Acute hypertension impairs endothelium-dependent vasodilatation

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1. Previous investigations have demonstrated an impaired endothelium-dependent vasodilatation (EDV) in patients with hypertension. The present study aimed to investigate if an acute rise in blood pressure to hypertensive levels impairs EDV in otherwise normotensive subjects.

2. Twenty-seven young, healthy, normotensive subjects were studied. Eight of these underwent evaluation of EDV and endothelium-independent vasodilatation (EIDV) by means of forearm blood flow measurements during local intra-arterial infusions of methacholine (2 and 4 µg/min) and sodium nitroprusside (5 and 10 µg/min), before and after 1 h of sustained hypertension, induced by noradrenaline given intravenously. Identical measurements were made in 11 subjects before and during concomitant local intra-arterial infusion of noradrenaline without change in blood pressure and eight subjects were studied during saline infusions.

3. One hour of sustained hypertension (diastolic blood pressure > 95 mmHg) significantly attenuated both forearm blood flow (17.4 ± 6.8 versus 27.4 ± 6.8 ml·min⁻¹·100 ml⁻¹ tissue at baseline, P < 0.05) and forearm vascular resistance decrease (3.2 ± 0.87 versus 7.4 ± 2.5 units at baseline, P < 0.05) during methacholine infusion. These attenuations were significantly more pronounced for methacholine than for sodium nitroprusside (P < 0.05). In contrast, local intra-arterial noradrenaline infusions impaired vasodilatation induced by methacholine and sodium nitroprusside to a similar extent. Saline infusions did not change either EDV or EIDV.

4. Thus, an acute rise in blood pressure to hypertensive levels induced by noradrenaline impaired EDV more than EIDV in otherwise normotensive subjects, while no such selective effect of local noradrenaline was seen, suggesting that a high blood pressure impairs endothelial vasodilator function.

INTRODUCTION

The vascular endothelium has been found to be an important modulator of vascular tone and peripheral blood flow. Nitric oxide (NO) and endothelin are two major endothelium-derived factors responsible for vasodilatation and vasoconstriction [1–7].

Most [8–15], but not all [16], investigators have found an association between essential hypertension and an attenuated endothelium-dependent vasodilatation (EDV). Whether this endothelial dysfunction is a primary or secondary phenomenon of the hypertensive disease is not known.

The present study aimed to investigate if an acute rise in blood pressure to hypertensive levels impairs the endothelial vasodilator function in young, otherwise normotensive subjects. The forearm blood flow (FBF) response to local intra-arterial infusions of methacholine (MCh, evaluating EDV) and sodium nitroprusside [SNP, evaluating endothelium-independent vasodilatation (EIDV)] was studied before and after 1 h of sustained acute hypertension induced by noradrenaline (NA).

MATERIAL AND METHODS

Subjects

Twenty-seven healthy, normotensive, non-smoking subjects (14 males and 13 females), aged 23 ± 3 years, were studied. All subjects were normotensive and were free of dyslipidaemia, diabetes mellitus or cardiovascular disorders.

Eight of these subjects underwent evaluation of EDV and EIDV by means of FBF measurements during local intra-arterial (brachial artery) infusions of MCh and SNP, mainly in the non-dominant arm, before and after 1 h of sustained hypertension, induced by individually titrated doses of NA given intravenously.

In order to evaluate the role of a direct, local endothelial effect of NA, identical measurements of EDV and EIDV were made in 11 subjects before and during concomitant local intra-arterial infusion of NA.

To control for any effects of the study design, four subjects received intravenous saline infusion and four received local intra-arterial saline infusion instead of NA.
Measurements of FBF

During the blood flow measurements, the subjects were supine in an air-conditioned room maintained at a constant temperature (20 °C). An arterial cannula was inserted into the brachial artery of one arm for regional infusions of MCh and SNP together with NA or saline.

The vasodilatory drug infusions were given during 5 min for each dose with a 20-min washout period between the different drugs. The infused doses were 2 and 4 μg/min for MCh and 5 and 10 μg/min for SNP. The drugs were given in a random order at a rate of 1 ml/min.

Before and at the end of the different doses of the two drugs, FBF was measured in both forearms by venous occlusion plethysmography as follows. A mercury-in-silastic strain gauge, connected to a calibrated plethysmograph, was placed at the upper third of the forearm, which rested comfortably slightly above the level of the heart. Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mmHg by a rapid cuff inflator; approximately 4 inflations per minute were made, each of about 7 s. The vasodilatory infusions were undertaken in one of the arms while the contralateral arm served as a control. Evaluations of FBF were made by calculation of the mean of five consecutive recordings.

