Role of nitric oxide towards vasodilator effects of substance P and ATP in human forearm vessels

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1. It has been shown in animals that substance P as well as acetylcholine releases endothelium-derived nitric oxide and evokes vasodilatation and that ATP-induced vasodilatation is partially mediated by nitric oxide. The aim of this study was to examine whether vasodilator effects of substance P and ATP are mediated by nitric oxide in humans.

2. In healthy volunteers (n = 33), we measured forearm blood flow by a strain-gauge plethysmograph while infusing graded doses of acetylcholine, substance P, ATP or sodium nitroprusside into the brachial artery before and after infusion of \( \text{N}^0 \)-monomethyl-\( \text{L} \)-arginine (4 or 8 \( \mu \text{mol/min} \) for 5 min). In addition, we measured forearm blood flow while infusing substance P before and during infusion of \( \text{L} \)-arginine (10 mg/min, simultaneously), or before and 1 h after oral administration of indomethacin (75 mg).

3. Acetylcholine, substance P, ATP or sodium nitroprusside increased forearm blood flow in a dose-dependent manner. \( \text{N}^0 \)-Monomethyl-\( \text{L} \)-arginine decreased basal forearm blood flow and inhibited acetylcholine-induced vasodilatation but did not affect substance P-, ATP-, or sodium nitroprusside-induced vasodilatation. Neither supplementation of \( \text{L} \)-arginine nor pretreatment with indomethacin affected substance P-induced vasodilatation.

4. Our results suggest that, in the human forearm vessels, substance P-induced vasodilatation may not be mediated by either nitric oxide or prostaglandins and that ATP-induced vasodilatation may also not be mediated by nitric oxide.

INTRODUCTION

To examine endothelial function in humans, acetylcholine and substance P are frequently used on the assumption that the vasodilator effects of both agents are mediated by release of nitric oxide from the endothelium. Previous studies have shown that acetylcholine-induced vasodilatation is impaired but substance P-induced vasodilatation is not in many pathological states including atherosclerosis [1], variant angina [2, 3] and congestive heart failure [4]. It has been implied that there may be selective impairment of muscarinic receptor-mediated release of nitric oxide from the endothelium in these pathological states.

It has been demonstrated in humans that acetylcholine-induced forearm vasodilatation is partially blocked by \( \text{N}^0 \)-monomethyl-\( \text{L} \)-arginine (\( \text{L} \)-NMMA), an inhibitor of nitric oxide synthesis [5-7], and that supplementation of \( \text{L} \)-arginine, a precursor of nitric oxide, augments acetylcholine-induced vasodilatation [6, 8]. These results confirm that acetylcholine-induced vasodilatation is mediated partially by release of nitric oxide in human forearm vessels. Another endothelium-dependent vasodilator, substance P, also causes forearm vasodilatation [4, 9, 10]. Substance P-induced vasodilatation may not be mediated by nitric oxide since animal experiments in vivo and in vitro suggest that substance P may cause endothelium-dependent vasodilatation mediated by endothelium-derived hyperpolarizing factor [11]. It is also possible that substance P-induced vasodilatation is mediated by endothelium-derived prostacyclin [12]. In a recent study in humans, Panza et al. [10] have shown that after infusion of \( \text{L} \)-NMMA the absolute values of forearm blood flow (FBF) induced by substance P were reduced. Thus, substance P-induced vasodilatation may also be mediated by nitric oxide in human forearm vessels. However, the decreases in resting blood flow by \( \text{L} \)-NMMA make the interpretation of the vasodilator effects of substance P difficult. Moreover, these findings need to be confirmed.

