isooamylbarbituric acid (Amytal; to inhibit mitochondrial respiration), and then rapidly sedimented, and the supernatant was removed, giving a cell concentration of roughly 25 mg protein/ml. Mineral oil (0.3 ml) was layered over the cell pellets, and myocytes were further incubated without shaking at 37°C for ~75 min.

To estimate cellular capacity for regenerating ATP, after 45 and 60 min of simulated ischemia, myocyte pellets were washed twice, resuspended (1–2 mg protein/ml) in medium containing pyruvate and glucose, and incubated for an additional 25 min in a shaking water bath at 37°C.

Analytical procedures. Cellular metabolites were analyzed after extraction in 2 N perchloric acid. Cardiac myocytes were separated from their suspending medium by rapid centrifugation of cells through 1-bromododecane into perchloric acid. The uppermost layer containing cell medium and the organic layer was removed, and the remaining acid extract containing cellular metabolites was neutralized with freon/triethylamine. Nucleotides and nucleosides in cell extracts were analyzed by HPLC. Metabolites were separated by elution from a Partisil 10 SAX column using a pH and phosphate gradient (8). cAMP was quantified in neutralized cell extracts by radioimmunoassay (1). Phosphocreatine and lactate were estimated according to Bergmeyer (5), and inorganic phosphate was measured by the method of Sanui (32). Myocyte protein was determined by the method of Lowry et al. (22).

Statistics. Values reported are means ± SE for n different myocyte preparations. Student’s-t-test was used when comparing two groups. In experiments involving three or more groups, values were evaluated using ANOVA followed by Dunnet’s test. Differences were considered significant at P < 0.05.
Table 1. Nucleotide and nucleoside content of isolated piglet myocytes

<table>
<thead>
<tr>
<th>Viability</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>RNA</th>
<th>NAD(H)</th>
<th>GTP</th>
<th>UTP</th>
<th>IMP</th>
<th>Ado</th>
<th>Ino</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>89.0</td>
<td>30.91</td>
<td>5.50</td>
<td>0.81</td>
<td>37.22</td>
<td>7.97</td>
<td>1.70</td>
<td>2.36</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>SE</td>
<td>±0.9</td>
<td>±0.83</td>
<td>±0.20</td>
<td>±0.07</td>
<td>±0.93</td>
<td>±0.22</td>
<td>±0.06</td>
<td>±0.15</td>
<td>±0.14</td>
<td>±0.09</td>
</tr>
</tbody>
</table>

Values represent means ± SE in nmol/mg protein for n = 35 myocyte preparations. RNA, total adenine nucleotides (ATP + ADP + AMP); NAD(H), pyridine nucleotides; Ado, adenosine; Ino, inosine.

RESULTS

Estimation of AMP deaminase in isolated cardiac myocytes. AMP deaminase activity was measured in situ in isolated neonatal piglet cardiac myocytes using a rapid ATP-depletion paradigm developed in this laboratory for adult rat ventricular cardiac myocytes (12). When cellular ATP stores are rapidly and synchronously depleted by treatment of cells with a combination of 3 mM Amytal to block mitochondrial electron transport, 5 mM iodoacetate to inhibit glycolysis, and 1 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP) to uncouple mitochondria and induce ATPase activity, within 2 min ATP is converted almost quantitatively to AMP and thereafter is degraded to either IMP and thereafter is degraded to either IMP

and adenosine (Fig. 1). The intracellular concentration of IMP was also decreased in rotenone-treated cells (0.72 ± 0.25 for aerobic cells, 0.35 ± 0.12 for PMA treated, 3.7 ± 0.06 vs. aerobic; †P < 0.05 vs. reoxygenated cells. PMA treated: 12.5 ± 0.5 nmol/mg; PMA + staurosporine treated: 6.0 ± 0.5 nmol/mg (P < 0.05 for PMA vs. PMA + staurosporine)), providing evidence that PKC phosphorylation was involved in the observed response.