Monitoring of blood pressure and central haemodynamics

Blood pressure (BP) was monitored by an automatic device (OMRON® HEM 705C, Tokyo, Japan) before the start of NA infusion, in connection with each FBF measurement and after 1 and 2 h of intravenous or intra-arterial NA infusions. Stroke volume and heart rate were monitored by a thoracic bioimpedance cardiograph (BioMed® NCCOM® 3 Cardiodynamic Monitor, Irvine, CA, U.S.A.) in parallel with the BP recordings.

Systemic NA infusions

Systemic NA infusions were given in a cannula inserted in an antecubital vein in the control arm. Since the pressor response to a certain dose of NA is known to show a great inter-individual variation, the dose was titrated individually in increments every fifth minute, in order to achieve a diastolic BP exceeding 95 mmHg. Other studies have shown that infusion rates of this magnitude result in arterial concentrations of NA in the range of 10–50 nmol/l [18,19]. After 1 h of sustained acute hypertension, infusion of NA was continued at the same dose for another hour, during which the local intra-arterial MCh and SNP infusions were repeated.

In five subjects in whom EDV was evaluated after EIDV, the MCh infusion was continued for 10 min after discontinuation of the NA infusion. During this period BP and FBF were measured every second minute.

Local NA infusions

In six subjects, local intra-arterial infusions of NA were given at a dose titrated in a stepwise increasing manner to define the highest possible dose without any effect on baseline FBF. This dose was thereafter chosen for continuous infusion during 1 h. Another five subjects received an intra-arterial infusion of NA, individually titrated to the maximal dose that could be given without causing systemic effects. This dose was thereafter infused during 1 h.

After 1 h of the local NA infusions, the intra-arterial infusions of MCh and SNP were repeated during concomitant NA infusion. Local intra-arterial NA was given at a maximal rate of 0.5 ml/min.

The control studies with intravenous or local intra-arterial saline infusion mimicked the NA studies with regard to infusion rates (both 0.5 ml/min), time intervals of infusions and all other measurements.

The study protocol was approved by the Hospital Ethics Committee and informed consent was obtained from all participants.
Calculations of haemodynamics

Mean artery pressure (MAP) was calculated as one-third of the pulse pressure (systolic BP minus diastolic BP) added to diastolic BP.

Cardiac index was calculated as stroke volume times heart rate indexed for body surface area.

Total peripheral vascular resistance index was calculated by the formula \(80 \times (MAP - 3)/\text{cardiac index}\) (in which 3 is the central venous pressure).

Forearm vascular resistance was given by MAP divided by FBF.

Statistical analysis

Differences within each subject were calculated by means of the Wilcoxon signed rank sum test (two-tailed). The results were also evaluated by calculating the area under the curve, and by analysis of variance (ANOVA) for repeated measurements with two within-individual variables, dose of vasodilator and the use of NA or not as a factor variable. \(P < 0.05\) was regarded as significant.

RESULTS

Systemic NA infusions

Individually titrated doses of NA given intravenously increased MAP from 83±6 to 116±10 mmHg (± S.D.) \((P < 0.001)\). Total peripheral vascular resistance index increased by 33±32% \((P < 0.05)\), while neither heart rate nor cardiac index changed significantly during the same period. These systemic haemodynamics were not significantly altered during the second hour of NA infusion during which EDV and EIDV were evaluated (Table 1).

Table I  Haemodynamic changes during systemic NA Infusion

<table>
<thead>
<tr>
<th></th>
<th>Before NA infusion</th>
<th>After NA infusion</th>
<th>1 h</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110±6</td>
<td>152±10*</td>
<td>151±13*</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70±7</td>
<td>96±10*</td>
<td>97±10*</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>69±8</td>
<td>58±10</td>
<td>56±11</td>
<td></td>
</tr>
<tr>
<td>Cardiac index (l·min⁻¹·m⁻²)</td>
<td>3.7±0.6</td>
<td>4.1±1.0</td>
<td>4.0±0.8</td>
<td></td>
</tr>
<tr>
<td>Total peripheral resistance index (dyn × s × m²/cm²)</td>
<td>1765±319</td>
<td>2331±563*</td>
<td>2373±554*</td>
<td></td>
</tr>
</tbody>
</table>

\(^*P < 0.05\).