Key words: acetylcholine, \( \text{L} \)-arginine, endothelium, \( \text{N}^0 \)-monomethyl-\( \text{L} \)-arginine, prostaglandin.
Abbreviations: ANOVA, analysis of variance; FBF, forearm blood flow; \( \text{L} \)-NMMA, \( \text{N}^0 \)-monomethyl-\( \text{L} \)-arginine; SNP, sodium nitroprusside.
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Accordingly, in order to determine precisely in human forearms whether substance P-induced vasodilatation is mediated by nitric oxide, we examined substance P-induced changes in FBF before and after infusion of \( L - \text{NMMA} \) at 4 \( \mu \text{mol/min} \) (a usual dose) or 8 \( \mu \text{mol/min} \) (a high dose) for 5 min or \( L - \text{arginine} \) supplementation. We compared the effects of \( L - \text{NMMA} \) on substance P-induced vasodilatation with those on acetylcholine (an endothelium-dependent vasodilator)- and sodium nitroprusside (SNP, an endothelium-independent vasodilator)-induced vasodilatation. Furthermore, to examine whether substance P-induced vasodilatation is mediated by nitric oxide in human forearm vessels since it has also been considered that ATP-induced vasodilatation is partially mediated by nitric oxide [13].

**METHODS**

**General procedure**

Studies were performed in healthy subjects (aged 26 ± 3 years, ranging from 18 to 67 years; 33 males and two females) who volunteered for the study. Subjects were mainly young (18–29 years of age), healthy male students at the university who volunteered for the study \( (n = 29) \). The other subjects \( (n = 6) \) in Experiment 4 were also volunteers (hospital employees) and were older (46–67 years of age) than the subjects in the other experiments. We were careful to exclude subjects with risk factors which impair endothelium-dependent vasodilatation. Each subject was screened by taking a careful history and physical examination in relation to risk factors of cardiovascular diseases such as hypertension, a family history of hypertension, smoking, diabetes mellitus, hypercholesterolaemia and also renal or hepatic diseases, and we confirmed they did not have such factors. If subjects were over 30 years old, an ECG, a chest X-ray and blood chemistry were also examined. They were all within normal limits. The protocol was explained, and informed written consent was obtained from each subject. The study was approved by the Ethical Committee for Human Study in our institution. The study was carried out with subjects in a supine position and in an air-conditioned room at a room temperature of 25–26°C. Under local anaesthesia with 2% procaine, the left brachial artery was cannulated with a 20-gauge intravascular over-the-needle poly(tetrafluoroethylene) catheter for drug infusion. The catheter was connected by a three-way stopcock to a pressure transducer (Viggo-Spectramed, Oxnard, CA, U.S.A.) for direct measurement of arterial pressure. The arterial line was kept open by infusing heparinized saline (0.1 ml/min) when no drug was being administered. Heart rate was obtained by counting pulse for a few minutes on arterial pressure recordings.

**Measurements of FBF**

FBF was measured by a mercury-in-silastic strain-gauge plethysmograph with a venous occlusion technique [4, 7, 8, 14]. The strain gauge was placed approximately 5 cm below the antecubital crease. FBF \( (\text{ml min}^{-1} 100 \text{ml}^{-1} \text{forearm}) \) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating a cuff on the upper arm. The pressure in the venous occlusion or congesting cuff on the upper arm was 40 mmHg. Circulation to the hand was arrested by inflating a cuff around the wrist. The wrist cuff was inflated before the determination of FBF and continuously throughout the measurements. Forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic pressure plus one-third of the pulse pressure in mmHg) by the FBF. These values are expressed as units throughout this report. An average of four flow measurements made at 15 s intervals, which were calculated by two authors independently, was used for later analysis.

**Protocols**

Forearm vascular responses to drugs were examined in the next five protocols which are schematically described in Fig. 1. After the placement of canulas and a strain-gauge plethysmograph, at least 15 min were allowed for subjects to become accustomed to the study condition before the experiments began. Five experiments were performed. Each experiment was carried out on different days and in different subjects.