Table 2. Effect of anoxia and reoxygenation on cellular metabolites

<table>
<thead>
<tr>
<th></th>
<th>ATP, nmol/mg</th>
<th>PCR, nmol/mg</th>
<th>Pi, % of control</th>
<th>NAD(H)</th>
<th>IMP, nmol/mg</th>
<th>Deenergized IMP, nmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>32.3±1.8</td>
<td>45.2±4.1</td>
<td>100</td>
<td>2.59±0.25</td>
<td>0.35±0.06</td>
<td>6.5±0.5</td>
</tr>
<tr>
<td>Argon/rotone</td>
<td>30.8±2.2</td>
<td>27.7±4.5</td>
<td>161±21.0</td>
<td>0.72±0.06</td>
<td>0.63±0.12</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>Reoxygenation</td>
<td>36.1±2.9</td>
<td>41.8±4.8</td>
<td>96.6±6.1</td>
<td>2.61±0.33</td>
<td>0.33±0.14</td>
<td>9.1±0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–10 myocyte preparations. Pi, content of aerobic myocytes was 9.8 ± 1.4 nmol/mg. †P < 0.05 vs. aerobic; †P < 0.05 vs. reoxygenated cells.
Stimulation of AMP deaminase by elevating cellular cAMP. Further studies indicated that piglet cardiac AMP deaminase activity was also increased after treatment with agents that raise intracellular cAMP levels. As shown in Fig. 3, myocytes challenged with increasing concentrations of the β-adrenergic agonist isoproterenol and then subsequently deenergized had elevated IMP. The dose-dependence curve for IMP formation yielded an EC50 of 1.5 × 10⁻⁸ M isoproterenol. In the presence of the nonspecific phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 100 µM), the EC50 of the isoproterenol titration curve was reduced to 1.1 × 10⁻⁸ M.

A plot of IMP formation in deenergized cells as a function of cellular cAMP content yielded a positive linear relationship for cAMP values <90 pmol/mg (r² = 0.9). In the presence of IBMX, concentrations of isoproterenol >10 nM raised intracellular cAMP to levels >100 pmol/mg. It should be noted that elevation of intracellular cAMP above 90–100 pmol/mg did not further promote IMP production, suggesting that activation by this pathway was saturated. Moreover, preincubation of myocytes with staurosporine [which also blocks cAMP-dependent protein kinase (PKA) but at a much higher inhibition constant than for PKC] reduced cAMP-mediated IMP formation by 68 ± 7% (cellular cAMP = 75 pmol/mg in these cells) but was much less effective under conditions in which cAMP content was quite high (39% inhibition in the presence of 100 µM IBMX + 10 nM isoproterenol; cAMP = 286 pmol/mg).

Stimulation of cells with 10 µM norepinephrine was also effective in raising both intracellular cAMP (control: 21.2 ± 1.9 pmol/mg; norepinephrine: 38.8 ± 2.4 pmol/mg; n = 3, P < 0.05) and, subsequently, IMP (55 ± 14% increase in IMP over control, n = 3, P < 0.05) in ATP-depleted piglet myocytes.

Adenosine modulation of cardiac AMP deaminase. Preincubation of piglet cardiac myocytes with 10 µM adenosine in the presence of 3 µM coformycin (to prevent adenosine deaminase-mediated degradation of adenosine), followed by simultaneous exposure to Amytal, iodoacetate, and CCCP, stimulated IMP formation nearly twofold from 5.6 ± 1.0 nmol/mg in control deenergized piglet cells to 11.5 ± 1.4 nmol/mg in the presence of adenosine (n = 7, P < 0.001).

ATP degradation pathways in ischemic piglet myocardium. Many of the interventions demonstrated to affect piglet cardiac AMP deaminase activity have also been...
reported to be involved in preconditioning adult ischemic hearts. However, it is not known what effect preconditioning agents have on nucleotide metabolism in ischemic neonatal swine myocardium; furthermore, the role of AMP deaminase has not been investigated. The next series of experiments were designed to characterize ATP-degradation pathways in newborn piglet myocardium and to determine whether pretreatment with adenosine and PMA, two activators of AMP deaminase, promoted deamination of AMP to IMP over dephosphorylation to yield adenosine.

The nucleotide profile of freeze-clamped normal newborn piglet myocardium is given in Table 3, and the time course of change in these metabolites with prolonged ischemia is depicted in Fig. 4. As ischemia progressed, ATP slowly declined from 34 to 2 nmol/mg protein over 60 min. Associated with the ATP loss was an initial rapid increase in ADP peaking between 5 and 10 min, followed by a gradual increase in AMP. Subsequently, AMP was further degraded to both adenosine and IMP, with levels of these latter metabolites stabilizing at 1–2 nmol/mg protein over 60 min.

Pretreatment of piglet hearts with 10 µM adenosine did not affect the rate of ATP decline during ischemia compared with that in control ischemic myocardium. However, AMP levels were significantly lower by 30 min (P < 0.05), and IMP content was higher compared with that in control ischemic myocardium (P < 0.05) (Fig. 4). In contrast to the findings in adenosine-treated myocardium, ATP-degradation pathways were not altered compared with those in control ischemic tissue by pretreatment of hearts with 100 nM PMA (Fig. 4).

