Intracoronary Administration of Adenosine Triphosphate Increases Myocardial Adenosine Levels and Coronary Blood Flow in Man

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Adenosine triphosphate (ATP) is reported to be released mainly from presynaptic vesicles and cardiomyocytes. The released ATP, which can be degraded to adenosine, may cause coronary vasodilation. However, there is no clear evidence that ATP is degraded to adenosine and causes coronary vasodilation in humans. The present study was undertaken to test whether intracoronary administration of ATP increases myocardial adenosine levels and coronary blood flow. In 11 patients, 3 doses of ATP (0.1, 0.2, and 0.4 mg) were injected into the left anterior descending coronary artery. The velocity of coronary blood flow was measured by Doppler flow probe, and the adenosine concentration in the coronary sinus blood was measured. We also continuously infused ATP (0.2 mg/min) for 1 min in another 10 patients. Coronary blood flow increased dose dependently soon after injection of ATP. Coronary arteriovenous differences in adenosine concentration increased [from 21±15 to 178±15 pmol/ml (p<0.05) 10 sec after the injection of ATP (0.4 mg)] and there were marked reductions in both aortic blood pressure and heart rate. The adenosine levels returned to baseline 20 sec after the injection of ATP, and aortic blood pressure and heart rate also recovered, although coronary blood flow remained increased. Furthermore, continuous infusion of ATP for 1 min increased coronary blood flow velocity and coronary arteriovenous differences in adenosine concentration from 25±14 to 71±13 pmol/ml (p<0.05) in 10 patients. These results indicate that intracoronary administration of ATP immediately increases coronary blood flow and the adenosine concentration of coronary venous blood, which returns to the baseline level thereafter. The differences in the time courses of increases in coronary venous adenosine levels and coronary blood flow after ATP injections suggest that vasodilatory mechanisms other than adenosine, eg, nitric oxide and prostaglandins, may also be involved in the ATP-induced coronary vasodilation. ATP may be used as a cardioprotective agent as well as adenosine.

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Adenosine triphosphate (ATP) is reported to be released mainly from presynaptic vesicles and cardiomyocytes. Intracoronary and intravenous administration of ATP may activate adenosine receptors via adenosine formed from the breakdown ATP, and directly activates P2y receptors. Both actions of ATP may contribute to coronary vasodilation. In patients with coronary artery diseases, it is reported that intracoronary administration of ATP causes coronary vasodilation, which is useful for the assessment of the coronary flow reserve. If intracoronary ATP administration increases adenosine levels in the heart, it may be a useful treatment for ischemic heart diseases. This is because adenosine attenuates the severity of myocardial ischemia and stunning, and limits infarct size. Indeed, adenosine has recently been recognized as an important mediator of the infarct size-limiting effect of ischemic preconditioning. However, there has been no trial to measure plasma adenosine concentration in coronary venous blood during intracoronary infusion of ATP in human hearts. This study was therefore undertaken to examine...
whether intracoronary ATP administration increases plasma adenosine concentration in coronary venous blood as well as coronary blood flow in human hearts.

Methods

Patients

This study involved 21 patients [17 men and 4 women; average age 57 years (range 41–71 years)] undergoing cardiac catheterization and coronary angiography for evaluation of coronary heart disease. Patients with unstable angina, acute myocardial infarction, or coronary heart disease with severe stenosis of the left main coronary artery were excluded. Eleven patients underwent protocol 1 for evaluation of the effect of a bolus injection of ATP. To avoid the marked systemic hypotension and bradycardia caused by a bolus injection of ATP (Adephos-L Kowa Injection, Kowa), 10 patients in protocol 2 also underwent a continuous infusion of ATP. Each patient signed a consent form for this study, which was approved by the institution review board.

Study Protocol

The patients were brought to the catheterization laboratory in a fasting state after premedication with 50 mg of hydroxyzin. Cardiac medications were withheld on the morning of the procedure. Standard left and right heart catheterizations, left ventriculography, and coronary angiography were performed using Judkin's technique in each patient. After the diagnostic cardiac catheterization, the NIH catheter (2F) was inserted into the coronary sinus for the sampling of coronary venous blood, and the pacing catheter (7F) was also positioned in the right ventricle for temporal pacing. The position of the NIH catheter was determined by an injection of 2–3 ml of the contrast medium (Iopamiron, Shering, Berlin, Germany), and was verified by repeated fluoroscopic examinations. Back-up demand pacing was prepared in case of decreased heart rate below 40 beats/min.