**Experiment 1.** In the first protocol, we examined forearm vasodilating responses to intra-arterial infusions of substance P and ATP at graded doses before and after intra-arterial infusions of \( L - \text{NMMA} \) at 4 \( \mu \text{mol/min} \) \( (n = 9) \). First, we examined FBF responses to intra-arterial infusions of substance P \( (0.4, 0.8, 1.6 \text{ and} \ 3.2 \text{ ng/min}) \) [4] or ATP \( (0.5, 1.0, 2.0 \text{ and} \ 3.0 \text{ pg/min}) \) [14] for 2 min at each dose. FBF reached the steady state by 1 min after starting infusion of the drugs. The order of series of drug infusion was alternated. Infusion of the second drug was begun at least 15 min after the termination of the first drug when FBF had returned to the baseline value. After complete recovery from the second drug infusion, \( L - \text{NMMA} \) was infused intra-arterially at 4 \( \mu \text{mol/min} \) for 5 min \( [5 - 7] \). Immediately after \( L - \text{NMMA} \) infusion, baseline parameters were obtained. Intra-arterial infusions of substance P and ATP were then performed in the same way as before \( L - \text{NMMA} \) infusion. Again, infusions of substance P and ATP were alternated. Fifteen
minutes after the final dose of the first drug, L-NMMA was infused again at 4 μmol/min for 5 min. Baseline parameters were obtained and infusion of the second drug was performed. FBF and arterial pressure were continuously monitored and recorded during drug infusion. The last 1 min measurements of FBF during infusion of each dose of the drug were used for later analysis.

Experiment 2. This protocol was performed in eight subjects. In this protocol, we determined whether infusion of L-NMMA at a higher dose (8 μmol/min) affected forearm vasodilating responses to intra-arterial infusions of substance P and acetylcholine because we did not see attenuated forearm vasodilating responses to substance P and ATP after infusion of L-NMMA (4 μmol/min) in Experiment 1, although previous studies from our laboratory and others have shown that this dose of L-NMMA can partially block acetylcholine-induced forearm vasodilatation [5-7]. We also determined the reproducibility of substance P-induced forearm vasodilatation in this group because there may be tachyphylaxis of substance P-induced vasodilatation [9]. First, we examined forearm vasodilating responses to intra-arterial infusions of substance P (0.8, 1.6 and 3.2 ng/min) and acetylcholine (4, 8 and 12 μg/min). The order of series of drug infusion was alternated. Intra-arterial infusion of substance P was then repeated before infusion of L-NMMA (Fig. 1).

Experiment 3. This protocol was performed in seven subjects. We examined forearm vasodilating responses to SNP (0.8, 1.6 and 3.2 μg/min), an endothelium-independent vasodilator, and substance P (0.8, 1.6 and 3.2 ng/min) before and after infusion of L-NMMA (8 μmol/min for 5 min). The order of series of drug infusion was alternated. Measurements of variables were performed in the same way as in Experiment 1.

Experiment 4. In this protocol in six subjects, we examined whether supplementation of L-arginine augmented substance P-induced vasodilatation. Substance P was infused at 0.25, 0.5, 1.0, 1.5 and 2.0 ng/min for 2 min at each dose. After complete recovery, L-arginine was infused at 10 mg/min. Ten minutes after starting infusion of L-arginine, forearm vasodilating responses to substance P were examined while L-arginine was simultaneously infused. We chose this dose of L-arginine because we have previously shown that it augments acetylcholine-induced forearm vasodilatation but does not affect resting FBF [8, 15]. Measurements of variables were performed in the same way as in Experiment 1. Although subjects in this experiment were older than those in the other experiments, we included them because we have previously shown that aging does not affect endothelium-dependent vasodilatation in the forearm [14].

Experiment 5. In this protocol in five subjects, we determined whether substance P-induced vasodilatation was mediated by prostaglandins. Substance P was infused at 0.8, 1.6 and 3.2 ng/min for 2 min at each dose. Indomethacin (75 mg) was then administered orally. Sixty minutes after indomethacin administration, infusion of substance P was repeated. Measurements of variables were performed in the same way as in Experiment 1.

Preparation of drugs

Substance P (Peptide Research Laboratories, Osaka, Japan) was diluted in physiological saline and was sterilized in the pharmaceutical section of our hospital. ATP, acetylcholine and SNP solution were prepared by dissolving 20 mg of ATP (Kowa, Nagoya, Japan), 100 mg of acetylcholine chloride (Daichi Pharmaceutical, Tokyo, Japan) and 10 mg of SNP (Wakou Junyaku Kogyo, Osaka, Japan) in physiological saline at a concentration of 5 μg/ml, 40 μg/ml and 4 μg/ml, respectively, immediately before use. Special care was taken not to expose SNP to light. For the infusion of L-arginine, commercially available L-arginine solution (0.1 g of L-arginine hydrochloride/ml; Morishita Pharma-
ceutical, Osaka, Japan) was used. L-NMMA was obtained from Clinalfa AG (Zurich, Switzerland) and was dissolved in physiological saline immediately before use.

