Characterization of ATP-Induced Vasodilation in the Human Forearm Vascular Bed

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Background Animal data indicate that ATP derived from aggregating thrombocytes or endothelium induces an endothelium-dependent vasodilator response that is mediated by P2Y-purinergic receptors and is reduced when high dosages are administered. This reduced vasodilator response to high ATP doses has been associated with the concomitant release of endothelium-derived contracting factors. In contrast to the endothelium-dependent vasodilator response, ATP as released from sympathetic nerve endings induces a P2X-purinergic receptor-mediated vasoconstrictor response that may contribute to the attenuated vasodilator response to high dosages of luminaly applied ATP. The dual action of ATP might be important in the pathophysiology of disease states characterized by an impaired endothelial function and increased thrombocyte aggregation. This study was performed to characterize the vascular response to ATP in humans.

Methods and Results The brachial artery was cannulated in 50 healthy male volunteers (age, 18 to 44 years) for drug infusion and measurement of mean arterial pressure. Forearm blood flow was recorded by venous occlusion strain-gauge plethysmography. ATP induced a dose-dependent vasodilator response that was significantly higher than the effect of equimolar adenosine infusion and that was not reduced by concomitant infusion of the P2X-purinergic receptor antagonist theophylline. The infusion of the NO synthase antagonist Nω-monomethyl-L-arginine (L-NMMA) reduced the averaged fall in forearm vascular resistance (FVR) to acetylcholine (−59±6% [mean±SEM] versus −42±8%; P<.05; N=10) but did not affect the vasodilator response to ATP (−68±3% versus −64±6%; P>.1; N=10) or sodium nitroprusside (SNP; −53±3% versus −49±4%; P>.1; N=6). The L-NMMA-induced increase in FVR appeared to be related to the type of vasodilator pretreatment, being 94.7±16.7%, 44.9±8.7%, and 40.8±7.3% for acetylcholine, ATP, and SNP pretreatment, respectively (P<.01 for acetylcholine versus ATP and SNP; P>.1 for ATP versus SNP). In contrast to animal data, high dosages of intra-arterially infused ATP (up to 1000 μg·100 mL forearm−1·min−1) did not reveal a reduction in the forearm vasodilator response but appeared to be similar to the maximal forearm vasodilation as observed during postocclusi-ve reactive hyperemia.

Conclusions These observations indicate that ATP induces a potent dose-dependent vasodilator response that is not mediated by P1-purinergic receptor stimulation or by the release of nitric oxide. Moreover, in healthy volunteers, the vasodilator response to high intra-arterial dosages of ATP is not reduced by the release of endothelium-derived contracting factors or by the stimulation of P2X-purinergic receptors on the smooth muscle cells. (Circulation. 1994;90:1891-1898.)

Key Words • adenosine • phosphates • nitric oxide • receptors, purinergic • pharmacology

