Forearm vasoconstriction in response to noradrenaline and $N^G$-monomethyl-L-arginine in essential hypertension

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ABSTRACT

A role for abnormal NO production in essential hypertension remains controversial. Blunted vasoconstriction of forearm resistance vasculature in response to $N^G$-monomethyl-L-arginine ($L$-NMMA; an inhibitor of NO biosynthesis), relative to the response to noradrenaline, has been reported in hypertensive patients and interpreted as evidence of reduced basal NO biosynthesis. We sought to determine whether reduced sensitivity of forearm vasculature to the vasoconstrictor action of $L$-NMMA relative to that of noradrenaline is a consistent finding in essential hypertension. We studied a group of patients ($n = 32$; blood pressure $176 \pm 4/102 \pm 2$ mmHg; means $\pm$ S.E.M.) and a group of healthy normotensive controls ($n = 32$; blood pressure $130 \pm 2/75 \pm 1$ mmHg). Noradrenaline ($60–240$ pmol $[\text{min}^{-1}]$) and $L$-NMMA ($1–4$ l mol $[\text{min}^{-1}]$) were infused into the brachial artery, and forearm blood flow was measured by venous occlusion plethysmography. The effects of each vasoconstrictor were similar in hypertensive and control subjects. The highest dose of $L$-NMMA reduced forearm blood flow by $0.75 \pm 0.12$ ml $[\text{min}^{-1}]$ $[\text{dl}^{-1}]$ in the control group and by $0.89 \pm 0.10$ ml $[\text{min}^{-1}]$ $[\text{dl}^{-1}]$ in the hypertensive group. The study had $90\%$ power (with $P = 0.05$) to detect a $10\%$ difference in forearm blood flow response between the hypertensive and control groups. We conclude that reduced sensitivity of forearm resistance vasculature to the vasoconstrictor action of $L$-NMMA is not a universal feature of essential hypertension. This argues against a primary role for reduced basal NO biosynthesis in skeletal muscle resistance vessels in the pathogenesis of essential hypertension.

INTRODUCTION

Nitric oxide (NO) is synthesized from l-arginine in vascular endothelial cells by a constitutive isofrm of NO synthase (eNOS). Basal release of NO plays a role in the control of resistance-vessel tone and arterial blood pressure. It has therefore been suggested that abnormal NO production plays a part in the pathogenesis of essential hypertension, but this remains controversial. Brachial artery infusion of drugs and hormones has been widely used to study resistance-vessel function in essential hypertension. Endothelial function has been investigated in this way either by using endothelium-dependent vasodilators (notably acetylcholine) to stimulate the l-arginine/NO pathway, or by infusion of $N^G$-monomethyl-l-arginine ($L$-NMMA) to inhibit basal NO biosynthesis.

Several studies have reported blunted vasodilator responses to acetylcholine in essential hypertension [1–3], although this is not a universal finding [4]. Vasocon-
strictor responses to brachial artery administration of L-NMMA may reflect basal release of NO under physiological conditions of pulsatile flow, which provides a mechanical stimulus to NO production. However, such studies have yielded conflicting results. Three studies reported that the mean vasoconstrictor response to L-NMMA was less in patients with essential hypertension than in normotensive controls [5–7]. Two other studies, focused primarily on vasodilator responses in essential hypertension, incidentally reported similar responses to L-NMMA in patients with essential hypertension and normotensive subjects [8, 9]. These studies employed relatively small numbers (less than 13 in each) of hypertensive patients. Determination of the vasoconstrictor response to L-NMMA was the primary aim in only one study [5], which was also the only one to employ a comparator vasoconstrictor (noradrenaline), an approach that increases the power of this method [10].

Because of the importance of the question of whether reduced basal NO biosynthesis is necessary for the development of essential hypertension, we have re-addressed this issue. We studied subjects with uncomplicated essential hypertension to determine whether reduced sensitivity of this vascular bed to the vasoconstrictor action of L-NMMA, relative to that of noradrenaline, is a consistent finding in essential hypertension.