Statistical analysis

The effects of L-NMMA, L-arginine and indomethacin on the resting values were analysed by paired t-test. The forearm haemodynamic values in response to drugs were analysed by one-way or two-way analysis of variance (ANOVA) when appropriate. All values are expressed as means ± SEM and \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Effects of L-NMMA on resting haemodynamic values

In Experiment 1, infusion of L-NMMA at a low dose (4 pmol/min for 5 min, \( n = 9 \)) decreased resting FBF (\( P < 0.05 \), by paired t-test) and increased forearm vascular resistance (\( P < 0.01 \)) (Table 1). In Experiments 2 (\( n = 8 \)) and 3 (\( n = 7 \)), we infused a high dose (8 pmol/min for 5 min) of L-NMMA which also decreased resting FBF (\( P < 0.01 \)) and increased resting forearm vascular resistance (\( P < 0.01 \)) (Tables 2 and 3). The magnitudes of decreases in FBF and increases in forearm vascular resistance evoked by the high dose of L-NMMA were greater (\( P < 0.05 \) by unpaired t-test) than those evoked by the low dose of L-NMMA. These doses of L-NMMA did not affect arterial pressure (Tables 1, 2 and 3) and heart rate (data not shown).

Effects of L-NMMA on drug-induced vasodilatation

Figure 2 shows representative plethysmographic recordings of FBF in a subject in response to intraarterial infusion of substance P at graded doses before and after infusion of L-NMMA at 4 \( \mu \)mol/min. Substance P increased FBF dose-dependently before and after infusion of L-NMMA in Experiments 1, 2 and 3 (\( P < 0.01 \), by one-way ANOVA). L-NMMA decreased resting FBF but did not alter FBF responses to substance P. Table 1

<table>
<thead>
<tr>
<th>Substance P (ng/min) ( (n = 9) )</th>
<th>P value by two-way ANOVA</th>
<th>ATP (ng/min) ( (n = 9) )</th>
<th>P value by two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 0.4</td>
<td>Before 0.8</td>
<td>Before 1.6</td>
<td>Before 3.2</td>
</tr>
<tr>
<td>MBP (mmHg) Before</td>
<td>83.6±3.1</td>
<td>84.1±3.5</td>
<td>82.7±3.1</td>
</tr>
<tr>
<td>After</td>
<td>84.1±2.7</td>
<td>84.4±2.4</td>
<td>83.0±2.4</td>
</tr>
<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹) Before</td>
<td>4.5±0.8</td>
<td>4.7±0.9</td>
<td>8.4±1.3</td>
</tr>
<tr>
<td>After</td>
<td>3.5±0.6</td>
<td>3.7±0.5</td>
<td>6.9±1.0</td>
</tr>
<tr>
<td>FVR (units) Before</td>
<td>22.3±3.6</td>
<td>21.3±3.1</td>
<td>11.2±1.4</td>
</tr>
<tr>
<td>After</td>
<td>27.6±3.7</td>
<td>26.3±3.7</td>
<td>13.2±1.4</td>
</tr>
</tbody>
</table>

Table 2. Forearm haemodynamics before and after infusion of L-NMMA (8 \( \mu \)mol/min). Abbreviations: MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; NS, not significant; before I, first infusion of substance P before L-NMMA; before 2, second infusion of substance P before L-NMMA; after, after L-NMMA. Data are means ± SEM.