At the end of the 19th century, Heidenhain was probably the first to show that endogenous compounds, as extracted from freshly prepared organs, can elicit circulatory effects such as vasodilation and a fall in blood pressure (BP) when infused intravenously in animals.1 In 1929, Drury and Szent-Györgyi2 recognized the importance of endogenous nucleotides in this response. Their impressive early report on the pharmacology of nucleotides in rats is considered the beginning of modern research on purine pharmacology.3 After a detailed review of the existing literature and their own observations, Burnstock et al4 proposed a subdivision of purinergic receptors. The major subdivision is between P1-purinergic receptors, with an endogenous agonist potency order of adenosine > adenosine-5'-monophosphate (AMP) > adenosine-5'-diphosphate (ADP) > adenosine-5'-triphosphate (ATP) and P2-purinergic receptors, with an endogenous agonist potency order of ATP > ADP > AMP > adenosine. Based on a different potency order of synthesized adenosine analogues and divergent effects on intracellular cAMP levels, an important second messenger of P2-purinergic receptor stimulation, P2-purinergic receptors are subdivided into A1- and A2-adenosine receptors.4 In the vascular wall, A2-adenosine receptors, located on endothelial and smooth muscle cells, are involved in the vasodilator response to adenosine.5 On sympathetic nerve endings, stimulation of the A1-adenosine receptor results in a reduced release of norepinephrine.6 Methylxanthines like theophylline and caffeine are competitive antagonists on both P1-purinergic receptor subtypes.7,9 Recently, this subdivision has been confirmed by molecular techniques.10 Largely on the basis of the rank order of agonist potency of structural analogues of ATP, two subtypes of the P2-purinergic receptor are generally accepted: the P2x- and the P2y-purinergic receptors.11-13 Under physiological circumstances, ATP is coreleased with norepinephrine from sympathetic nerve endings.14 The subsequent stimulation of P2x-purinergic receptors on vascular smooth muscle cells is thought to contribute to the sympathetic nervous system–mediated vasoconstriction.14 Additionally, ATP is released from endothelial cells and aggregating thrombocytes, resulting in a P2y-purinergic receptor–mediated endothelium-
TABLE 1. Baseline Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Total Group</th>
<th>Time-Control Study</th>
<th>ATP + Theophylline</th>
<th>ATP + L-NMMA</th>
<th>ACh + L-NMMA</th>
<th>SNP + L-NMMA</th>
<th>High-Dosed ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>50</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>12</td>
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<tr>
<td>Age, y</td>
<td>27.7±8.0</td>
<td>26.0±7.9</td>
<td>25.0±8.7</td>
<td>31.1±7.6</td>
<td>28.7±8.2</td>
<td>29.2±9.8</td>
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</tr>
<tr>
<td>Weight, kg</td>
<td>75.8±8.3</td>
<td>74.4±8.8</td>
<td>72.4±11.0</td>
<td>75.8±7.0</td>
<td>77.4±9.1</td>
<td>78.1±4.4</td>
<td>75.8±9.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>182.6±6.6</td>
<td>178.9±4.7</td>
<td>184.9±6.6</td>
<td>181.4±5.5</td>
<td>183.9±5.8</td>
<td>182.2±9.6</td>
<td>183.4±7.4</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.8±2.3</td>
<td>23.3±3.1</td>
<td>21.1±1.9</td>
<td>23.1±2.02</td>
<td>22.9±2.9</td>
<td>23.6±1.6</td>
<td>22.5±2.1</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hgt</td>
<td>127.0±8.0</td>
<td>124.2±11.0</td>
<td>128.0±12.2</td>
<td>126.8±7.7</td>
<td>126.5±5.0</td>
<td>131.3±5.9</td>
<td>126.2±8.0</td>
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<tr>
<td>Diastolic blood pressure, mm Hgt</td>
<td>68.2±9.7</td>
<td>69.0±9.5</td>
<td>69.5±13.5</td>
<td>69.0±8.0</td>
<td>67.7±10.1</td>
<td>68.0±10.4</td>
<td>66.8±10.3</td>
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<tr>
<td>Heart rate, bpm†</td>
<td>65.2±10.0</td>
<td>63.3±10.6</td>
<td>67.3±8.6</td>
<td>56.4±9.1*</td>
<td>69.6±8.7</td>
<td>71.0±12.4</td>
<td>65.7±7.1</td>
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<tr>
<td>Forearm vascular resistance, AU</td>
<td>58±34</td>
<td>48±16</td>
<td>47±28</td>
<td>43±18</td>
<td>62±44</td>
<td>49±7</td>
<td>73±32</td>
</tr>
<tr>
<td>Forearm volume, L</td>
<td>1.10±0.14</td>
<td>1.03±0.15</td>
<td>1.08±0.17</td>
<td>1.08±0.14</td>
<td>1.11±0.15</td>
<td>1.18±0.16</td>
<td>1.10±0.12</td>
</tr>
</tbody>
</table>

L-NMMA indicates N⁵-monomethyl-L-arginine; ACh, acetylcholine; SNP, sodium nitroprusside; bpm, beats per minute; and AU, arbitrary units. Values are mean±SD.

*P<.05 for comparison with the other L-NMMA groups, the ATP + theophylline group, and the high-dosed ATP group.
†Blood pressure was measured in the supine position with the Riva-Rocci Technique using Korotkoff sounds I and V for systolic and diastolic blood pressure, respectively. Heart rate was measured by pulse frequency counting (radial artery).

Dependent vasodilator response that opposes the effect of thromboxane-derived vasoconstricting agents like thromboxane A₂ and serotonin.15-17 Thus, endogenously released ATP might have an important role in physiology and pathophysiology, and it is of interest to characterize the effects of ATP in vivo in humans. Up to now, our pharmacological knowledge on ATP is based largely on in vitro data from animal studies. Human in vivo data about the direct vascular effects of ATP are scarce. Although the vasodilator effect of ATP in humans has been described before,18,19 the mechanism of action is largely unknown. Of course, endothelial P₂-, purinergic receptors are expected to be involved in the vasodilator response to ATP, but this response may also be mediated by adenosine receptors,20 because ATP is rapidly degraded to adenosine21,22 and because ATP has weak adenosine receptor agonist properties.22,23 In this report, a series of experiments is presented to elucidate the mechanism of action of the vasodilator property of ATP in humans.

This study focuses on three questions in particular: (1) What is the involvement of P₂-purinergic receptor stimulation in the ATP-induced forearm vasodilatation? (2) Is nitric oxide (NO) involved in the forearm vasodilator response to ATP? (3) Is there evidence for an ATP-mediated release of endothelium-derived contracting factors (EDCFs) or P₂-purinergic receptor stimulation?