In protocol 1, ATP was diluted with saline to adjust the final concentration of 0.2 mg/ml. Three doses of ATP (0.1, 0.2, and 0.4 mg) were randomly administered by bolus intracoronary injection at 5-min intervals in each patient. Blood from the aorta and the coronary sinus were sampled simultaneously for blood gas analysis and the measurement of adenosine concentrations before and after ATP administration in these patients. In 6 and 5 patients, blood was sampled 10 and 20 sec after the injections of ATP, respectively. Arterial blood pressure and coronary blood flow velocity were measured and 12-lead electrocardiograms were recorded 5, 10, 20, 30, and 60 sec after the ATP injection.

In protocol 2, 0.2 mg of ATP diluted with 10 ml of saline was administered by 1-min intracoronary infusion (the injection rate, 10 ml/min). Blood from the aorta and the coronary sinus for the measurement of adenosine concentration was sampled before the ATP infusion, 20 and 40 sec after the onset of ATP administration, and 2 min after the discontinuation of ATP infusion. Arterial blood pressure and coronary blood flow velocity were measured and 12-lead electrocardiograms were recorded at the same time as the blood sampling.

Measurements of Coronary Blood Flow Velocity

Coronary blood flow velocity was measured with a 0.018-inch Doppler angioplasty guide wire (Flowire, Cariometrics, Mountain View, CA, USA). As previously described and validated by Doucette et al., the Doppler angioplasty guide wire was a 175-cm-long, 0.014-inch-diameter flexible steerable guide wire with a 12-MHz piezoelectric ultrasound transducer integrated into the tip. The forward-directed ultrasound beam diverged in a 27° arc from the long axis, and coronary blood flow velocity was measured at the 6-decibel round-tip points of the ultrasound beam pattern. We set a pulse repetition frequency of >40 kHz, pulse duration of 0.83 msec, and sampling delay of 6.5 msec for the standard for clinical use. The system was coupled to a real-time spectrum analyzer, a videocassette recorder, and a video page printer. The quadrant Doppler audio signals were processed by the spectrum analyzer using the on-line fast Fourier transformation to provide a scrolling gray-scale spectral display. The frequency response of the system calculated approximately 90 spectra per second. Mean coronary blood flow velocity (CBFV) was defined as the average of peak coronary blood flow velocity in 1 cardiac cycle.

Measurements of Myocardial Metabolites

Oxygen differences in coronary arterial and venous blood (AVO₂D) were assessed by the difference between coronary arterial and venous oxygen contents.

The method used for adenosine measurement has been reported previously. Briefly, 1 ml of blood was drawn into a syringe containing 0.5 ml of dipyridamole (0.02%), 100 ml of of 2'-deoxycoformycin (0.1 mg/ml), and EDTA (500 mmol/L) to block both uptake of adenosine by red blood cells and degradation of adenosine. Without these chemicals, adenosine levels become undetectable (<6.0 pmol/ml). After centrifugation, the supernatant was obtained and the
adenosine content was determined by radioimmunoassay. Adenosine in the plasma (100 ml) was succinylated by 100 ml of dioxane containing succinic acid anhydride and triethylamine. After a 20-min incubation, the mixture was diluted with 100 ml of adenosine 2', 3'-O-disuccinyl-3-[125I]iodotyrosine methyl ester (0.5 pmol) and 100 ml of diluted anti-adenosine serum. The mixture was kept in a cold water (4°C) bath for 18 h, and a second antibody solution (goat anti-rabbit IgG antiserum, 500 ml) was added. After incubation at 4°C for 1 h, unreacted materials were removed by centrifugation at 2,500 g at 4°C for 20 min. The radioactivity remaining in the tube was counted by a gamma counter. The amount of adenosine degradation during this blood sampling procedure has been reported to be negligible.\textsuperscript{13,14}

**Statistical Analysis**
Statistical analysis was performed by ANOVA. When the analysis using ANOVA reached significance levels, we obtained significant levels for each of paired data using the Bonferroni test.\textsuperscript{15} The changes in time courses in hemodynamic parameters and adenosine concentrations were tested by repeated-measures ANOVA. All values were expressed as means±SEM, and p<0.05 was considered significant.