<table>
<thead>
<tr>
<th>Substance P (ng/min) ( (n = 8) )</th>
<th>P value by two-way ANOVA</th>
<th>Acetylcholine (ng/min) ( (n = 8) )</th>
<th>P value by two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 0.4</td>
<td>Before 0.8</td>
<td>Before 1.6</td>
<td>Before 3.2</td>
</tr>
<tr>
<td>MBP (mmHg) Before</td>
<td>84.5±2.3</td>
<td>82.9±2.2</td>
<td>82.0±2.7</td>
</tr>
<tr>
<td>Before</td>
<td>85.8±2.3</td>
<td>84.0±2.8</td>
<td>84.1±2.8</td>
</tr>
<tr>
<td>After</td>
<td>88.5±2.8</td>
<td>88.0±2.6</td>
<td>86.7±3.0</td>
</tr>
<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹) Before</td>
<td>4.2±0.9</td>
<td>7.1±1.0</td>
<td>11.6±1.3</td>
</tr>
<tr>
<td>Before</td>
<td>4.7±1.6</td>
<td>8.4±2.8</td>
<td>13.5±3.2</td>
</tr>
<tr>
<td>After</td>
<td>2.6±0.7</td>
<td>4.9±0.9</td>
<td>8.6±1.0</td>
</tr>
<tr>
<td>FVR (units) Before</td>
<td>25.0±3.9</td>
<td>14.0±3.0</td>
<td>7.7±0.9</td>
</tr>
<tr>
<td>Before</td>
<td>26.0±4.4</td>
<td>15.2±3.9</td>
<td>8.0±1.5</td>
</tr>
<tr>
<td>After</td>
<td>41.7±5.6</td>
<td>21.1±3.3</td>
<td>11.2±2.4</td>
</tr>
</tbody>
</table>
Vasodilatation by substance P and ATP

Table 3. Forearm haemodynamics before and after infusion of L-NMMA (8 pmol/min). Abbreviations: MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; NS, not significant. Data are means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Substance P (ng/min) (n = 7)</th>
<th>P value by two-way ANOVA</th>
<th>SNP (µg/min) (n = 7)</th>
<th>P value by two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.8</td>
<td>1.6</td>
<td>3.2</td>
</tr>
<tr>
<td>MBP (mmHg) Before</td>
<td>86.9 ± 4.8</td>
<td>84.4 ± 3.0</td>
<td>85.8 ± 4.8</td>
<td>86.4 ± 5.2</td>
</tr>
<tr>
<td>After</td>
<td>88.3 ± 4.2</td>
<td>87.0 ± 4.1</td>
<td>86.7 ± 4.0</td>
<td>87.0 ± 4.0</td>
</tr>
<tr>
<td>FBF (ml/min/100ml⁻¹) Before</td>
<td>6.1 ± 1.8</td>
<td>9.4 ± 2.2</td>
<td>12.1 ± 2.6</td>
<td>15.5 ± 2.7</td>
</tr>
<tr>
<td>After</td>
<td>4.5 ± 1.5</td>
<td>7.2 ± 1.7</td>
<td>10.5 ± 2.0</td>
<td>11.9 ± 1.6</td>
</tr>
<tr>
<td>FVR (units) Before</td>
<td>23.0 ± 6.2</td>
<td>11.8 ± 2.6</td>
<td>8.6 ± 1.7</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>After</td>
<td>32.4 ± 8.7</td>
<td>16.4 ± 4.1</td>
<td>10.0 ± 2.2</td>
<td>8.2 ± 1.5</td>
</tr>
</tbody>
</table>

summarizes forearm haemodynamic values in response to substance P and ATP before and after infusion of L-NMMA at 4 µmol/min. Table 2 summarizes forearm haemodynamic values in response to substance P and acetylcholine, and Table 3 summarizes forearm haemodynamic values in response to substance P and SNP, before and after infusion of L-NMMA at 8 µmol/min. Infusion of any drug did not alter arterial pressure (Tables 1, 2 and 3) and heart rate (data not shown). After infusion of L-NMMA, the absolute values of FBF in response to any drug were less and the absolute values of forearm vascular resistance were greater than those before infusion of L-NMMA (Tables 1, 2 and 3). Because L-NMMA decreased resting FBF and increased resting vascular resistance, the responses to substance P, ATP, acetylcholine and SNP were normalized by resting values and compared before and after infusion of L-NMMA as the percentage changes. The percentage increases in

Fig. 2. Representative plethysmographic recordings showing changes in FBF during intra-arterial infusion of substance P before and after infusion of L-NMMA. The slope indicates FBF. Substance P causes progressive increases in FBF. Infusion of L-NMMA decreased resting FBF but did not alter responses to substance P.