Methods

After approval from the local ethics committee, a total of 50 healthy male volunteers signed written informed consent statements before participation in the study. Their demographic characteristics are summarized in Table 1. Before the start of the study, the subjects were asked to abstain from caffeine-containing products for at least 36 hours and to refrain from smoking for at least 12 hours. Furthermore, subjects were asked to discontinue any food intake 2 hours before the start of the experiment. All experiments were performed in the afternoon with the subject in the supine position. After local anesthesia (xylocaine 2%), the left brachial artery was cannulated with a 20-gauge catheter (Angiocath, Deseret Medical, Becton Dickinson) for both intra-arterial drug infusion (automatic syringe infusion pump, type STC-521, Terumo) and BP recording (Hewlett Packard GmbH). Forearm blood flow (FBF) was registered in both forearms by ECG-triggered venous occlusion plethysmography using mercury-in-Silastic strain gauges (Hokanson EC4, D.E. Hokanson). The upper-arm collecting cuffs were simultaneously inflated with a rapid cuff inflator (Hokanson E-20). At least 1 minute before FBF measurements were made, hand circulation was occluded by inflation of wrist cuffs to 200 mm Hg. FBF was recorded three times per minute. All experiments were started at least 30 minutes after intra-arterial cannulation. In all experiments, total infusion rate was kept constant at 100 μL · 100 mL forearm⁻¹ · min⁻¹. Saline and all drug dosages were infused for 5 minutes.

Involvement of P₂-Purine (Adenosine) Receptor Stimulation in ATP-Induced Forearm Vasodilator Response

In 12 subjects, the vasodilator effect of equimolar dosages of intra-arterially infused adenosine and ATP were compared. Additionally, the effect of intra-arterially infused theophylline, a competitive P₂-purinergic receptor antagonist, on the vasodilator response to ATP was studied. The experiment started with the measurement of baseline FBF during saline infusion (NaCl 0.9%). Fig 1 shows the course of FBF and the schedule of the several drug infusions. The effect of two increasing dosages of adenosine (0.5 and 1.5 μg · 100 mL forearm⁻¹ · min⁻¹, equivalent to 2 and 6 nmol · 100 mL⁻¹ · min⁻¹) was compared with that of saline infusion. Fifteen minutes after the last adenosine infusion, saline was infused again. Now, the effect of four increasing dosages of ATP (0.1, 0.3, 1.0, and 3.0 μg · 100 mL forearm⁻¹ · min⁻¹, equivalent to 0.2, 0.6, 2, and 6 nmol · 100 mL⁻¹ · min⁻¹) were compared with saline infusion. Because prolonged occlusion of hand circulation can cause discomfort, with subsequent effects on BP and heart rate (HR), a 10-minute rest was allowed between the second and third ATP infusions. Forty-five minutes after the last ATP infusion, saline infusion and the four increasing ATP dosages were repeated in six subjects ("time-control study"). In the other six subjects, saline infusion and the four increasing ATP dosages were repeated with concomitant infusion of theophylline (100 μg · 100 mL⁻¹ · min⁻¹, equivalent to 1.5 μg · 100 mL⁻¹ · min⁻¹) every 20 minutes. After a 30-minute washout period, the experiment was repeated with the other fixations.
Involvement of NO in ATP-Induced Forearm Vasodilator Response

We studied the effect of intra-arterially infused N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA), a competitive NO synthase antagonist, on the vasodilator response to ATP (N=10), to the NO-dependent vasodilator acetylcholine (N=10), and to sodium nitroprusside (SNP, N=6), an NO donor that does not stimulate NO synthase activity.\textsuperscript{25} Acetylcholine, a proven NO-dependent vasodilator in the human forearm,\textsuperscript{26,27} was used to ascertain the effectiveness of our experimental setup to demonstrate antagonistic properties of L-NMMA (positive-control experiment). L-NMMA reduced FBF, which might nonspecifically affect the subsequent response to a vasodilator stimulus. Therefore, the interaction between SNP and L-NMMA was also studied (negative-control experiment). Apart from the effect of L-NMMA on the aforesaid vasodilator response to acetylcholine, ATP, and SNP, the forearm vasocostrictor response to L-NMMA itself was analyzed to find out whether this was related to the effect of the previously infused vasodilator substances on NO synthase.