After 1 h of sustained acute hypertension [mean dose (range) of NA 16.7±8.4 (6.7-33.3) \(\mu\)g/min], baseline FBF increased significantly \((P < 0.05)\). However, the increase in FBF induced by MCh during acute hypertension was significantly lower when compared with the corresponding MCh-induced increase during normotensive conditions \((P < 0.05\) at both doses, Figure 1). On the other hand, the FBF response to SNP was not significantly altered by the NA-induced hypertension (Figure 2). This difference in response of FBF to NA for MCh and SNP was significant \((P < 0.05)\). After 1 h of sustained hypertension, although baseline forearm vascular resistance (FVR) was unaltered, MCh infusion evoked a less pronounced reduction in FVR when compared with the reduction achieved during normotension (Figure 1). Also the reduction in FVR seen during SNP infusion was significantly attenuated during acute hypertension when compared with that achieved during normotensive conditions (Figure 2). However, the attenuation of the reduction in FVR induced by acute hypertension was significantly more pronounced for MCh than for SNP infusion \((P < 0.05)\).

The reduction in EDV by systemic NA infusion was highly reproducible as it was seen in all of the subjects. The reported significance values regarding the sys-
systemic effects of NA on MCh-induced and SNP-induced vasodilatation were the same regardless of whether the statistical analysis was performed with Wilcoxon signed rank sum test, the area under the curve approach or with ANOVA for repeated measurements with two within-individual variables, dose of vasodilator and the use of NA or not as a factor variable.

No significant relations were observed between the dose of systemic NA infused and the degree of vasodilatory attenuation during either MCh or SNP infusion.

A rapid decline in MAP and an increase in MCh-induced FBF were seen after termination of NA infusion (Figure 3).

**Local NA infusions**

The local NA infusions, at the maximal dose without inducing any change in baseline FBF [mean (range) 9.0 ± 15 (1.7–40) ng/min], caused no significant changes in central haemodynamics (MAP +1 ± 2 mmHg, heart rate – 1 ± 2 beats/min). Neither was any significant alteration in FBF response to MCh or SNP observed (Table 2).

When NA was given at a dose [mean 3.3 ± 1.7 (1.7–5.0 μg/min)] inducing minimal effects on MAP (+2 ± 1 mmHg) and heart rate (–5 ± 4 beats/min) during 1 h, baseline FBF decreased by about 60% and baseline FVR was more than doubled (Table 2). Local NA infusion at this vasoconstrictive dose caused significantly lower FBF during both MCh and SNP infusions, when compared with the values noted before the local infusion of the pressor drug (both \( P < 0.05 \), Table 2). However, the FBF responses to MCh and SNP were equally affected by the local vasoconstrictive dose of NA.

The reported significance values regarding the local effects of NA on MCh-induced and SNP-induced vasodilatation were the same regardless of whether the statistical analysis was performed with Wilcoxon signed rank sum test, the area under the curve approach or with ANOVA for repeated measurements with two within-individual variables, dose of vasodilator and the use of NA or not as a factor variable.

The relative attenuation of both the MCh- and the SNP-induced FBF was closely correlated to the vasoconstrictive NA dose given locally (\( r = 0.81, \ P < 0.05 \) and \( r = 0.95, \ P < 0.001 \) respectively).

**Saline infusions**

None of the systemic haemodynamic parameters measured, or the MCh- or SNP-induced vasodilatation, was significantly affected by the local intra-arterial or intravenous saline infusions. The variation in FBF was 5 ± 4% between saline and baseline measurements.

No significant differences in the responses to local or systemic NA were seen between men and women. The FBF responses to MCh and SNP during local or systemic NA infusion were not significantly related to forearm circumference (mean 276 ± 23 mm, \( r = 0.07 – 0.11 \) during the different conditions) or length (mean 262 ± 15 mm, \( r = 0.03 – 0.12 \) during the different conditions).

<table>
<thead>
<tr>
<th>Time after termination of the NA infusion (min)</th>
</tr>
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<tbody>
<tr>
<td><img src="image-url" alt="Graph" /></td>
</tr>
</tbody>
</table>

**Figure 3** Development of MAP (○) and FBF (●) during infusion of MCh (4 μg/min) in the first 8 min after termination of acute hypertension induced by systemic NA infusion.