Fig. 3. Percentage changes in FBF evoked by intra-arterial infusion of graded doses of substance P and ATP before (○) and after (●) infusion of a low dose of L-NMMA (4 µmol/min for 5 min)
FBF evoked by substance P, ATP and SNP did not differ before and after intra-arterial infusion of L-NMMA [Figs 3 and 4(bottom)]. However, the percentage increases in FBF evoked by acetylcholine were significantly attenuated after infusion of L-NMMA ($P<0.05$, by two-way ANOVA) [Fig. 4(top)].

Reproducibility of substance P-induced vasodilatation

In Experiment 2, the magnitudes of vasodilator responses to the first and second series of infusions of substance P were similar (Fig. 5).

Effects of l-arginine on substance P-induced vasodilatation

In Experiment 4, intra-arterial infusion of l-arginine did not alter resting arterial pressure, FBF, forearm vascular resistance (Table 4) and heart rate (data not shown). l-Arginine did not alter vasodilator responses to substance P.

Effects of indomethacin on substance P-induced vasodilatation

In Experiment 5, oral administration of indomethacin (75 mg) did not alter resting arterial pressure,
FBF, forearm vascular resistance (Table 5) and heart rate (data not shown). Forearm vasodilating responses to substance P did not differ before and after indomethacin administration.

**DISCUSSION**

The major purpose of the present study was to determine whether substance P-induced vasodilatation in human forearms is mediated by nitric oxide by examining the effects of L-NMMA (a blocker of nitric oxide synthesis) and L-arginine (the precursor of nitric oxide) on substance P-induced changes in forearm haemodynamics. We and other investigators have previously demonstrated that L-NMMA at 4 µmol/min blocks [5-7], and L-arginine at 8-10 mg/min augments [8, 15], acetylcholine-induced forearm vasodilatation, and that neither L-NMMA nor L-arginine affects SNP-induced vasodilatation [6, 15]. These results confirm that acetylcholine-induced vasodilatation is mediated by nitric oxide in humans. Using the same probes, we determined whether substance P-induced vasodilatation is mediated by nitric oxide in human forearms. Intra-arterial infusion of L-NMMA inhibited acetylcholine-induced vasodilatation but did not alter substance P-induced vasodilatation. Supplementation of L-arginine did not augment substance P-induced vasodilatation. Thus, our results suggest that there may be a smaller nitric oxide component to substance P-induced vasodilatation in the forearm resistance vessels of healthy humans.

**Table 4. Forearm haemodynamics before and after infusion of L-arginine.** Abbreviations: MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; NS, not significant. Data are means ± SEM.

<table>
<thead>
<tr>
<th>Substance P (ng/min) (n = 6)</th>
<th>Control</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
<th>P value by two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>Before</td>
<td>85.5 ± 4.0</td>
<td>84.5 ± 3.3</td>
<td>84.0 ± 3.8</td>
<td>84.7 ± 3.4</td>
<td>84.0 ± 3.8</td>
<td>83.8 ± 3.6</td>
</tr>
<tr>
<td>After</td>
<td>88.2 ± 4.7</td>
<td>86.3 ± 4.1</td>
<td>85.7 ± 4.0</td>
<td>85.8 ± 4.6</td>
<td>86.2 ± 4.1</td>
<td>86.3 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹)</td>
<td>Before</td>
<td>4.3 ± 0.6</td>
<td>5.7 ± 0.8</td>
<td>8.0 ± 0.9</td>
<td>10.4 ± 1.0</td>
<td>12.2 ± 1.6</td>
<td>12.3 ± 1.5</td>
</tr>
<tr>
<td>After</td>
<td>4.5 ± 0.8</td>
<td>6.3 ± 1.1</td>
<td>6.8 ± 1.4</td>
<td>10.6 ± 1.9</td>
<td>13.1 ± 2.0</td>
<td>14.3 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>FVR (units)</td>
<td>Before</td>
<td>22.0 ± 4.0</td>
<td>16.2 ± 2.7</td>
<td>11.1 ± 1.3</td>
<td>8.4 ± 0.7</td>
<td>7.3 ± 0.8</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>After</td>
<td>22.3 ± 3.8</td>
<td>15.5 ± 2.7</td>
<td>14.0 ± 1.9</td>
<td>9.1 ± 1.5</td>
<td>7.1 ± 0.8</td>
<td>6.7 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5. Forearm haemodynamics before and after oral administration of indomethacin.** Abbreviations: MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; NS, not significant. Data are means ± SEM.