The experiment started with the measurements of baseline FBF during saline infusion. The effects of three increasing dosages of ATP (0.3, 3, and 10 \(\mu\)g \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)) were compared with that of saline infusion. Because prolonged occlusion of hand circulation can cause discomfort, with subsequent effects on BP and HR, a 10-minute rest was allowed between the first and second ATP infusions. Forty-five minutes after the last ATP infusion, the vasoactive effect of L-NMMA infusion (0.1 mg \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)) was compared with that of saline infusion. Subsequently, the lowest ATP dose was infused (0.3 \(\mu\)g \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)) together with infusion of L-NMMA (0.05 mg \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)). Ten minutes thereafter, L-NMMA was infused again (0.1 mg \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)), immediately followed by the middle and the highest ATP dosages (3 and 10 \(\mu\)g \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)) again together with the lower L-NMMA dose (0.05 mg \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)). In the acetylcholine group, acetylcholine (0.5, 2, and 8 \(\mu\)g \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)) was substituted for ATP. In the SNP group, SNP (0.02, 0.2, and 0.6 \(\mu\)g \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)) was substituted for ATP. Since SNP is diluted in glucose 5%, in the SNP group, glucose 5% was substituted for NaCl 0.9%. Otherwise, the same protocol was performed in the three groups.

Effect of High Dosages of Intra-Arterially Infused ATP on Forearm Vascular Tone

In vitro data suggest that high dosages of ATP reveal a vasoconstrictor component compared with the effect of lower ATP dosages.\textsuperscript{28,29} Both P\textsubscript{2}-purinergic receptor stimulation and release of EDCF\textsuperscript{s} may be involved in this response. To study these possible mechanisms in humans, the effects of high dosages of intra-arterially infused ATP were studied in 12 subjects. The effects of four increasing intra-arterially infused ATP dosages (10, 30, 100, and 300 \(\mu\)g \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\)) were compared with that of saline infusion. To avoid discomfort, a 10-minute rest was allowed between the second and third ATP infusions. After the first 6 experiments, an interim analysis was performed, revealing no decreased vasodilator response at the highest dosage. In the subsequent 6 experiments, 1000 \(\mu\)g ATP \cdot 100 mL forearm\(^{-1}\) min\(^{-1}\) was infused immediately after the fourth ATP infusion. To be sure that maximal vasodilatation occurred in response to the ATP infusions, maximal vasodilatation was measured during postocclusive reactive hyperemia according to the well-established method of Pedrinelli et al.\textsuperscript{30,31} A cuff applied to the left upper arm was inflated to 300 mm Hg for 13 minutes. During the last minute of ischemia, the subjects were asked to perform repeated hand contractions. Immediately after desufflation of the upper-arm cuff, FBF measurements were started for at least 2 minutes with occluded hand circulation. The lowest forearm vascular resistance (MAP\(\times\)FBF) was considered to represent maximal vasodilatation.

Drugs and Solutions

ATP solutions were freshly prepared from 2-mL ampoules containing 20 mg ATP as disodium salt (Striadyne, Wyeth Laboratories) and were diluted in NaCl 0.9%. L-NMMA acate and acetylcholine chloride (Sigma Chemical Co) were reconstituted on the morning of the study day from a sterile lyophilized powder, passed through a 0.2-\(\mu\)m Millipore filter, and diluted in NaCl 0.9%. Adenosine (Sigma) was freshly prepared from 10-mL ampoules containing 20 mg adenosine with NaCl 0.9% as solvent. Theophylline solutions were freshly prepared from 10-mL ampoules containing 24 mg/mL ephedrine (Euphyllin, BYK Nederland) and diluted in NaCl 0.9%. SNP was reconstituted immediately before the start of the experiment from a sterile lyophilized powder, diluted in glucose 5% (Nipride, Roche Nederland), and protected against light.

Statistical Analysis

Mean arterial BP (MAP) was measured continuously during each recording of FBF and averaged per FBF measurement. Forearm vascular resistance (FVR) was calculated from simultaneously measured MAP and FBF (MAP\(\times\)FBF) and expressed as arbitrary units (AU). The calculated FVRs obtained during each 5 minutes of saline infusion or during the last 2 minutes of each drug infusion were averaged to one value. Drug-induced effects were expressed as percentage of change from preceding saline infusion or antagonist infusion. The percentage changes in FVR to each dosage of a vasodilator substance were averaged to one value both before and during antagonist infusion. These two values were compared to assess the effect of an antagonist. All results are mean\(\pm\)SEM unless indicated otherwise. To avoid multiple comparison, within-subject effects were assessed by Friedman two-way nonparametric ANOVA first and further analyzed.
with the Wilcoxon paired signed rank test if appropriate. Likewise, differences between groups were assessed with the Kruskal-Wallis nonparametric one-way ANOVA and further analyzed with the Mann-Whitney U test if appropriate; \( P < .05 \) (two-sided) was considered statistically significant.

**Results**

**Involvement of \( P_1 \)-Purine (Adenosine) Receptor Stimulation in the ATP-Induced Forearm Vasodilator Response**

Apart from the dosage schedule, Fig 1 shows the course of FBF during infusion of adenosine and ATP. FBF in the control arm was not significantly affected during any of the infusions.