<table>
<thead>
<tr>
<th>Table 2 Endothelium-dependent and endothelium-independent vasodilatation before and during local intra-arterial infusions of NA in low (n = 5) and high dose (n = 6)</th>
</tr>
</thead>
</table>

NA was given locally in the forearm during 1 h at an individually titrated maximal dose without change in FBF ('low dose') and at an individually titrated maximal dose without systemic haemodynamic effects ('high dose'). *\( P < 0.05 \) compared with before local infusion of high dose.

<table>
<thead>
<tr>
<th>Forearm blood flow (ml/min-1 · 100 ml tissue)</th>
<th>Before NA infusion</th>
<th>During NA infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.0 ± 0.8</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>MCh, 2 μg/min</td>
<td>20 ± 3.3</td>
<td>20 ± 2.5</td>
</tr>
<tr>
<td>MCh, 4 μg/min</td>
<td>24 ± 3.6</td>
<td>23 ± 3.2</td>
</tr>
<tr>
<td>SNP, 5 μg/min</td>
<td>18 ± 5.1</td>
<td>18 ± 3.0</td>
</tr>
<tr>
<td>SNP, 10 μg/min</td>
<td>25 ± 5.6</td>
<td>23 ± 3.8</td>
</tr>
<tr>
<td>High dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.6 ± 2.1</td>
<td>1.9 ± 1.1*</td>
</tr>
<tr>
<td>MCh, 2 μg/min</td>
<td>21 ± 3.9</td>
<td>9.8 ± 4.0*</td>
</tr>
<tr>
<td>MCh, 4 μg/min</td>
<td>25 ± 3.5</td>
<td>14 ± 4.6*</td>
</tr>
<tr>
<td>SNP, 5 μg/min</td>
<td>14 ± 2.6</td>
<td>5.4 ± 2.4*</td>
</tr>
<tr>
<td>SNP, 10 μg/min</td>
<td>19 ± 3.8</td>
<td>8.8 ± 4.6*</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Forearm vascular resistance (mmHg/ml min-1 · 100 ml tissue)</th>
<th>Before NA infusion</th>
<th>During NA infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21 ± 4.1</td>
<td>22 ± 6.6</td>
</tr>
<tr>
<td>MCh, 2 μg/min</td>
<td>4.2 ± 0.8</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>MCh, 4 μg/min</td>
<td>3.4 ± 0.6</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>SNP, 5 μg/min</td>
<td>4.9 ± 1.8</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>SNP, 10 μg/min</td>
<td>3.5 ± 1.2</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>High dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22 ± 11</td>
<td>55 ± 23*</td>
</tr>
<tr>
<td>MCh, 2 μg/min</td>
<td>41 ± 0.7</td>
<td>11 ± 6.1*</td>
</tr>
<tr>
<td>MCh, 4 μg/min</td>
<td>3.4 ± 0.4</td>
<td>7.1 ± 3.3*</td>
</tr>
<tr>
<td>SNP, 5 μg/min</td>
<td>6.1 ± 1.3</td>
<td>19 ± 9.9*</td>
</tr>
<tr>
<td>SNP, 10 μg/min</td>
<td>4.5 ± 1.3</td>
<td>13 ± 7.8*</td>
</tr>
</tbody>
</table>
During the infusions with MCh and SNP FBF in the contralateral arm did not change significantly ( < 5%) compared with the level measured before the infusions. Thus, the basal FBF was stable during the separate experimental conditions (before NA, during local infusion of NA and during systemic NA infusion) and the results were therefore not corrected for any deviations in basal FBF.

**DISCUSSION**

The present results demonstrate that acute hypertension induced by NA infusion in healthy normotensive subjects significantly impaired EDV more than EIDV in the forearm circulation.

This finding suggests that an acute rise in BP could have a negative effect on EDV. This is in agreement with other investigators showing an impaired EDV in subjects with hypertension [8–15]. However, not all patients with essential hypertension show an attenuated EDV and one study using various muscarine receptor agonists could not find any impaired EDV in patients with essential hypertension [16]. Thus, although the impairment in EDV was seen in all of the subjects who experienced an acute elevation of BP in the present study, it is obvious that this finding cannot be found in all subjects with a chronic elevation in BP.

It is not likely that the impairment of EDV is merely a consequence of NA acting directly on endothelial function, since no selective attenuation of EDV was seen during local infusion of NA in the forearm. At a dose without vasoconstrictive effects, intra-arterial NA infusion caused no significant alteration in FBF response, either to MCh or SNP. When a vasoconstrictive dose of NA was given locally, a non-specific general suppression of the vasodilatory process in a dose-dependent manner was observed, during which the vascular responses to MCh and SNP were similarly affected. In agreement with the present findings, John et al. [20] found that EDV and EIDV were equally impaired by local exposure of the rabbit aorta to NA at different concentrations. The latter is further supported by the finding in the present study that the relationships between the locally given dose of NA and EDV or EIDV were almost identical.

