Partially Endothelium-Dependent Vasodilator Effect of Adenosine in Rat Aorta

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SUMMARY The vasodilator effect of adenosine on the contraction induced by phenylephrine, an \(\alpha\)-adrenergic receptor agonist, was investigated in the isolated rat aorta. We found that the effect of adenosine was greater in intact aortas than in endothelium-denuded preparations. Denuding caused a parallel shift of the dose-response curve of adenosine to the right by a factor of five in comparison with intact aorta. This finding indicates that the relaxing effect of adenosine is partially endothelium-dependent in rat aorta. The mechanism of action of adenosine on vascular smooth muscle was also investigated in receptor-mediated and voltage-dependent calcium influx experiments performed with the addition of phenylephrine and high potassium concentrations, respectively. Although adenosine significantly inhibited only the tonic phase of the contraction induced by phenylephrine (10\(^{-5}\) M), it did so to both the fast and slow phases of the contraction produced by high potassium concentrations (75 mM) with no preferential difference. In comparison to verapamil, a calcium entry blocker, adenosine behaved in a manner similar to that of verapamil in counteracting the constriction induced by either phenylephrine or potassium. We conclude that the vasodilator effect of adenosine is partially endothelium-dependent and that the mechanism of this effect may involve the inhibition of calcium influx and the release of an endothelium-derived relaxing factor.

(Hypertension 11: 514-518, 1988)

KEY WORDS  • adenosine • phenylephrine • high potassium • rat aorta

SEVERAL lines of evidence have shown that adenosine does not require the presence of endothelial cells to elicit the relaxation of isolated arteries. Furchgott and Zawadzki\(^1\) reported that the removal of endothelial cells in rabbit aorta did not interfere with its relaxation by adenosine and adenylic acid. De Mey and Vanhoutte\(^2\) confirmed these reports in canine femoral arteries. By contrast, Gordon and Martin\(^3\) found that endothelium-denuded preparations of pig aorta reduced the degree of relaxation produced by adenosine. Therefore, whether the endothelium was related to the relaxing effect of adenosine was not completely clear.

To clarify these discrepancies, a series of experiments was designed to determine if an endothelium-dependent vasodilator effect of adenosine was involved in rat aorta. In addition, the mechanism of the relaxation effect of adenosine on vascular smooth muscle was also investigated by using phenylephrine (PE) and high potassium concentrations (75 mM) in receptor-mediated and voltage-dependent calcium influx experiments, respectively.

Materials and Methods

Contractile Experiments

Adult Sprague-Dawley rats of either sex, weighing 230 to 280 g, were decapitated, and the thoracic aorta was removed and cleaned of all loosely adherent tissues. Pairs of aortic rings 2 mm long were cut from the area close to the aortic arch. The endothelium was removed from one of each pair by rubbing with a wooden stick. The rings were then mounted under a tension of 1 g between parallel hooks in a 20-ml organ bath containing a physiological salt solution, the composition of which was (mM) NaCl, 118; KCl, 4.7; NaHCO\(_3\), 25; KH\(\text{PO}_4\), 1.2; MgCl\(_2\), 1.25; CaCl\(_2\), 2.5; and glucose, 11, maintained at 37°C and bubbled with a mixture of 95% O\(_2\) and 5% CO\(_2\). The mechanical activity was recorded isometrically by means of a Grass FTO3C force-displacement transducer (Quincy, MA, USA). The signal from the transducer was displayed on a Grass Model 7D polygraph. After an equilibration period of 45 minutes before a maximal vasoconstriction was elicited with PE (10\(^{-5}\) M), carba-
cholec (10^{-5} M) was added for 5 minutes to ensure that the endothelium had been completely removed (Figure 1). This procedure was repeated two times in each preparation. Drugs were then removed from the chambers by several washes with physiological salt solution, and the tension was allowed to return to baseline. Tissues were allowed to reequilibrate for 30 minutes after the drug response to ensure maximum washout of the drug and to minimize the possibility of receptor desensitization.

An isotonic high potassium solution (KKS) containing 75 mM K+ solution was prepared by substituting 70 mM NaCl with an equimolar amount of KCl. This concentration of KKS was shown to induce a maximum contraction in the rat aortic ring. After an equilibration period of 20 minutes, the solution was removed from the chambers by several washes with physiological salt solution and the tension was allowed to return to baseline.

Tension was induced by PE (10^{-5} M) or KKS (75 mM K+) in intact aortic rings and rings devoid of endothelium, and cumulative dose-response curves were constructed for adenosine. The influence of vasodilators of various concentrations on the contractile response was determined by preincubating aortas with these drugs for 5 minutes before and during exposure to 10^{-5} M PE or KKS (75 mM) solution.