Baseline FVR was 47 ± 6 AU in the cannulated arm. In 12 subjects, the effects of two adenosine dosages and four ATP dosages were studied first. FVR of the infused arm during the first and second saline infusions did not significantly differ (47 ± 6 and 53 ± 9 AU, respectively, \( P = NS; N = 12 \)). ATP decreased FVR by 17 ± 12%, 37 ± 11%, 52 ± 7%, and 60 ± 7% for 0.1, 0.3, 1, and 3 \( \mu \)g ATP \( \cdot 100 \) mL forearm \( \cdot min \) \(^{-1} \), respectively (\( N = 12 \); \( P <.05 \) for the three highest dosages). The FVR in the control arm was not significantly affected. In Fig 2, the forearm vascular effects of two equimolar adenosine and ATP dosages (2 and 6 nmol \( \cdot 100 \) mL \( \cdot min \) \(^{-1} \)) are compared. Both ATP dosages induced significantly more forearm vasodilation than their equimolar adenosine counterparts (37 ± 11% versus 9 ± 6% for 2 nmol \( \cdot 100 \) mL forearm \( \cdot min \) \(^{-1} \); 60 ± 7% versus 21 ± 7% for 6 nmol \( \cdot 100 \) mL forearm \( \cdot min \) \(^{-1} \); \( N = 12 \); \( P <.05 \) for both comparisons).

In 6 subjects, the ATP infusions were repeated 45 minutes later. The vasodilator response to ATP did not significantly differ between the two infusion periods: the averaged responses in FVR were \(-46 ± 8\%\) and \(-45 ± 10\%\) in the infused arm for the first and second ATP infusions, respectively (\( P = NS \)). In the control arm, vascular resistance remained unchanged (averaged response in FVR, 2±5% and 8±14% during the first and second infusion periods, respectively; \( N = 6 \), \( P > .1 \)). In the other 6 subjects, the ATP infusions were repeated with concomitant infusion of theophylline. FVRs during the second saline infusion and the theophylline infusion were 54 ± 16 and 54 ± 17 AU, respectively (\( P = NS; N = 6 \)). FVR during theophylline infusion did not differ significantly from FVR during the third saline infusion of the time-control study group (54 ± 17 versus 62 ± 8 AU; \( P = NS \), \( N = 6 \) for both groups). Thus, theophylline did not significantly affect baseline FVR. Likewise, theophylline did not significantly affect the ATP-induced forearm vasodilator response: the averaged responses in FVR were \(-38 ± 12\%\) and \(-38 ± 10\%\) for ATP infusions with saline infusion and theophylline, respectively (\( P = NS; N = 6 \)). In the control arm, no changes in FVR were observed.

**Involvement of NO in the ATP-Induced Forearm Vasodilator Response**

FBF in the control arm was not affected by the various drug infusions. Fig 3 depicts the effect of each dosage of the three vasodilator substances on FVR in the infused arm before and during L-NMMA infusion. In the infused arm, for each vasodilator substance, a
Table 2. Effect of L-NMMA on Baseline Forearm Vascular Resistance and Vasodilator Response to ATP, ACh, and SNP

<table>
<thead>
<tr>
<th>Vasodilator</th>
<th>% Rise in FVR to First L-NMMA Infusion</th>
<th>% Rise in FVR to Second L-NMMA Infusion</th>
<th>Averaged % Response in FVR to Vasodilator</th>
<th>Averaged % Response in FVR to Vasodilator (+ L-NMMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused arm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>64±13†</td>
<td>95±17†</td>
<td>−59±6*</td>
<td>−42±8*§</td>
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<tr>
<td>ATP</td>
<td>37±10*</td>
<td>45±9*</td>
<td>−68±3*</td>
<td>−64±6*</td>
</tr>
<tr>
<td>SNP</td>
<td>33±11*</td>
<td>41±7*</td>
<td>−53±3*‡</td>
<td>−49±4*</td>
</tr>
<tr>
<td>Control arm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>5±4</td>
<td>16±9</td>
<td>1±5</td>
<td>5±4</td>
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<tr>
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<td>−3±5</td>
<td>6±5</td>
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<tr>
<td>SNP</td>
<td>5±2</td>
<td>−8±11</td>
<td>6±5</td>
<td>19±10</td>
</tr>
</tbody>
</table>

L-NMMA indicates N⁰-monomethyl-L-arginine; ACh, acetylcholine; SNP, sodium nitroprusside; and FVR, forearm vascular resistance. Values are mean±SEM.