Table 3. Metabolite content of piglet myocardium

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>ΣAN</th>
<th>NAD(H)</th>
<th>GTP</th>
<th>UTP</th>
<th>IMP</th>
<th>Ado</th>
<th>Ino</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>34.3</td>
<td>11.07</td>
<td>2.39</td>
<td>47.8</td>
<td>9.82</td>
<td>1.69</td>
<td>2.17</td>
<td>0.73</td>
<td>1.30</td>
<td>2.30</td>
</tr>
<tr>
<td>SE</td>
<td>±1.5</td>
<td>±0.79</td>
<td>±0.36</td>
<td>±2.0</td>
<td>±0.36</td>
<td>±0.10</td>
<td>±0.25</td>
<td>±0.12</td>
<td>±0.11</td>
<td>±0.38</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol/mg protein for 12 hearts. Hearts from newborn piglets were perfused with bicarbonate-buffered Krebs-Henseleit medium for 15–20 min. Chunks of ventricular myocardium were then freeze-clamped and analyzed for tissue nucleotide and nucleoside content.

Fig. 4. Time courses of metabolite changes in piglet myocardium exposed to global ischemia. Hearts from newborn piglets were perfused with Krebs-Henseleit buffer alone (Con) or containing 10 µM Ado or 100 nM PMA for 8 min. Ventricles were rapidly sectioned into small chunks, sealed in zipper-lock bags, and further incubated at 37°C. Metabolites were acid extracted from freeze-clamped tissue and assayed by HPLC as described in Methods. Data points are means ± SE for 3–5 piglet hearts for each treatment. ΣAN, total adenine nucleotides. *P < 0.05 compared with control myocardium.
chunks of piglet myocardial tissue. It was then important to determine whether IMP production was similarly affected in isolated neonatal piglet myocytes incubated under conditions simulating in vivo ischemia.

Isolated piglet myocytes were suspended in substrate-free medium, pretreated for 10 min with either 20 µM adenosine or 300 nM PMA, and finally pelleted to simulate ischemic conditions. Under these conditions, \( \sim 340 \pm 90 \text{ nmol/mg} \) lactate accumulated in control cells after 45 min of ischemia. Lactate production was not different in either adenosine- or PMA-pretreated myocytes (340 \( \pm \) 120 nmol·mg\(^{-1}\)·45 min\(^{-1}\) for both adenosine and PMA ischemic cells). Cellular viability decreased from 86 to 71% after 60 min of ischemia in all incubations.

Subjecting isolated piglet myocytes to "simulated ischemia" resulted in a steady loss of total adenine nucleotides and ATP with corresponding increases in AMP, inosine, and adenosine (Fig. 5). Consistent with results from intact myocardial tissue, myocyte adenosine content rose by \( \sim 2 \text{ nmol/mg} \). Intracellular inosine increased from 0.5 \( \pm \) 0.3 to 5.9 \( \pm \) 1.3 nmol/mg after 60 min, whereas \( \sim 13 \text{ nmol/mg} \) inosine was recovered in the extracellular medium at 60 min.

As observed with piglet myocardial chunks, pretreatment of isolated myocytes with 300 nM PMA before the onset of ischemia did not alter the pattern of nucleotide degradation from control ischemic cells (Fig. 5). There was a tendency for both AMP and IMP to be higher relative to levels in control ischemic cells, but increases did not reach significance. Similarly, preincubation with 20 µM adenosine (plus 3 µM coformycin) before cells were pelleted did not affect the rate of ATP decline (Fig. 5); however, considerably more AMP was converted to adenosine (\( P < 0.05 \)). Again, increases in IMP did not reach statistical significance compared with levels in control ischemic cells.

When ischemic cells were resuspended in fresh substrate containing medium and incubated for an additional 25 min, ATP and total adenine nucleotides were restored to 21 and 27 nmol/mg, respectively. These values were not affected by PMA or adenosine pretreatment (data not shown).

Studies were then performed using 1 mM adenosine in the preincubation medium before exposure of myocytes to simulated ischemia. Declines in ATP were not different compared with data in control ischemic myocytes, yet AMP degradation to adenosine and IMP was substantially elevated after adenosine pretreatment (\( P < 0.05 \) vs. degradation in control ischemic cells) (Fig. 6), and intracellular inosine was 3 nmol/mg lower after 60 min of ischemia (control = 4.9 \( \pm \) 0.8 nmol/mg protein; adenosine treated = 2.0 \( \pm \) 0.2 nmol/mg protein). Despite these changes in AMP-degradation patterns, ATP and total adenine nucleotides were restored to the same extent in both control and adenosine-pretreated myocytes.
treated ischemic myocytes when pelleted cells were resuspended in glucose- and pyruvate-containing medium (Fig. 6). In addition to rephosphorylation of AMP and ADP, it appears that reamination of IMP and/or phosphorylation of adenosine also contributed ~2–4 nmol/mg ATP; however, it was not possible to distinguish between these two pathways.