**Determination of Fast and Slow Responses**

The initial rapid portion of the contractile response, which was linear with time, was defined as the fast component, as described by Bohr and Hurwitz et al. The duration of the fast component in response to PE or KKS was about 20 seconds. The remaining portion of the response was designated as the slow component and was 95% complete after 5 minutes.

**Drugs**

PE HCl, carbachol, carbamylcholine chloride (carbachol), and adenosine hemisulfate were purchased from Sigma Chemical (St. Louis, MO, USA). Verapamil was supplied by the manufacturer (Knoll AG, Ludwigshafen, FRG).

**Data Analysis**

The contractile force was expressed as milligrams of force per milligram of tissue weight. Data were expressed as means ± SE. Tests of significance were performed using Student's t test. Values of p < 0.05 and p < 0.01 were accepted as significant differences. The concentration of an antagonist producing 50% of the maximal response for that agonist (EC50 value) was estimated from its concentration-effect curve.

**Results**

**Partially Endothelium-Dependent Relaxing Effect of Adenosine in Rat Aorta**

Ring segments of aorta, with and without the presence of endothelium, were induced to contract with PE (10^{-6} M). Adenosine (10^{-5}-3 \times 10^{-3} M), added cumulatively, relaxed both types of aortic rings in a dose-dependent manner (Figure 2A). Figure 2B shows the dose-response curves for the action of adenosine in both preparations. The maximum relaxation of PE-induced tone was 85 ± 3%. In preparations devoid of endothelium, the EC50 value of adenosine on the inhibition of contractions induced by PE was about five-fold higher than that determined in intact preparations.

**Effect of Adenosine on the Biphasic Response Induced by Phenyphrine**

As shown in Figure 3, contractions in response to 10^{-5} M PE were separated into phasic and tonic phases (fast and slow components, respectively), and the effect of increasing concentrations of adenosine on each phase of contraction was determined. Attenuation of the contractile responses by adenosine (10^{-4}-10^{-3} M) pretreatment was due primarily to inhibition of the tonic (slow) phase of contraction induced by PE. The phasic (fast) phase was not significantly inhibited except at 10^{-3} M adenosine (p < 0.05), whereas the tonic phase was attenuated completely by the same concentration of adenosine (p < 0.01).

**Effect of Adenosine on the Biphasic Response to Potassium Depolarization**

Concentration-response curves for the inhibitory effect of adenosine on fast and slow phases in the isolated rat aortas with endothelium are shown in Figure 4. Both the fast and the slow phases of the contractile response induced by high potassium concentrations (75 mM) were attenuated by adenosine (10^{-4}-2 \times 10^{-3} M) pretreatment. The concentration of adenosine required to inhibit the slow phase of high potassium contraction was about 5 \times 10^{-4} M (p < 0.05). At the same concentration, there was no significant inhibition of the fast phase except at 2 \times 10^{-3} M (p < 0.05). However, there was no detectable significant interaction between the type of response (slow or fast) and the dose of adenosine as analyzed by two-factor analysis of variance.

**Effect of Verapamil on Contractile Response Induced by Phenylephrine and High Potassium Concentrations**

Figure 5A illustrates that verapamil, a calcium entry blocker, completely attenuated the tonic phase of the contractile response induced by PE. Furthermore, both...
FIGURE 2. Comparison of the dose-response relationship to adenosine between an intact ring (+ EC) and a ring devoid of endothelium (− EC). A. When tone had stabilized, a cumulative dose-response relationship was established beginning at $5 \times 10^{-2}$ M and increasing to $2 \times 10^{-3}$ M. The integers 5, 4, and 3 mark the negative values of the logarithmic concentration (molar) for adenosine. To the right of each marking are the multiples of said concentration. Thus, $5 \times$ between 5 and 4 denotes a concentration of $5 \times 10^{-5}$ M; whereas the same $5 \times$ between 4 and 3 signifies $5 \times 10^{-4}$ M. B. Dose-response relationship showing the effect of adenosine in aortic rings with (•) and without (△) endothelium present. Values are means ± SE. Double ($p < 0.01$) asterisk indicates significant differences in the relaxation by adenosine between a pair of control and denuded preparations.

phases of contractions induced by high potassium concentrations were abolished by verapamil ($5 \times 10^{-5}$ M; Figure 5B). Based on this evidence, the relaxing effect of adenosine, which is similar to that of verapamil, may be the blocking of extracellular calcium influx.