*Statistically significant difference from baseline (P<.05); †statistically significant difference from ATP and SNP groups (P<.05); ‡statistically significant difference with ATP group (P<.05); §statistically significant effect of L-NMMA on vasodilator response (P<.05).

dose-dependent vasodilation was observed. Besides a smaller effect of the lowest SNP dosage, the three vasodilator substances induced a comparable vasodilator response. Only the acetylcholine-induced vasodilator response could be antagonized significantly by L-NMMA. The effect was most pronounced for the lower dosages. Table 2 tabulates the effect of L-NMMA on forearm vascular tone and the averaged responses to vasodilator substances before and during L-NMMA infusion. The averaged percentage responses in FVR to all three vasodilator substances were −59±6%, −68±3%, and −53±3% for acetylcholine, ATP, and SNP, respectively. During concomitant infusion of L-NMMA, the averaged responses in FVR were −42±8%, −64±6%, and −49±4% for the acetylcholine, ATP, and SNP groups, respectively (P<.05 for the effect of L-NMMA on acetylcholine; P=NS for the effect of L-NMMA on ATP and SNP). Besides a small increase during the second SNP infusion with L-NMMA (35.7±14.8%; P<.05 versus baseline), FVR in the control arm was not significantly affected for any of the vasodilators.

Within each group, the vascular resistances during the three saline infusions were not significantly different, indicating that vascular tone had returned toward baseline levels before the second infusion of a vasodilator substance and before the first L-NMMA infusion. Additionally, baseline FVR did not differ significantly between the three groups. During the first L-NMMA infusion, FVR increased in all three groups (P<.05 for the acetylcholine [N=10], ATP [N=10], and SNP [N=6] groups). In the acetylcholine group, this vasconstrictor response differed significantly from the other two groups, being 64.3±12.7% versus 37.2±9.7% and 33.3±10.7% for the ATP and SNP groups, respectively (P<.05 for the acetylcholine group versus each other group; P>0.1 for the ATP group versus the SNP group). These group differences were more pronounced for the second L-NMMA infusion. In the ATP group, the increase in FVR did not differ significantly from the SNP group, being 44.9±8.7% and 40.8±7.3% for the ATP and SNP groups, respectively (ATP versus SNP group, P=1.0; first versus second L-NMMA infusion, P>1 for both groups). However, in the acetylcholine group, L-NMMA now tended to induce a more pronounced increase in FVR of 94.7±16.7% (P<.01 versus ATP and SNP groups; P=.09 versus first L-NMMA infusion).

Group differences in vasoconstrictor response to L-NMMA may have confounded the effect of L-NMMA on the response to vasodilator substances. To investigate this possibility, a correlation between the vasoconstrictor response to L-NMMA and the L-NMMA-induced reduction in averaged percentage response in FVR to acetylcholine and ATP was analyzed for each agonist separately. This analysis revealed that with an increasing vasoconstrictor response to L-NMMA, the inhibition of agonist-induced vasodilation was reduced (r=−.56, P=0.09 for acetylcholine and r=−.66, P<.05 for ATP). Therefore, it is unlikely that the reduced vasoconstrictor response to L-NMMA in the ATP group masked a possible inhibitory effect of L-NMMA on ATP-induced vasodilation.

Effect of High Dosages of Intra-Arterially Infused ATP on Forearm Vascular Tone

Baseline FVR was 73±10 AU. Fig 4 shows the percentage response in FVR during the four increasing ATP dosages. In the control arm, FVR was not significantly affected (Friedman ANOVA, P=.1; N=12). In the infused arm, FVR was 9±2, 8±3, 6±2, and 5±2 AU during infusion of 10, 30, 100, and 300 μg ATP·100 mL⁻¹·min⁻¹, respectively (P<.01 versus baseline for all dosages, N=12). These values are distorted because of one outlier with an FVR in the infused arm of 77 AU during saline infusion and 26, 35, 32, and 28 AU during the four increasing ATP dosages, respectively. After exclusion of this outlier, FVR was 8±2, 5±1, 4±1, and 2±0.2 AU for the four increasing ATP dosages, respectively (P<.01 versus baseline for all dosages, N=11). Thus, up to 300 μg ATP·100 mL forearm⁻¹·min⁻¹, FVR was continuously reduced. In six subjects, this dose-response curve was extended to 1000 μg ATP·100 mL forearm⁻¹·min⁻¹, which was infused immediately
after 300 μg ATP · 100 mL forearm⁻¹ · min⁻¹. On average, FVR was reduced to 3±1 AU during infusions of 1000 μg ATP · 100 mL forearm⁻¹ · min⁻¹, respectively (P=NS for 300 versus 1000 μg ATP · 100 mL forearm⁻¹ · min⁻¹, N=6). In four of the six subjects, FVR in the control arm increased slightly during the highest ATP dosage (P>1). The minimal FVR during postocclusive reactive hyperemia was 3±0.2 AU and did not differ significantly from FVR during infusion of 300 (N=12) or 1000 μg ATP · 100 mL forearm⁻¹ · min⁻¹ (N=6).