<table>
<thead>
<tr>
<th>Substance P (ng/min) (n = 5)</th>
<th>Control</th>
<th>0.8</th>
<th>1.6</th>
<th>3.2</th>
<th>P value by two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>Before</td>
<td>89.3 ± 4.1</td>
<td>89.3 ± 4.0</td>
<td>86.1 ± 3.7</td>
<td>86.3 ± 3.8</td>
</tr>
<tr>
<td>After</td>
<td>92.3 ± 3.9</td>
<td>91.2 ± 3.7</td>
<td>91.1 ± 3.4</td>
<td>91.1 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹)</td>
<td>Before</td>
<td>3.4 ± 0.6</td>
<td>5.9 ± 0.5</td>
<td>9.6 ± 1.8</td>
<td>12.2 ± 1.3</td>
</tr>
<tr>
<td>After</td>
<td>3.4 ± 0.8</td>
<td>5.9 ± 0.9</td>
<td>9.2 ± 1.7</td>
<td>11.3 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>FVR (units)</td>
<td>Before</td>
<td>30.0 ± 6.6</td>
<td>15.5 ± 1.3</td>
<td>9.8 ± 1.6</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>After</td>
<td>31.2 ± 6.7</td>
<td>16.6 ± 2.4</td>
<td>1.1 ± 2.3</td>
<td>9.3 ± 2.2</td>
<td></td>
</tr>
</tbody>
</table>
We performed this experiment because acetylcholine and substance P are frequently used to examine endothelial function in humans on the assumption that both drugs cause vasodilatation by releasing nitric oxide from the endothelium. It has been reported in many pathological states including atherosclerosis [1], variant angina [2, 3] and heart failure [4] that acetylcholine-induced vasodilatation is impaired but substance P-induced vasodilatation is preserved in the coronary vasculature or forearm vasculature. These findings are interpreted as selective impairment of muscarinic receptors to release nitric oxide although there may be enhanced smooth muscle sensitivity to the muscarinic effects of acetylcholine in the case of large coronary artery. In a recent study, Panza et al. [10] reported that patients with essential hypertension have impaired endothelium-dependent vasodilator responses to both acetylcholine and substance P, indicating that the endothelial abnormality in this condition is not restricted to the muscarinic receptor. In their study, substance P-induced vasodilatation was reduced after infusion of L-NMMA (4 μmol/min for 5 min) in normal subjects. Thus, it was suggested that substance P-induced vasodilatation is also mediated by release of nitric oxide from the endothelium. We cannot fully explain the differences between the results of their study and ours. However, based on the following findings, we think that substance P-induced forearm vasodilatation may not be mediated by the release of nitric oxide from the endothelium. First of all, we used not only a low dose but also a high dose of L-NMMA to inhibit nitric oxide formation. Previous investigators used only the low dose of L-NMMA (4 μmol/min for 5 min) [6–8, 10]. In this study, before L-NMMA infusion, acetylcholine and substance P similarly increased FBF from 4.3±1.1 to 15.6±3.0 ml min⁻¹ 100 ml⁻¹ and from 4.7±1.6 to 17.1±4.5 ml min⁻¹ 100 ml⁻¹, respectively (Table 2). The high dose of L-NMMA (8 μmol/min for 5 min) significantly attenuated increases in FBF induced by acetylcholine (from 2.5±0.6 to 5.4±1.3 ml min⁻¹ 100 ml⁻¹), whereas substance P still increased FBF from 2.6±0.7 to 10.7±1.7 ml min⁻¹ 100 ml⁻¹ after L-NMMA infusion (Table 2). Secondly, the normalized FBF (the percentage changes) was used for analysis. The high dose of L-NMMA attenuated acetylcholine-induced increases in normalized FBF but did not affect substance P-induced increases [Fig. 4(top)]. Thirdly, we examined vasodilator responses to SNP (an endothelium-independent vasodilator) before and after infusion of L-NMMA because it was possible that changes in resting forearm vessel tone induced by L-NMMA would affect the degree of vasodilatation to substance P. Decreases in resting FBF after infusion of L-NMMA were similar for SNP and substance P (Table 3), and relative vasodilatation (normalized FBF responses) to both agents was similar [Fig. 4(bottom)]. Finally, although it has been shown that supplementation with l-arginine augments acetylcholine-induced vasodilatation [7, 8, 10], supplementation with l-arginine did not augment substance P-induced vasodilatation in this study. Taken together, we think that substance P-induced forearm vasodilatation is mediated mostly by other mechanisms such as endothelium-dependent hyperpolarizing factor [11, 16, 17] rather than nitric oxide. It is also possible, however, that there may be a small component of nitric oxide to substance P-induced vasodilatation.