METHODS

Subjects
Healthy volunteers were recruited by local advertisement in Southeast London, and otherwise healthy patients with uncomplicated essential hypertension were recruited consecutively from the Guy’s Hospital hypertension clinic. None of these subjects had taken part in our earlier studies of responsiveness to acetylcholine in essential hypertension [4]. Subjects were diagnosed on the basis of blood pressure readings taken during three or more consecutive clinic visits when diastolic blood pressure exceeded 100 mmHg. Blood pressure was measured by sphygmomanometry in the clinic after 5 min seated. Secondary causes of hypertension, metabolic abnormalities and evidence of end-organ damage were sought by history, physical examination and laboratory testing, including echocardiography, urinalysis, measurements of plasma creatinine and electrolytes, and further biochemical and imaging studies to exclude underlying causes of hypertension when clinically indicated. Healthy volunteers were screened by history and physical examination. Serum total cholesterol and triacylglycerols were measured in all subjects following an overnight fast. Subject characteristics are summarized in Table 1.

Protocol
The protocol was similar to that in the only previous study comparing vasoconstrictor responses caused by L-NMMA with responses to noradrenaline in untreated essential hypertension [5]. It was approved by both the Guy’s Hospital and the St Thomas’ Hospital Research Ethics Committees, and all subjects gave written informed consent. Anti-hypertensive treatment was discontinued 2 weeks before the study. No subjects were taking any medication at the time of the study. Studies were performed in a quiet temperature-controlled laboratory (24–26°C). A 27-gauge unmounted steel needle (Cooper’s Needle Works, Birmingham, U.K.), sealed with dental wax to an epidural cannula, was inserted into the left brachial artery using less than 1 ml of 1% lignocaine hydrochloride to provide local anaesthesia. Drugs were dissolved in 0.9% NaCl, and saline or drug solution was infused at 1 ml·min⁻¹ using a constant-infusion pump.

Forearm blood flow was measured in both arms simultaneously by venous occlusion plethysmography [11] with electrically calibrated temperature-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n = 32)</th>
<th>Hypertensive subjects (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>20/12</td>
<td>20/12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.4 ± 1.86</td>
<td>45.2 ± 1.87</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>28 ± 2.2</td>
<td>28 ± 0.6</td>
</tr>
<tr>
<td>Previous treatment (yes/no)</td>
<td>—</td>
<td>21/11</td>
</tr>
<tr>
<td>Ethnicity (W/B/I)</td>
<td>27/4/1</td>
<td>25/6/2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130 ± 2</td>
<td>176 ± 4 *</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 ± 1</td>
<td>102 ± 2 *</td>
</tr>
<tr>
<td>Total cholesterol (mmol·l⁻¹)</td>
<td>4.99 ± 0.19</td>
<td>5.39 ± 0.14</td>
</tr>
<tr>
<td>Non-smoker/smoker</td>
<td>27/5</td>
<td>27/5</td>
</tr>
<tr>
<td>Baseline blood flow (ml·min⁻¹·dl⁻¹)</td>
<td>2.33 ± 0.23</td>
<td>2.99 ± 0.30</td>
</tr>
</tbody>
</table>

*P < 0.0001 compared with values in control subjects.

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compensated strain gauges [12]. During measurements, the hands were excluded from the circulation by inflation of wrist cuffs to 180 mmHg or to 20 mmHg greater than systolic arterial pressure, whichever was greater. Upper-arm collecting cuffs were inflated to 40 mmHg. Basal blood flow was measured following saline infusion for at least 12 min. This was followed by cumulative infusions of noradrenaline (Levophed; Sanofi Winthrop, Guildford, Surrey, U.K.) at doses of 60, 120 and 240 pmol min⁻¹ (each dose for 6 min). Saline (0.9% NaCl) was then infused for 18 min, and a second series of baseline measurements was recorded before cumulative infusions of L-NMMA (Clinalfa, Laufelfingen, Switzerland) at doses of 1, 2, and 4 μmol min⁻¹ (each dose for 6 min). Forearm blood flow was measured for the final 3 min of each infusion period. Flow was recorded for approx. 10 s in every 15 s, and the mean of the final five measurements of each recording period was used for analysis. Blood flow was expressed as ml min⁻¹ 100 ml⁻¹ forearm volume.

