Energetic effects of adenosine on vascular smooth muscle

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Barron, John T., and Liping Gu. Energetic effects of adenosine on vascular smooth muscle. Am. J. Physiol. Heart Circ. Physiol. 278: H26–H32, 2000.—Adenosine (Ado) is a naturally occurring compound that has several important cardiovascular actions, including activation of ATP-sensitive K⁺ channels in vascular smooth muscle, vasorelaxation, and an effect to alter glucose metabolism of cardiac muscle. The metabolic effects of Ado on vascular smooth muscle have not been defined and were examined in this study. Porcine carotid artery strips were incubated in the presence and absence of 0.5 mM Ado. Compared with the control, Ado had no effect on glucose uptake, glucose oxidation, or fatty acid (octanolate) oxidation. Ado suppressed glycolysis but enhanced glycogen synthesis. Relative to the rate of glycolysis, Ado increased lactate production. Ado stimulated O₂ consumption by 52 ± 10%, altered the activities of the tricarboxylic acid cycle and malate-aspartate shuttle, and increased the content of ATP, ADP, AMP, and phosphocreatine. Alteration in the metabolic variables by Ado could not be attributed to diminished energy requirements of reduced resting muscle tone of the arterial strips. Relaxation of the arterial strips in response to Ado were abolished in arteries incubated under hypoxic conditions (95% N₂-5% CO₂). Hypoxia was associated with increased ADP content. It is concluded that Ado affected glucose metabolism indirectly. The metabolic and energetic effects of 0.5 mM Ado are mediated by alterations in the concentrations of AMP, ATP, and phosphorylation potential (AMP/ADP).

metabolism: hypoxia; adenosine nucleotides; adenosine 5'-triphosphate-sensitive potassium ion channel

ADENOSINE IS A NATURALLY occurring compound that is elaborated in the myocardium in response to hypoxia and under conditions in which there is increased myocardial demand for O₂. Adenosine is principally formed on degradation of intracellular ATP when high-energy phosphate use exceeds its formation (5, 14, 30, 32). ATP is hydrolyzed to ADP and then to AMP when high-energy phosphate reserves are compromised. Through the action of 5'-nucleotidase, AMP is hydrolyzed to adenosine, which then diffuses into the interstitial space (19, 27). Adenosine may also be formed directly in the interstitial space by ecto-5'-nucleotidase catabolism of adenosine nucleotides originating from platelets and vascular endothelial cells (26). Other sources of adenosine are also possible (30). In any case, the adenosine so formed exerts several important cardiovascular actions that may be viewed as cardioprotective and compensatory mechanisms to correct the imbalance between myocardial O₂ supply and demand. These mechanisms include negative inotropic and negative chronotropic effects on the myocardium and an effect to decrease atrioventricular node conduction velocity. Perhaps the most important physiological action of adenosine is to relax arterial smooth muscle (30); the resultant decrease in resistance of the coronary vascular bed would augment coronary blood flow and increase delivery of O₂ to the myocardium, thus ameliorating the metabolic derangements caused by relative O₂ deprivation.

Direct metabolic actions of adenosine on heart muscle metabolism have also been reported (1, 16, 27). Adenosine stimulates the uptake of glucose in normoxic rat hearts by a mechanism that is independent of the action of insulin (1). The increased glucose uptake and attendant increased glycolysis has been postulated to be important in maintaining regional contractile function during regional myocardial ischemia caused by critical coronary stenosis (12). This cardioprotective effect of adenosine was shown to be independent of its vasodilatory action. These and other cardioprotective effects of adenosine on metabolic variables may also play a role in its involvement in ischemic preconditioning of the myocardium (3).

The effect of adenosine on metabolism of vascular smooth muscle, particularly glucose metabolism, has not been examined. Such studies are of interest because both contractile reactivity and vasorelaxation of vascular smooth muscle have been reported to be specifically dependent on glucose metabolism (36). Metabolic modulation of vascular smooth muscle tone may also involve modulation of the resting membrane potential, which may be partially governed by the activity of the ATP-sensitive K⁺ (KₐTP) channel (24). It is of interest that activation of KₐTP channels may be at least partially responsible for the vasorelaxation produced by adenosine (11, 24, 28). These are important considerations because of the homeostatic role of adenosine in normalizing blood flow to the myocardium and other tissues under conditions in which normal metabolic processes are compromised. Accordingly, we investigated the effect of adenosine on several metabolic variables of vascular smooth muscle under oxygenated and hypoxic conditions.