Discussion

There are contradictory reports about the role of vascular endothelium in the response of arteries to adenosine. On the one hand, for rabbit aorta and dog femoral artery, the concentration-dependent relaxation by adenosine 5'-monophosphate (AMP) and adenosine ($10^{-3}$ to $10^{-5}$ M) was not affected by the removal of endothelial cells.$^{1,2}$ On the other hand, in pig aorta, a major part of the relaxation by AMP and adenosine was reported to be endothelium-dependent.$^3$ In the present study, we observed a third possibility, that of a partial dependence on endothelium, in rat aorta. As shown in Figure 2, the vasodilator effect of adenosine was greater in intact aortas than in preparations devoid of endothelium preparations. Furthermore, the concentration of adenosine required to induce relaxation in an intact aorta was lower than that needed for denuded preparations (see Figure 2). These results showed that the vasodilator effect of adenosine was partially endothelium-dependent. These discrepancies may reflect species differences.

We also found (see Figure 2) that the baseline ten-
sion after PE in denuded rings was much greater than that in intact rings in about 80% of preparations. This finding was in agreement with several other studies using isolated arterial preparations in which endothelium removal was found to enhance the vasoconstrictor response to norepinephrine and other α-adrenergic receptor agonists. This increased potency of PE after endothelium removal has been attributed to loss of endothelium-derived relaxing factor (EDRF). However, can this change in the response to PE in denuded preparation itself alter the dose-response curve to adenosine? The effect of vasodilators on the dilation of vascular smooth muscle is dependent on vascular tonicity. Generally, the greater the constriction, the greater the dilation, unless the vascular tone reaches the maximum. This was true in our studies of vasodilation involving nitroglycerin (unpublished data, 1987). The concentration of PE used in our experiments was below the maximum. Therefore, although the removal of the endothelium could alter the aorta's response to PE, this factor might not shift its dose-response curve to adenosine. In fact, the curve might remain the same.

The mechanism for this partial dependency on endothelium for the vasodilator action is still not clear. Our data are consistent with the notion that adenosine may release an EDRF, which relaxes the smooth muscle. It is also possible that EDRF itself is a modulator that regulates the ability of adenosine to vasodilate. These two possible effects need to be clarified.

The subdivision of postjunctional α-adrenergic receptors into α₁-adrenergic and α₂-adrenergic subtypes is now generally accepted. In the present study PE, an α₁-adrenergic agonist, was used to induce contraction in intact rat aorta; this system was used as a model to investigate the mechanism of adenosine-induced vasodilation. Activation of α₁-adrenergic receptors by PE produces contraction through increased cytoplasmic concentration of calcium. The nature of the contractile response is biphasic, consisting of a fast and a slow component. The fast component (phasic phase) of the contraction is due to the mobilization of intracellular calcium, whereas the slow component (tonic phase) is directly dependent on an influx of extracellular calcium. As shown in Figure 3, adenosine selectively attenuated the tonic phase more than the phasic phase. In other words, the relaxing effect of adenosine may predominantly prevent the influx of extracellular calcium rather than inhibit the mobilization of intracellular calcium.

The contraction in response to high potassium concentrations was also biphasic. Initially, a rapid, highly transitory increase in tension (fast phase) developed and was followed by a second, slower increase in tension (slow phase). Hurwitz et al. and Hogestatt and Andersson suggested that the underlying basis for the development of a separate fast phase and a slow phase of the mechanical response was the functional operation of two distinctly different types of calcium channels in the plasma membranes of smooth muscle. As shown in Figure 4, adenosine attenuated both the fast and slow phases of the contractile response in the presence of high potassium. There was no significant interaction between the type of response (slow and fast) and the dose of adenosine. Thus, our data showed that a possible mechanism for the relaxing effect of adenosine on vascular smooth muscle is the inhibition of calcium influx.

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** Effect of increasing concentrations of adenosine on the fast (A) and slow (B) phases of high potassium-induced contractions. The contractile force is expressed as milligrams of tension per milligram of tissue. Vertical lines represent SEM (n = 8). Single (p<0.05) and double (p<0.01) asterisks indicate significant differences between responses in the absence and presence of adenosine. The concentration of potassium was 75 mM.

![Figure 5](http://hyper.ahajournals.org/)

**Figure 5.** Typical illustrations of contractile responses in rat aortas induced by (A) phenylephrine (PE; 10⁻⁵ M) and (B) KCl (75 mM). The left panel (control) illustrates responses in the absence of verapamil, and the right panel illustrates responses in the presence of verapamil (5.5 × 10⁻⁵ M).
In conclusion, the present study demonstrated that the relaxing effect of adenosine in rat aorta is partially endothelium-dependent. Adenosine significantly attenuated contractions induced by both PE and high potassium concentrations. The results of this study suggest that the mechanism of the vasodilator effect of adenosine may be due to both inhibition of calcium influx and the release of EDRF.

References
Partially endothelium-dependent vasodilator effect of adenosine in rat aorta.
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Hypertension. 1988;11:514-518
doi: 10.1161/01.HYP.11.6.514

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