**DISCUSSION**

**Role of purine nucleotide cycle in cardiac energy metabolism.** In skeletal muscle, purine nucleotide cycle activity increases with workload; however, there is no evidence for a similar stimulation in rat hearts subjected to a high workload (34). Nevertheless, in substrate-deprived anoxic rat hearts, adenine nucleotide degradation is diverted from adenosine formation and IMP becomes the most prominent end product (16). Furthermore, significant elevations in IMP are also observed during ischemia in adult rat (16), guinea pig (31), and rabbit hearts (30), especially in the presence of adenosine deaminase inhibitors. By contrast, adult canine and swine hearts accumulate little IMP during ischemia (9, 29). Similarly, isolated rat and chick cardiac myocytes produce considerable IMP when adenine nucleotides are acutely degraded by inhibition of glycolytic and mitochondrial pathways for ATP production (2, 8, 12, 25, 26). Furthermore, reamination of IMP contributes significantly to the resynthesis of ATP when metabolic inhibition is relieved (2, 8).

Degradation of AMP to IMP rather than to adenosine would have several advantages in heart tissue. The release of NH$_3$ when AMP is deaminated may counter H$^+$ accumulation associated with ATP hydrolysis. In contrast to the freely permeable adenosine, IMP does not readily penetrate the cell membrane and, thus, is retained in the myocardium on reperfusion and is available for ATP regeneration. Furthermore, a complete turn of the purine nucleotide cycle permits synthesis of guanine nucleotides from the larger adenylate pool and, in addition, provides fumarate, fuel for the citric acid cycle.

Inhibition of AMP deaminase in glycolyzing myocytes. In agreement with results in neonatal piglet myocytes, this laboratory has previously reported that AMP deaminase activity is reduced in nonrespiring, glycolyzing adult rat cardiac myocytes. In support of these findings are several publications (12, 26) demonstrating that AMP degradation to IMP predominates when glycolytic activity is blocked. In comparison with aerobic cells, phosphocreatine is reduced, NADH levels are increased, and phosphate and glycolytic intermediates are elevated in anoxic myocytes maintaining ATP stores through glycolysis. It is not known which products or intermediates of glycolysis inhibit AMP deaminase.

![Fig. 6. Effect of 1 mM Ado on metabolite content of piglet myocytes exposed to simulated ischemia and reperfusion. Myocytes were preincubated with no addition (Con) or 1 mM Ado for 10 min and then concentrated to 25 mg protein/ml and subjected to simulated ischemia as described in METHODS. After 45 and 60 min, an aliquot of cells was resuspended in fresh substrate-containing medium and incubated further for 25 min at 1.5 mg protein/ml (dashed lines). Values are means ± SE for 6 myocyte preparations. *P < 0.05 compared with control myocytes.](http://ajpheart.physiology.org/)

**Fig. 6.** Effect of 1 mM Ado on metabolite content of piglet myocytes exposed to simulated ischemia and reperfusion. Myocytes were preincubated with no addition (Con) or 1 mM Ado for 10 min and then concentrated to 25 mg protein/ml and subjected to simulated ischemia as described in METHODS. After 45 and 60 min, an aliquot of cells was resuspended in fresh substrate-containing medium and incubated further for 25 min at 1.5 mg protein/ml (dashed lines). Values are means ± SE for 6 myocyte preparations. *P < 0.05 compared with control myocytes.
nase in situ, although inorganic phosphate and a variety of phosphate compounds alter the kinetics of the purified enzyme in vitro (4, 23).

Modulation of AMP deaminase by PKC. This laboratory was the first to demonstrate that adult rat cardiac AMP deaminase is stimulated by $\alpha_1$-adrenergic agonists (12) and, furthermore, that enzyme activation is mediated through PKC (13). Similarly, piglet cardiac AMP deaminase activity is also increased by activation of PKC with phorbol esters. Moreover, we have evidence (10) that enzyme purified from piglet ventricles is phosphorylated by PKC. Accordingly, the maximum activity ($V_{\text{max}}$) of purified cardiac piglet AMP deaminase is increased twofold after phosphorylation. By contrast, phosphorylation of rat skeletal muscle (37) and rabbit heart (35) AMP deaminase by PKC decreases the $K_m$ but does not affect the $V_{\text{max}}$.