METHODS

Procurement and preparation of porcine carotid strips for study in organ baths were as described (6–10). Sufficient passive stretch was applied to the arterial strips to simulate...
100 mmHg mean arterial pressure (4, 32). The initial tension applied to each strip was ~45 g. The arterial strips were functionally denuded of endothelial cells by gentle rubbing of the intimal surfaces. Tension on the strips was monitored with force transducers. The strips were incubated in normal physiological salt solution containing (in mM) 118 NaCl, 20 NaHCO3, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.6 CaCl2, and 5.6 glucose at 37°C. The medium was aerated with a gas mixture of 95% O2-5% CO2 for 1 h, after which time the passive tension was re-adjusted, and the arteries incubated for an additional 90 min. The tension of the strips at 90 min was taken as the basal resting tension. At this point adenosine was introduced into the organ bath as were different radiolabeled substrates. The incubation continued for an additional 90 min, during which time aliquots of incubation medium were withdrawn at various times for determinations of metabolic rates. The total incubation time was 180 min. At the end of experimentation, the arteries were removed, blotted, weighed, and frozen in liquid nitrogen. In some experiments, to simulate hypoxia, the incubation medium was aerated with 95% N2-5% CO2.

Glucose uptake, glycolysis, and glucose oxidation were determined based on production of H2O from metabolism of [2-3H]glucose, [5-3H]glucose, and [6-3H]glucose, respectively. The use of these different tritiated isotopes of glucose to gauge the metabolic fate of glucose has been validated as previously reported (8).

The H2O present in aliquots of incubation medium was separated from the remaining labeled substrate using anion exchange column chromatography as previously described in detail (6). Similarly, the oxidation of fatty acid was assessed using 0.5 mM octanoate and [8-3H]octanoate.

The consumption of O2 was measured as described previously (6). Perchloric acid extracts of frozen porcine carotid arteries were prepared as described (19). The following intracellular metabolites present in the extract were measured using NAD-linked enzymatic fluorometric assays: ATP, ADP, AMP, phosphocreatine, citrate, oxaloacetate, malate, α-ketoglutarate, aspartate, glutamate, glycerol 3-phosphate (G-3-P), and dihydroxyacetone phosphate (DHAP). Glycogen in whole tissue homogenates was measured as described (20).

All chemicals and enzymes were purchased from Sigma. [2-3H]glucose and [6-3H]glucose were purchased from NEN, [5-3H]glucose from Amersham, and [8-3H]octanoate from American Radio-Chemicals (St. Louis, MO).

Statistics. When comparing means of two groups, Student’s t-test was used. One-way ANOVA followed by the Bonferroni procedure was used in comparing means of three or more groups. A P < 0.05 was significant. Unless indicated otherwise, n refers to the number of experiments; there were at least two different arteries from different animals in each experiment.

**RESULTS**

Oxygen consumption. The consumption of O2 by porcine carotid artery strips was measured in control arteries and in arteries incubated with either 0.1, 0.5, or 1.0 mM adenosine. O2 consumption in control arteries was 0.21 ± 0.03 µmol·g⁻¹·min⁻¹ (n = 4); adenosine incubation resulted in a progressive use in O2 consumption with time so that by the end of 90 min incubation O2 consumption increased by 25 ± 5, 52 ± 10, and 33 ± 8% at 0.1, 0.5, and 1.0 mM adenosine, respectively (n = 4). Adenosine at a concentration of 0.5 mM produced the largest increase in O2 consumption (P < 0.05, Fisher’s LSD multiple comparisons test), and therefore this concentration was used throughout this study. At a concentration of 0.5 mM adenosine O2 consumption was 0.33 ± 0.06 µmol·g⁻¹·min⁻¹ (n = 4, P < 0.0001), whereas the rate of O2 consumption in control arteries in the absence of adenosine was 0.21 ± 0.03 µmol·g⁻¹·min⁻¹ (n = 4, P < 0.0001).