Discussion

In vitro studies have demonstrated pharmacological effects of ATP that might have important physiological and pathophysiological implications. ATP released from sympathetic nerve endings at the adventitial side of a blood vessel results in a vasoconstrictor response, mediated by so-called P₂₅-purinergic receptors on smooth muscle cells. On the other hand, luminal released ATP from aggregating thrombocytes or the endothelium itself stimulates endothelial P₃-purinergic receptors, resulting in an NO-mediated vasodilator response. To assess the relevance of these in vitro observations, we now characterized the vascular in vivo effects of ATP in men. The perfused forearm technique was used because this model has been validated previously as a method to study direct vascular effects in humans. In this model, the possibility of measuring FBF in the noninfused arm has methodological advantages because it enables us to detect systemic actions of the infused drugs, such as baroreflex activation, that could potentially counteract the local effects.

Our results demonstrate that ATP induces vasodilation even at low concentrations in the nanomolar range, which are thought to occur under physiological conditions. This vasodilator response appeared to be unrelated to P₁-purinergic receptor stimulation and NO release. The vasodilator response was not reduced at high ATP infusion rates.

Involvement of P₁-Purine (Adenosine) Receptor Stimulation in the ATP-Induced Forearm Vasodilator Response

The vascular effects of equimolar dosages of ATP and adenosine, the degradation product of ATP with the highest P₁-purinergic receptor agonist activity, demonstrate that P₁-purinergic receptor stimulation hardly contributes to the ATP-induced vasodilation. This view is further supported by the fact that theophylline did not affect the vasodilator response to ATP. Since theophylline has been shown to antagonize the forearm vasodilator response to adenosine, the present observation rules out that P₁-purinergic receptor stimulation is involved in the ATP-induced forearm vasodilator response. This is in agreement with most in vitro studies. In theory, theophylline could have inhibited intracellular phosphodiesterase activity. Since cAMP is thought to be an important second messenger of A₁-purinergic receptor stimulation, this effect could have counteracted the P₁-purinergic receptor-antagonizing action of theophylline. This problem has been addressed by others using the forearm vasodilator response to theophylline as a marker of phosphodiesterase inhibition. They found that theophylline infusions up to 100 μg · 100 mL⁻¹ · min⁻¹ did not affect forearm vascular tone but significantly antagonized the adenosine-induced vasodilation. In our study, the same theophylline dosage was used without any effect on vascular tone. Therefore, it is very unlikely that inhibition of phosphodiesterase has contributed to the results.

In view of the extensive literature on in vitro vascular effects of ATP, the exclusion of P₁-purinergic receptor involvement strongly suggests the existence of the P₃-purinergic receptor in the human forearm vascular bed. However, the involvement of a recently suggested "pyrimidine receptor" cannot be excluded. Specific P₁-purinergic receptor antagonists are needed to resolve these problems in P₁-purinergic receptor classification.

Involvement of NO in the ATP-Induced Forearm Vasodilator Response

L-NMMA, a competitive inhibitor of NO synthase without affinity for the muscarine receptor, was used in this study to inhibit the formation of vascular NO. Acetylcholine is known to stimulate NO synthase both in vitro and in vivo in the human forearm. Therefore, this agonist was used as a positive control. SNP is regarded as an NO donor that directly stimulates soluble guanylate cyclase without involvement of NO synthase, and therefore it was a suitable negative control in this study part.

The vasodilator response to acetylcholine was reduced significantly by L-NMMA, indicating that the dosage of L-NMMA used in this study is able to inhibit NO synthase significantly. In contrast, the ATP-induced vasodilator response could not be inhibited by concomitant L-NMMA infusion, despite a similar degree of vasodilation induced by ATP and acetylcholine. This finding supports the argument that NO does not significantly contribute to ATP-induced vasodilation. The SNP-induced forearm vasodilation was not affected by concomitant L-NMMA infusion, ruling out the possibility that agonist-induced vasodilation was affected by L-NMMA–evoked precontraction. During the second series of SNP infusions, FVR in the control arm increased temporarily. A possible systemic effect of the concomitant L-NMMA infusion is not likely, because it was not observed in the ATP and acetylcholine groups and because FVR in the control arm returned to baseline levels during the last administration of SNP.
with concomitant L-NMMA infusion. Whatever the cause might be, it did not seriously affect the results obtained from the infused arm, since it was only a small change in FVR that did not significantly differ from FVR fluctuations in the control arm during SNP administration with concomitant saline infusion.