Statistical analysis
Data were summarized as means ± S.E.M. The ratio of blood flow in the infused forearm to that in the non-infused forearm was calculated for each measurement period and used for analysis in addition to absolute forearm blood flow, to control for external influences such as state of arousal (which affects both arms similarly). Vasoconstrictor responses were expressed both as the absolute decrease in forearm blood flow (ml min⁻¹ dl⁻¹) and as the percentage decrease in the blood flow ratio (infused/control arm) relative to the immediately preceding baseline value, for comparison with previous studies that have used one or other of these measures. Repeated-measures analysis of variance was used to seek an interaction between drug (L-NMMA and noradrenaline) and hypertension in determining vascular responses. Effects of potentially confounding factors (gender, age and total cholesterol) were examined by incorporating these as co-variates. All P values are two-sided, and values of < 0.05 were considered to indicate statistical significance.

RESULTS
Forearm blood flow in the non-infused arm did not change significantly during infusion of noradrenaline or L-NMMA in either group of subjects, confirming that these drugs did not have systemic effects at these doses.

Noradrenaline and L-NMMA produced significant dose-dependent decreases in forearm blood flow both in hypertensive patients and in normotensive control subjects. The decrease in blood flow from baseline in response to each constrictor was similar in hypertensive patients and normotensive controls, whether expressed as the absolute decrease in blood flow (Figure 1a; noradrenaline, P = 0.74; L-NMMA, P = 0.47) or as the percentage decrease in the forearm blood flow ratio (Figure 1b; noradrenaline, P = 0.18; L-NMMA, P = 0.58).

DISCUSSION
The present finding that forearm blood flow responses to noradrenaline are not significantly different in hypertensive and normotensive subjects requires comment. It agrees with some previous observations [5,13], but differs...
from another early careful study using similar techniques in hypertensive and normotensive subjects [14], in which increased sensitivity to noradrenaline was observed in hypertensive subjects. When responses are expressed as the percentage decrease in the blood flow ratio (infused/control arm), there is a non-significant trend towards smaller responses in hypertensive subjects (Figure 1b), as was also apparent in a study of treated hypertensive patients [13]. The reason for these differences is not clear. Patients studied by Doyle and colleagues [14] had anti-hypertensive medication withdrawn only 24 h before the study, and the authors considered the possibility that the increased vascular response to noradrenaline that they observed was caused by residual effects of treatment. However, they rejected this possibility, because similar findings were observed in an untreated group of patients [14]. In subsequent work, it was found that the effective concentration of intra-arterial noradrenaline that increases forearm vascular resistance by 30% is similar in hypertensive and normotensive subjects, whereas responses to higher doses of noradrenaline and other vasoconstrictors are non-specifically increased in hypertensive patients, probably because of structural changes in resistance arteries [15]. There is evidence of reduced sympathetic activity and neuronal re-uptake, and of structural changes, in forearm resistance vessels of patients with longstanding essential hypertension [16]. Small gluteal or omental arteries in hypertensive patients have increased reactivity to noradrenaline compared with arteries in normotensive controls in terms of wall tension, but not in terms of active media stress [17]. It seems likely that vasoconstrictor sensitivity to noradrenaline will depend critically on the duration, severity and prior treatment of hypertension acting through structural changes in the media/lumen ratio of small resistance arteries, reduced neuronal uptake and possibly other mechanisms. Unrecognized characteristics of this kind may underlie this difference between our study and that of Doyle and colleagues [14].

The present observations do not confirm previous findings of reduced sensitivity to the vasoconstrictor action of L-NMMA in patients with essential hypertension, whether assessed alone [6] or relative to the effects of noradrenaline [5]. We used a protocol similar to that in the only previous study comparing vasoconstrictor responses caused by L-NMMA with responses to noradrenaline in essential hypertension [5], in order to permit direct comparison with that study. A theoretical disadvantage of a protocol in which noradrenaline and L-NMMA are infused sequentially is that L-NMMA causes persistent vasoconstriction. Noradrenaline is therefore infused first during each study. There is no evidence that this influences sensitivity to the subsequent infusion of L-NMMA [5], and a protocol where the comparator vasoconstrictor is infused on the same occasion as L-NMMA reduces variability. The size of our study was such that it had 90% power (with $P = 0.05$) to detect the $> 10\%$ difference in the decrease in forearm blood flow between the hypertensive and control groups reported previously [5]. It is therefore unlikely that we missed a true difference of this magnitude, and the main conclusion of the present study is that a substantially reduced sensitivity to L-NMMA of forearm resistance vasculature is not an invariable feature of essential hypertension. This has important implications for understanding the role of the NO/L-arginine system in the pathogenesis of essential hypertension.