Glucose metabolism. The uptake of glucose under normoxic conditions in the presence and absence of adenosine is shown in Fig. 1A. Glucose uptake in control arteries (8.8 ± 0.7 µmol/g at 90 min, n = 4) was not different from that in arteries treated with adenosine [9.4 ± 0.5 µmol/g at 90 min, n = 4, not significant (NS)]. Figure 1B shows the effect of adenosine on glycolysis. Adenosine suppressed glycolysis (5.4 ± 0.6 µmol/g at 90 min, n = 6, P < 0.05) compared with control (8.7 ± 0.5 µmol/g at 90 min, n = 4). The production of lactic acid (15.7 ± 1.2 µmol/g at 90 min vs. 18.11 ± 1.2 µmol/g at 90 min, n = 4, NS) and the oxidation of glucose (0.88 ± 0.004 µmol/g at 90 min vs. 0.78 ± 0.016 µmol/g at 90 min, n = 4, NS) were not different in the presence or absence of adenosine. The content of glycogen in control arteries was 5.2 ± 0.3 µmol/g (n = 14) but was significantly increased in arteries treated with adenosine (7.2 ± 0.2 µmol/g, n = 8, P < 0.04).

Fatty acid oxidation. Octanoate is a medium chain fatty acid that is readily soluble in physiological salt solutions and is readily oxidized by porcine carotid

![Graph A](image1.png)  ![Graph B](image2.png)

**Fig. 1.** Effect of adenosine on glucose uptake (A) and glycolysis (B). Adenosine concentration, 0.5 mM. Points represent means ± SE, n = 4–6 experiments. ●, Control; ○, presence of adenosine.
Arteries (6, 10). Octanoate present in the incubation medium at a concentration of 0.5 mM was oxidized at a rate of 0.63 ± 0.02 µmol/g at 90 min (n = 6) in control arteries and at 0.57 ± 0.04 µmol/g at 90 min (n = 6, NS) in the presence of adenosine. Thus adenosine had no effect on fatty acid oxidation.

Adenosine nucleotides and phosphocreatine. The tissue concentrations of ATP, ADP, AMP, and phosphocreatine (PCR) were measured in oxygenated arteries to ascertain whether the increase in O2 consumption induced by adenosine was accompanied by a change in high-energy phosphates (Table 1). Incubation of arterial strips for 90 min with adenosine resulted in significant increases in ATP, ADP, AMP, and an approximately fourfold increase in PCR. Note that although the concentration of ADP measured in perchloric acid extract represents both free ADP and ADP bound to cellular elements (e.g., actin) in vivo, it is reasonable to assume that total free ADP increased with adenosine incubation. The assumption that free ADP was increased is also supported by the fact that AMP was elevated, which has been reported to reflect an increase in free ADP (27). It is apparent that adenosine incubation caused a generalized increase in synthesis and in the size of the adenine nucleotide pool.

Tricarboxylic acid cycle and malate-aspartate shuttle. The increase in O2 consumption and marked alteration in PCR and adenine nucleotides indicated that adenosine altered the energy charge of the tissue. Therefore, the effect of adenosine on the tricarboxylic acid (TCA) cycle, the final common pathway in oxidative metabolism, was examined. Accordingly, the concentrations of metabolite intermediates of the TCA cycle and subsidiary transaminase reactions were measured in the presence and absence of adenosine, as shown in Table 2. There was a pronounced decrease in the level of citric acid, but a pronounced increase in the level of malate and a modest increase in a-ketoglutarate. Aspartate and a-ketoglutarate metabolites of the TCA cycle and a reactant of the malate-aspartate shuttle (Fig. 2) was significantly reduced. These results indicate that adenosine had a significant effect on the pathways of oxidative metabolism and mitochondrial energetics under normoxic conditions.

Because adenosine altered the concentration of the metabolites of the malate-aspartate shuttle, it was possible that the NADH redox state of the cytoplasm might be correspondingly altered. The malate-aspartate shuttle is responsible for clearing NADH-associated reducing equivalents from the cytoplasm (see Discussion). Accordingly, the NADH redox state of the cytoplasm was assessed by examining the disposition of the cytosolic G-3-P/DHAP metabolite redox couple. An increase in the concentration ratio of [G-3-P]/[DHAP] indicates an increase in the concentration of free NADH/NAD in the cytoplasm. Table 3 shows that the G-3-P/DHAP concentration ratio and attendant redox potential (Eh) was significantly increased in adenosine-treated arteries. The G-3-P/DHAP redox potential was calculated using 1.3 × 10⁻⁴ as the equilibrium constant for G-3-P dehydrogenase (8, 34).