**Results**

**Systemic and Coronary Hemodynamics**
Bolus intracoronary administrations (protocol 1) of ATP decreased heart rate (control: 78±4, 80±3, 79±3/min; peak: 60±4, 52±4 (p<0.05), 47±4/min (p<0.05) at 0.1, 0.2, and 0.4 mg of ATP, respectively) and systolic and diastolic aortic blood pressure (control: 108±7/62±3, 111±6/65±3, 108±5/63±3 mmHg; peak: 85±6/48±3 (p<0.05), 78±7/44±3 (p<0.05), 67±5/36±2 mmHg (p<0.05) at 0.1, 0.2, and 0.4 mg of ATP, respectively). CBFV was markedly increased (control, 57±15, 53±13, 56±14 cm/min; peak, 97±22 (p<0.05), 90±18 (p<0.05), 90±17 cm/min (p<0.05) at 0.1, 0.2, and 0.4 mg of ATP, respectively). The percent changes in mean aortic pressure and CBFV following injections of ATP are shown in Fig 1.

Continuous intracoronary administration of ATP (protocol 2) decreased neither heart rate (control, 78±7; 20 sec, 78±6; and 40 sec, 79±7/min) nor systolic and diastolic blood pressure (control, 140±8/80±5; 20 sec, 136±8/71±5; and 40 sec, 136±8/72±5 mmHg). The changes in hemodynamic parameters were markedly attenuated (p<0.05) compared with those in protocol 1. CBFV [control, 26±4; 20 sec, 51±5 (p<0.05); 40 sec, 50±5 cm/sec (p<0.05)] increased significantly. The serial changes in mean aortic pressure and CBFV are shown in Fig 2.

**AVO\textsubscript{2}D and Plasma Adenosine Concentration**
Intracoronary bolus administration of ATP (protocol 1) decreased AVO\textsubscript{2}D [control: 9.1±1.9, 9.1±2.0, 9.5±1.2 ml/dl; after: 6.1±1.8 (p<0.05), 5.4±1.6 (p<0.05), 5.9±2.0 ml/dl (p<0.05) at 0.1, 0.2 and 0.4 mg of ATP, respectively]. Fig 3 shows coronary arteriovenous differences in adenosine concentration ([AVD(Ado)]. When the blood was sampled 10 sec after intracoronary administration of 0.1, 0.2, and...
Intracoronary Administration of ATP

![Graph showing percent changes in mean aortic blood pressure (mean AoP) and coronary blood flow velocity (CBFV) as a result of an intracoronary continuous infusion of ATP for 1 min. During administration of ATP, CBFV increased significantly without remarkable change in mean AoP.]

Fig 2. Percent changes in mean aortic blood pressure (mean AoP) and coronary blood flow velocity (CBFV) as a result of an intracoronary continuous infusion of ATP for 1 min. During administration of ATP, CBFV increased significantly without remarkable change in mean AoP.

![Graph showing changes in coronary arteriovenous differences in adenosine concentration [AVD(Ado)] before and after administration of ATP. AVD(Ado) increased 10–20 sec after intracoronary injection of ATP and returned to baseline in 20–30 sec.]

Fig 3. Changes in coronary arteriovenous differences in adenosine concentration [AVD(Ado)] before and after administration of ATP. AVD(Ado) increased 10–20 sec after intracoronary injection of ATP and returned to baseline in 20–30 sec.

0.4 mg of ATP, AVD(Ado) increased. AVD(Ado) returned to baseline when blood was sampled 20 sec after intracoronary administration of ATP. Adenosine concentrations in aortic blood before and 10 sec after the injections of ATP were 50.3±14.8 and 50.5±11.6 pmol/ml (0.1 mg ATP), 58.0±13.4 and 56.8±10.5 pmol/ml (0.2 mg ATP), and 57.7±1 5.5 and 47.7±12.0 pmol/ml (0.4 mg ATP), respectively. Furthermore, adenosine concentrations in aortic blood before and 20 sec after the injections of ATP were 41.8±6.7 and 55.0±2 6.1 pmol/ml (0.1 mg ATP), 46.2±1 9.0 and 54.8±16.4 pmol/ml (0.2 mg ATP), and 53.2±15.8
and 41.2 ± 15.9 pmol/ml (0.4 mg ATP), respectively. There were no significant differences in the adenosine concentrations in aortic blood throughout protocol 1.

Fig 4 shows coronary arteriovenous differences in AVD(Ado) during continuous intracoronary administration of 0.2 mg/min ATP for 1 min (protocol 2). When the blood was sampled during intracoronary administration of ATP, AVD(Ado) was significantly increased [control, 24.6 ± 13.6; 20 sec, 69.0 ± 20 (p < 0.05); 40 sec, 70.5 ± 13.1 pmol/ml (p < 0.05)]. AVD(Ado) returned to the baseline levels (14.0 ± 5.1) when blood was sampled 2 min after the discontinuation of intracoronary administration of ATP.