If the vasodilatation evoked by substance P is not mediated by nitric oxide, the interpretation of the discordant effects between acetylcholine and substance P would be different. Although we could not determine the mechanisms underlying substance P-induced vasodilatation in humans, results in animals in vitro and in vivo suggest that substance P may release endothelium-derived hyperpolarizing factors [11, 16, 17] and relax vascular smooth muscle. Thus, from our results, it may be possible that preserved substance P-induced vasodilatation in some pathological states is caused by intact vasodilator mechanisms involving endothelium-derived hyperpolarizing factors, although we cannot measure the membrane potential in the human vascular endothelium in vivo.

Because the plethysmographic measurements allowed us to examine forearm vasodilator responses of resistance vessels but not those of conduit vessels, we were unable to determine whether vasodilatation in response to substance P in the conduit vessels was mediated by nitric oxide. Chester et al. [18, 19] demonstrated that substance P-induced vasodilatation of excised human epicardial coronary arteries was inhibited by L-NMMA. We do not know the mechanism underlying the different effects of L-NMMA on substance P-induced vasodilatation in forearm resistance vessels and epicardial conduit vessels. It is possible that the contribution of nitric oxide to substance P-induced vasodilatation may differ between vascular beds. In fact, it has been reported that substance P caused disparate effects on systemic and coronary haemodynamics in conscious dogs, i.e. vasodilatation in the systemic circulation and vasoconstriction in the coronary circulation [20], and that the substance P-mediated relaxing effect was more potent in distal than in proximal arterial segments in human epicardial coronary arteries [21]. Therefore, these data suggest that the contribution of nitric oxide to substance P-induced vasodilatation may depend on the organ or the size of the arteries.

We also examined whether substance P-induced vasodilatation is mediated by vasodilatory prostanoids in the human forearm by examining FBF responses to substance P before and after indomethacin. Substance P increased FBF but substance P-induced forearm vasodilatation was not affected by indomethacin. From this evidence, we concluded that vasodilatory prostanoids may not contribute to substance P-induced vasodilatation. Because
vasodilatation was probably not due to the inade-
quately dose as it was shown previously that plasma
concentrations of indomethacin reached levels of 0.9
to 4 μg/ml at 1 h after oral administration of 50 mg
[22], concentrations sufficient to inhibit production
of prostaglandins [23]. In addition, this dose of
indomethacin did not change resting FBF in humans
[24].

L-NMMA did not affect ATP-induced vasodilata-
tion in this study. Although we did not test whether
a higher dose of L-NMMA inhibited ATP-induced
vasodilatation and whether supplementation of
L-arginine augmented ATP-induced vasodilatation,
it is not likely from the same lines of discussion that
ATP-induced vasodilatation in the human forearm is
mediated by nitric oxide. Results of animal experi-
ments also suggest that ATP relaxes vascular smooth
muscle by directly acting on the adenosine receptors
of vascular smooth muscle [25–27].

CONCLUSION

In forearm vessels of the healthy subjects, intra-
artrial infusion of L-NMMA did not affect subst-
tance P- and ATP-induced vasodilatation. In addi-
tion, supplementation of L-arginine did not aug-
ment, and pretreatment of indomethacin did not
attenuate, substance P-induced vasodilatation. Thus,
our results suggest that substance P-induced
vasodilatation may be mediated by neither nitric
oxide nor prostaglandins in the human forearm and
that ATP-induced vasodilatation may not be
mediated by nitric oxide.

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