Vasorelaxation. The reduction in resting tone of porcine carotid strips in response to adenosine under normoxic conditions in which the substrate composition of the medium was altered is given in Fig. 3. The arteries were incubated for 90 min under the conditions given in the figure, after which time they were treated with adenosine for an additional 90 min. The resting tension of the strips just before challenge with adenosine was taken as the basal tension. Table 4 gives the absolute level of resting tension on the arterial strips under different metabolic conditions before challenge with 0.5 mM adenosine. The tension on the strips after the additional 90-min exposure to adenosine was recorded. Resting tension in the control arteries had decreased 2.1 g from baseline over the additional 90-min incubation. Challenge with adenosine resulted in a significant reduction in resting tension by 5.5 g or a change of 7.6 g compared with control.
Adenosine had important effects on oxidative metabolism and mitochondrial energetics in vascular smooth muscle under normoxic conditions. Adenosine stimulated increased production of high-energy phosphates and other phosphatic metabolites (Table 2). The concentration of ATP in arterial strips subjected to passive stretch was unchanged compared with control (1.37 ± 0.07 µmol/g; n = 8, NS), in agreement with a previous study indicating that ATP levels in resting arterial smooth muscle are not reduced during periods of hypoxia (23). The maintained level of ATP was accompanied by augmented anaerobic glycolysis. Under oxygenated conditions, the rate of glycolysis was 0.10 ± 0.01 µmol·g⁻¹·min⁻¹ (n = 4) but it increased to 0.18 ± 0.01 µmol·g⁻¹·min⁻¹ (n = 4, P < 0.0001) under hypoxic conditions; anaerobic glycolysis was unchanged by adenosine (0.18 ± 0.01 µmol·g⁻¹·min⁻¹; n = 4, NS).

The basal resting tension of hypoxic arterial strips was not different from that of oxygenated strips (Table 2). Nevertheless, vasodilatory responses to adenosine were abolished, as shown in Fig. 3.

**DISCUSSION**

Adenosine had important effects on oxidative metabolism and mitochondrial energetics in vascular smooth muscle under normoxic conditions. Adenosine stimu-
Fig. 3. Response to adenosine under various metabolic and substrate conditions. Arterial strips were equilibrated in incubation medium containing different substrates, and basal resting tension was recorded after 90 min. Subsequently 0.5 mM adenosine was introduced into muscle bath, and tension was again recorded after an additional 90-min incubation. Bars are change (means ± SE; n = 8–36 arteries from different animals) in tension from the basal level after 90-min adenosine treatment. In normoxic control, arteries were allowed to equilibrate in normal medium in absence of adenosine for an additional 90 min; after which time resting tension was recorded. There was small but significant (P < 0.05) rise in tension in control arteries over 90-min period. *P < 0.05 compared with control; †P < 0.05 compared with normoxic + adenosine.

Table 4. Basal resting arterial tension under different metabolic conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Absolute Resting Tension, g</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Glucose + O2 (control)</td>
<td>27.4 ± 1.5</td>
<td>32</td>
</tr>
<tr>
<td>Glucose-free + O2</td>
<td>28.8 ± 2.0</td>
<td>15</td>
</tr>
<tr>
<td>Glucose + octanoate + O2</td>
<td>35.3 ± 1.7*</td>
<td>20</td>
</tr>
<tr>
<td>Glucose, hypoxic</td>
<td>24.0 ± 1.2</td>
<td>26</td>
</tr>
</tbody>
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Values are means ± SE, n = no. of different arteries from different animals. *P < 0.05.

lactate acid were unchanged, glycolysis was suppressed and glycogen synthesis was enhanced. The mechanism of these effects is likely related to the effects of adenosine on mitochondrial energetics. The elevated concentration of ATP caused by adenosine may exert feedback inhibition on phosphofructokinase (PFK), the rate-controlling enzyme of glycolysis. Because glucose uptake remained constant, the metabolite intermediates of the glycolytic pathway would accumulate proximal to the point of inhibition of PFK. Glucose-6-phosphate is one such intermediate, which when elevated stimulates glycolysis synthesis. In fact, it is calculated that —2–3 µmol/l less glucose traversed the glycolytic pathway in the presence of adenosine, which accounts for the increase of ~2 µmol glucosyl U/g in the content of glycogen. Thus the glucose units taken up, but not traversing the glycolytic pathway, were diverted to intracellular storage in the form of glycogen.

Despite reduction of the rate of glycolysis by adenosine, lactate production was not diminished. This implies that the rate of conversion of lactate from pyruvate was augmented. The equilibrium reaction: lactate + NAD = pyruvate + NADH (catalyzed by lactate dehydrogenase) would be displaced to the left, favoring the formation of lactate when the cytoplasmic [NADH]/[NAD] ratio is increased. The demonstration that the concentration ratio of the metabolite redox couple [G-3-P]/[DHAP] significantly increased confirms the assumption that the cytoplasmic NADH redox
potential and [NADH]/[NAD] were increased by adenosine. Previous studies have demonstrated that cytoplasmic [NADH]/[NAD] is in equilibrium with [G-3-P]/[DHAP] and with [lactate]/[pyruvate] in vascular smooth muscle (8, 9); an increase in [G-3-P]/[DHAP] indicates that [NADH]/[NAD] in the cytosol is elevated (8, 9, 25). Thus relative to the rate of glycolysis, adenosine augmented lactate production.