In all three groups, L-NMMA induced a significant increase in FVR, confirming that L-NMMA in the dosages used was able to inhibit baseline NO production.26 This effect of L-NMMA differed between the three groups, indicating an influence of agonist pre-treatment on NO synthase activity. During the second L-NMMA infusion, 10 minutes after the lowest agonist infusion, these between-group differences were more pronounced, supporting this view (see Table 2). Since SNP does not stimulate NO synthase, the effect of L-NMMA in the SNP group can be regarded as unaffected by the previous infusions of SNP. In the acetylcholine group, the vasoconstrictor response to L-NMMA was significantly higher than in the SNP group, indicating that NO synthase was still activated 50 minutes after the last acetylcholine infusion, although FVR had returned to baseline levels. In the ATP group, L-NMMA-induced vasoresistance was similar to that observed in the SNP group during both the first and second L-NMMA infusions. In combination with the lack of effect of L-NMMA on ATP-induced vasodilation, these results indicate that ATP does not stimulate NO synthase activity in the human forearm vascular bed. In theory, kinetic differences between acetylcholine and ATP could explain the between-group differences for the effect of L-NMMA on FVR. However, both acetylcholine and ATP have a short half-life of a few seconds because of rapid degradation by choline esterase and ectonucleotidases, respectively. Furthermore, both substances induce a similar amount of vasodilation, and in both the ATP and acetylcholine groups, FVR had returned to baseline levels before L-NMMA was infused. Therefore, kinetic differences between the two agonists are not able to explain the divergent effect of L-NMMA in the acetylcholine and ATP groups. Probably, NO synthase remained activated after acetylcholine infusion without continuous muscarine receptor stimulation.

One might argue that the group differences in L-NMMA–evoked contractions reflect group differences in NO synthase sensitivity to L-NMMA. However, this would assume a positive correlation between L-NMMA–induced vasoconstriction and the inhibitory effect of L-NMMA on the forearm vasodilator response to acetylcholine and ATP. In contrast, a negative correlation was observed. Therefore, we believe that our results are not relevantly confounded by group differences in NO synthase sensitivity to L-NMMA. In addition, the existence of a confounding factor is not likely, because the study groups consisted of healthy male volunteers very alike with respect to age, BP, and baseline FVR.

The present observation that ATP does not activate NO synthase in the human forearm contrasts with most in vitro studies.29,43,44 An important question remains: How is the ATP-induced vasodilation mediated in the human forearm? With regard to the endothelium, in vitro studies indicate that besides NO, prostacyclin is also released after endothelial stimulation with ATP and may be involved in a vasodilator response.45,46 Other, yet unidentified, endothelium-derived relaxing factors like the “endothelium-derived hyperpolarizing factor” may be involved as well.47,48 Therefore, the present finding does not exclude the possibility that ATP induces its vasodilator response in the human forearm by an endothelium-dependent mechanism. On the other hand, some in vitro studies have demonstrated an endothelium-independent component in the ATP-induced vasodilator response.38,39,49 The ATP-dependent potassium channel may be involved in ATP-induced vasodilation.50 Further research is needed to determine the precise role of the endothelium in the ATP-induced forearm vasodilator response.

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In vitro studies have shown a reduced vasodilator response to high dosages of ATP.13,28,29 Two possible mechanisms have been proposed: simultaneous stimulation of P2X purinergic receptors50 and concomitant release of EDCFs.28 In the present study, these in vitro observations could not be reproduced in the human forearm. Maximal ATP-induced vasodilation did not differ from postocclusive reactive hyperemia, confirming a previous observation.51 Postocclusive reactive hyperemia can be regarded as a condition of maximal and total vascular relaxation.30,31 The vasodilator response to ATP was not reduced even at the very high dosage of 1000 μg ATP · 100 mL forearm−1 · min−1, indicating that the vasodilator effect of high intra-arterial dosages of ATP is not reduced by the release of EDCFs. The presence of an intact endothelium and the existence of a highly active vascular ectophosphatase system could have prevented ATP from reaching the vascular smooth muscle cells.52,53 Therefore, the present observations do not exclude the existence of P2X purinergic receptors in the human forearm vasculature.

In conclusion, intra-arterial infusion of ATP induces a forearm vasodilator response that is not mediated by P2X purinergic receptor stimulation or by NO release. In healthy volunteers, the effect of high doses of ATP is not reduced by the potential release of EDCFs or stimulation of P2X purinergic receptors. The exact mechanism of the vasodilator response to ATP in the human forearm remains to be elucidated. However, our results convincingly indicate that the intravascular release of ATP or ADP from thrombocytes is not expected to induce vasoconstriction in a healthy vascular system. This, of course, does not exclude a vasoconstrictor response to interstitially released ATP.

Acknowledgments

This study was financially supported by the Dutch Heart Foundation (grant No. 90.301). The authors wish to express their gratitude to Dr K.J. Duyn, Wyeth Laboratories, Hoofddorp, the Netherlands, for his generous gift of Striadyne.

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Circulation. 1994;90:1891-1898
doi: 10.1161/01.CIR.90.4.1891

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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