Thus, the effects of acute hypertension induced by NA given systemically seem to comprise a moderate impairment of vasodilatation in general, possibly mediated by the local effect of NA, together with a more selective attenuation of EDV, induced by the increased BP. This assumption is in accordance with the experimental results of Rubanyi [21], who showed that a raised transmural pressure suppressed EDV in the canine carotid artery.

It may be argued that the increased baseline FBF seen during acute hypertension in the present study might contribute to the weakened MCh response by a diluting effect. However, this could not explain the selective effect of acute hypertension on the MCh response as MCh has been considered a more stable compound than SNP. Moreover, our previous observations in normotensive subjects do not support the assumption that the MCh response is dependent on baseline FBF.

The present study did not explore the mechanisms responsible for the negative effect of acute hypertension on EDV. Several local or systemic vasoregulatory mechanisms might be involved in this response.

Harder et al. [22] demonstrated that a raised transmural pressure in the cat cerebral artery resulted in the release of an endothelium-derived contracting factor (EDCF) with a short duration of effect. Several investigators have found that the attenuation of EDV seen as BP rises during the first months of life in the spontaneous hypertensive rat is mediated by a cyclooxygenase-dependent, short-lived EDCF [23–26] able to counteract the vasodilatory action of NO [27]. In humans, a cyclooxygenase-dependent EDCF is at least in part responsible for the impaired EDV seen in essential hypertension [11].

Another potentially interesting substance in this context is endothelin-1 (ET-1), a powerful vasoconstrictor counter-regulating the vasodilatory NO system, synthesized primarily by endothelial cells [4–7]. Hishikawa et al. [28] recently found that release of ET-1 from human endothelial cells was increased during high-pressure conditions in vitro. Even if no convincing evidence for elevated plasma ET-1 levels in patients with essential hypertension has emerged [29–32], previous investigations indicate that ET-1 is primarily released abluminally and that plasma levels only reflect luminal spillover [33]. Thus, the measurement of circulating ET-1 levels probably gives a vague picture of the exposure of the vascular smooth muscle cells to this primarily paracrine substance.

Previous investigations concerning the direct effect of NA on endothelial cells and the vasodilatory processes have produced ambiguous results. NA has been shown to stimulate NO release [34] even at doses not causing vasoconstriction, presumably by stimulating α-adrenergic receptors on the endothelial cells [35–37]. On the other hand, there is also evidence that NA can promote ET-1 production in endothelial cells [4].

Thus, previous investigations do not provide any evidence for a selective impairment of EDV by NA. A non-specific interference with the vascular smooth muscle in general seems more likely, which is consistent with the present results.

It has previously been shown that stimulation of endothelial muscarine receptor causes release of NO [38]. Both acetylcholine and methacholine have been used as muscarinic receptor agonists for this purpose. Conflicting results exist as to whether or not N-monomethyl-L-arginine, a blocker of NO synthesis, could blunt the vasodilatation induced by methacholine [39,40]. However, what is evident from these kinds of studies is that N-monomethyl-L-arginine does not completely block the vasodilatation evoked by methacholine or acetylcholine, suggesting that muscarine receptor activation in part causes vasodilatation.
by other means than NO release. Activation of muscarine receptors has been shown to induce release of other vasoactive substances, such as prostanoids, endothelin and an as yet unidentified endothelium-derived hyperpolarizing factor [41]. Furthermore, a direct effect on vascular smooth muscle cells by muscarine receptor agonists cannot be excluded. Thus, it is not certain that the results obtained in the present study are only a consequence of the action of a rise in BP on NO production. Several other endothelium-derived systems could be involved and further studies are needed to clarify this matter.

In conclusion, although a significant relationship between the diastolic BP level and EDV in the coronary microvasculature of patients with hypertension has been reported [13] and the reversal of secondary hypertension has been shown to normalize EDV [11], the present study provides the first direct evidence that a rise in BP to hypertensive levels impairs EDV in humans.

As can be seen in Figure 3, the reversal of this effect is very rapid. However, whether or not an impaired EDV is involved in the development of essential hypertension is another question that remains to be answered.

REFERENCES


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