Effect of raising intracellular cAMP on AMP deaminase. The present data establish a positive correlation between intracellular cAMP levels and activity of AMP deaminase in subsequently deenergized piglet myocytes. This effect is partially inhibited by a blockade of protein kinase activity with staurosporine, suggesting that phosphorylation is involved. However, incubation of purified piglet cardiac AMP deaminase with PKA does not alter its kinetics (10). Furthermore, neither rabbit cardiac nor rat skeletal muscle AMP deaminase is phosphorylated by PKA (35, 37). In addition, cAMP did not directly affect purified piglet cardiac AMP deaminase activity (unpublished data). Thus the mechanism whereby increased intracellular cAMP levels enhance in situ AMP deaminase activity remains to be elucidated.

Adenosine stimulation of AMP deaminase. The present study indicates that incubation of piglet myocytes with adenosine increases IMP formation in subsequently deenergized cells. Deamination of AMP is also favored in ischemic piglet myocardium pretreated with adenosine. Activation of AMP deaminase by adenosine, the product of the competing reaction for degradation of AMP (dephosphorylation via 5'-nucleotidase), may be one mechanism whereby the cell maintains tight control over intracellular adenine nucleotide concentration. Previously we have reported (14) adenosine stimulation of AMP deaminase in adult rat heart myocytes. Although the mechanism of action of adenosine was not determined, we did rule out sarcolemmal membrane receptor mediation because nucleoside transport inhibitors prevented the increase in activity; furthermore, adenosine-receptor antagonists did not block the effect. In addition, a direct effect of adenosine on partially purified cardiac AMP deaminase was not observed (14). However, adenosine is known to act intracellularly at P-site receptors that are coupled to pertussis toxin-sensitive G proteins (15); therefore, it is likely that a second messenger, perhaps a kinase, is involved.

Effect of adenosine pretreatment on ischemic myocardium. Both adenosine (7, 18) and PKC activation (40) have been implicated in the preconditioning response of ischemic hearts. Moreover, direct application of the nucleoside or PKC activators afford protection to ischemic myocardium. Phenylephrine, an $\alpha_1$-adrenergic agonist, and adenosine, as well as preconditioning, augments recovery of developed tension in ventricular strips prepared from human and rat hearts (6). Inhibitors of adenosine deaminase also attenuate ischemic injury and enhance restoration of ATP and phosphocreatine in guinea pig hearts (31). Protective effects of adenosine during ischemia have been linked to membrane adenosine receptors in the pig (19), rabbit (3, 36), rat, and human (6). Furthermore, ATP-sensitive $K^+$ channels may also be involved in adenosine protection (38).

Although adenosine has been shown to be cardioprotective in ischemic adult myocardium (for review, see Ref. 7), its effect on neonatal tissue is less clear. Immature hearts contain less 5'-nucleotidase and are not as well protected by cardioplegia (30). Neonatal pig hearts perfused with the nucleoside transport inhibitor drafalazine have significantly reduced ischemic contracture and release of creatine kinase and improved recovery of left ventricular systolic pressure compared with control ischemic hearts (33). However, reductions in ATP and phosphocreatine at the end of 90 min of no-flow ischemia were not different. Similarly, preconditioning reduced acidosis and intracellular Na accumulation and improved intracellular Ca$^{2+}$ functional recovery in newborn rabbit hearts, yet it did not affect ischemic and posts ischemic levels of phosphocreatine and ATP (20). By contrast, in older pigs, infusion of adenosine before ischemia slowed ATP loss and accelerated its recovery on reperfusion (39), but it did not alter changes in perfusion pressure. Moreover, adult swine hearts preperfused with the nucleoside transport inhibitor R-75231 had increased interstitial adenosine levels during ischemia and reduced infarct size compared with these findings in control ischemic hearts (24). It remains to be determined whether preperfusion with adenosine would enhance recovery of myocardial performance in ischemic piglet hearts; however, our data indicate that energy stores are not favorably affected.

In summary, I have established that piglet cardiac AMP deaminase is stimulated by activating PKC, increasing intracellular cAMP, and increasing adenosine. Furthermore, pretreatment with adenosine, but not phorbol esters or $\beta$-adrenergic agonists (data not shown), promotes degradation of AMP to IMP in ischemic myocytes. However, these treatments did not alter recovery of ATP in the postischemic period.

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