Another possibility that could account for at least some of the augmentation of lactate production is that adenosine itself could have been metabolized. Adenosine contains a sugar phosphate moiety which, on intracellular catabolism of adenosine, could enter the glycolytic pathway and be converted to lactate.

It is likely that alteration in the disposition of the metabolites of the TCA cycle and subsidiary reactions produced by adenosine was responsible for the increase in the cytoplasmic NADH redox potential. Several metabolite intermediates of the TCA cycle are also reactants of the malate-aspartate shuttle (Fig. 2). The malate-aspartate shuttle functions to transport and clear NADH-associated reducing equivalents from the cytoplasm into the mitochondrial matrix because NAD and NADH are impermeable to the inner mitochondrial membrane (18, 22). The shuttle operates through concerted action of several coupled metabolite translocases in the inner mitochondrial membrane, which includes the malate-citrate translocase (18) (Fig. 2). The pronounced reduction in the level of mitochondrial citrate would limit coupled exchange with malate, which is formed in the cytoplasm in one of the component reactions of the shuttle (Fig. 2). Consequently, there would be less malate available within the mitochondrial matrix to serve as a metabolite precursor to other intermediates of the TCA cycle (oxaloacetate and citrate) and, by extension, the malate-aspartate shuttle. The activity of the shuttle would be reduced, resulting in accumulation of reducing equivalents in the cytoplasm. This hypothesis is consistent with the combined observations of the markedly reduced concentration of citrate yet markedly elevated concentration of malate, a diminished concentration of aspartate, an increase in cytoplasmic NADH redox potential, and relative increase in lactate production.

Hypoxic conditions. The rate of glycolysis under hypoxic conditions was, as expected, substantially higher than that under oxygenated conditions. Adenosine had no effect on the rate of anaerobic glycolysis. This is consistent with the assertion that adenosine has no direct effect on glucose metabolism apart from its effect on oxidative metabolism. However, the vasorelaxation responses to adenosine were virtually abolished in arterial strips made hypoxic. The basal resting tone on the arterial strips under oxygenated and hypoxic conditions was the same so the decreased responsiveness to adenosine cannot be ascribed to an already diminished resting tone which would attenuate further vasorelaxation induced by adenosine. Among other mechanisms, vasorelaxation in response to adenosine is due in part to activation of \( K_{\text{ATP}} \) channels in the sarcolemma (11, 24, 28). \( K_{\text{ATP}} \) channels have been reported to be present in a variety of blood vessels and different smooth muscles (24). It is assumed that they are also present in porcine carotid artery because vasorelaxation responses to adenosine were inhibited by the specific \( K_{\text{ATP}} \) channel blocker glibenclamide. Normal or high levels of ATP promote channel closure, whereas low levels of ATP increase the probability of channel opening (2, 33). It was demonstrated that, although the ATP content of the arterial strips was not reduced during hypoxia, the content of ADP increased significantly. It is tempting to speculate that the increase in ADP played a role in the diminished responsiveness to adenosine. The role ADP plays in \( K_{\text{ATP}} \) channel opening is not clearly defined, especially with respect to the level of ATP (2, 33); however, high levels of ADP can inhibit channel opening (2). (It should be noted that the concentration of ADP in hypoxic arterial strips was elevated before challenge with adenosine.)

A decrease in intracellular \( \text{pH} \) caused by increased anaerobic glycolysis is another possible contributing factor to reduced \( K_{\text{ATP}} \) channel responsiveness (21); and the blunted responsiveness to adenosine during hypoxia may be caused by other factors independent of the status of \( K_{\text{ATP}} \) channel activity. Nevertheless, the physiological significance of this finding is that adenosine, whether formed endogenously or administered exogenously, may be a less effective vasodilator in vascular beds that have been rendered ischemic for prolonged periods.

We thank Carolynn Nichols for typing this manuscript. This work was supported by National Heart, Lung, and Blood Institute Grant HL-47329.

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Received 9 April 1999; accepted in final form 8 July 1999.

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