Discussion

The present study revealed that intracoronary injection of ATP increases the adenosine concentration of coronary venous blood as well as coronary flow velocity. This observation suggests that the coronary vasodilatation resulting from ATP injection may be at least partly attributable to the ATP breakdown product, adenosine.

**ATP and Coronary Circulation: Role of Adenosine**

Belardinelli et al. showed that 5 μmol of ATP administered into the coronary artery was almost completely degraded during a single passage through the isolated perfused guinea pig heart, and only 2.5% of the infused ATP was recovered in the effluent as ATP. This observation is consonant with previous reports showing that 97—99% of infused ATP is hydrolyzed during a single passage through the coronary vasculature. Indeed, Roca-Tesoni and Borghini showed, using the isolated perfused rat heart, that infusions of 5 μmol of ATP, ADP, and AMP increase the concentrations of adenosine in the effluent to the same extent. However, there are no reports investigating how much adenosine is produced as a result of intracoronary ATP administration in human. Here, we have shown that ATP administration increases coronary venous adenosine concentrations dose dependently.

In the present study, a marked increase in the plasma adenosine concentration of coronary venous blood
was observed 10 sec after intracoronary administration of ATP, and returned to baseline levels 20 sec after injection. The peak concentration of adenosine after ATP injection may be higher than we found, because (1) we did not sample coronary sinus blood immediately after injection of ATP owing to technical limitations and (2) adenosine in the blood may be degraded in the sampling catheter. The time courses of changes in adenosine concentrations after injection of ATP were not necessarily correlated with the time courses of coronary hyperemic flow. Indeed, increases in coronary blood flow velocity were still observed 30 sec after the injection of ATP, although adenosine concentrations in the coronary venous blood had returned to the baseline level. One explanation for this discrepancy is that factors other than adenosine are involved in coronary vasodilation as a result of ATP injections. Indeed, ATP activates Pγ receptors, which increases the production of NO and prostaglandins. These chemical mediators may be involved in the hyperemic flow in the late phase (20–60 sec). Furthermore, although increases in the doses of ATP increased adenosine concentration in coronary venous blood, increases in coronary blood flow velocity were maximal at 0.1 mg of ATP. This may suggest that (1) an increase in adenosine concentration sufficient to cause maximal coronary vasodilation is achieved even at a dose of 0.1 mg of ATP and (2) factors other than adenosine are involved in coronary vasodilation during infusion of ATP. Indeed, we have shown in preliminary studies using canine heart that coronary hyperemic flow as a result of intracoronary and intravenous administration of ATP is attributable to adenosine and NO.

ATP and Systemic Hemodynamics

It has long been recognized that ATP induces negative chronotropic and inotropic effects. The negative chronotropic action of ATP appears to be similar to that of adenosine. Indeed, administration of 3 doses of ATP in protocol 1 decreased heart rate, which corresponds well to previous experimental observations. Indeed, administration of ATP has been used clinically for the management of supraventricular tachycardia. ATP also has negative inotropic effects in the rat ventricle. In the present study, aortic blood pressure was decreased as a result of the administration of ATP. This may be attributable to decreases in myocardial contraction and decreases in systemic vascular resistance. Although we observed transient systemic vascular effects caused by intracoronary injections of ATP, fortunately no severe complication was observed in the present study. Furthermore, the continuous infusion of ATP caused only slight changes in systemic cardiovascular effects with marked increases in adenosine concentration in coronary venous blood. This result suggests that increases in coronary venous adenosine are attributable not to systemic cardiovascular effects, eg, systemic hypotension, but to the degradation of ATP. A continuous administration of ATP may be more appropriate in order to elevate adenosine levels in the heart without causing marked changes in systemic cardiovascular effects.

Clinical Implications

Timely reperfusion of the ischemic myocardium is thought to be essential for the treatment of acute myocardial infarction. However, reperfusion injury has been postulated to attenuate the beneficial effects of reperfusion. On the other hand, adenosine is reported to attenuate ischemic and reperfusion injury. Olafsson et al demonstrated that an intracoronary administration of adenosine markedly decreases infarct size in the canine model subjected to 90 min of regional ischemia. The present study clearly demonstrates that intracoronary administration of ATP markedly increases the differences in the adenosine levels between coronary venous and arterial blood without causing any decrease in heart rate and blood pressure. Thus, ATP administration may attenuate ischemia and reperfusion injury. Furthermore, administration of ATP may increase NO production in the heart. As NO as well as adenosine contributes to cardioprotection, intracoronary administration of ATP may be suitable for treatment of ischemic heart disease, although further clinical investigations are necessary.

References

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