eNOS-knockout mice have a higher mean arterial blood pressure than control wild-type littersmates [18]. In healthy human subjects, systemic administration of L-NMMA increases total systemic vascular resistance and increases arterial blood pressure [19]. There is thus convincing evidence for involvement of the L-arginine/NO pathway in the physiological regulation of resistance vessel tone and arterial blood pressure. It has been suggested [1–3,5,6] that decreased NO biosynthesis could contribute to increased peripheral vascular resistance in essential hypertension, but there is no evidence for a linkage of the eNOS gene with essential hypertension [20]. Indeed, a mutation of the eNOS gene that codes for a substitution of Asp for Glu at position 298 is reported to be a risk factor for coronary artery disease in an East Anglian population (odds ratio 4.7 for homozygotes; 95% confidence interval 2.5–7.7 compared with controls) [21]. There was no association between this mutant gene and arterial blood pressure, suggesting that subtle abnormalities in endothelial NO biosynthesis may influence atherosclerosis and/or thrombosis without affecting blood pressure, although the observed linkage between the eNOS gene variant and coronary disease may not have been causal.

Data obtained from experimental models of hypertension are conflicting. Endothelium-dependent relaxation in response to acetylcholine is impaired in aortae from rats with genetic hypertension, both the New Zealand strain [22] and the spontaneously hypertensive rat (SHR) [23,24]. However, systemic administration of acetylcholine reduces blood pressure similarly in SHRs and normotensive controls [25,26]. Reversible loss of endothelium-dependent relaxation of isolated aortae occurs in the salt-sensitive Dahl rat [27], salt/DOCA (deoxycorticosterone acetate), renovascular and coarctation-induced animal models of hypertension [28], suggesting that in these models endothelial dysfunction is secondary to hypertension rather than primarily involved in its pathogenesis. Haemododynamic responses to inhibition of basal NO synthesis are similar in SHRs compared with controls [25,29,30], although NO production appears to be decreased in isolated resistance vessels from hypertensive rats [31].

Evidence from studies on human essential hypertension is even more difficult to assess, because of genetic
and acquired differences (e.g. in cholesterol or body habitus) additional to arterial blood pressure in human populations. Such differences may themselves influence endothelial function independent of arterial blood pressure, or may otherwise influence responses to agonists. For instance, sensitivity to acetylcholine, which is rapidly hydrolysed in the blood, is increased in subjects with short forearms [32], and responses to high doses of acetylcholine are influenced by the biosynthesis of vasoconstrictor prostanoids [24,33] and are relatively resistant to inhibition by \( L \)-NMMA [34]. Consequently, studies such as the present one of basal rather than acetylcholine-stimulated NO biosynthesis are important.

Previous small-scale studies of the effects of \( L \)-NMMA on forearm resistance vasculature have suggested that essential hypertension is sometimes [5–7], but not always [8,9], associated with reduced basal NO biosynthesis. The present study demonstrates that a blunted response to \( L \)-NMMA relative to noradrenaline is not a necessary feature of essential hypertension in a larger group of moderately severely hypertensive patients. These findings, together with our earlier observation that acetylcholine-stimulated vasodilatation can be preserved in essential hypertension [4], provide strong evidence that reduced endothelial NO biosynthesis is not a primary contributor to the pathogenesis of essential hypertension.

The reasons for the differences from some earlier studies are uncertain. In contrast with studies of untreated patients, hypertensive patients \((n = 11)\) studied during treatment with angiotensin-converting enzyme inhibitors or a calcium antagonist had forearm blood flow responses to noradrenaline and to \( L \)-NMMA that were similar to those of normotensive control subjects [13]. Responses to \( L \)-NMMA have been reported to increase during treatment with either of these classes of drugs [35]. A previous study whose main object was to examine basal NO-mediated dilation in hypertension used only patients that had never been treated [5]. Other studies that reported blunted responses to \( L \)-NMMA used previously treated patients in whom anti-hypertensive medication was stopped 2 weeks before study [6,7]. In the present study, 21 patients had received anti-hypertensive treatment, which was discontinued 2 weeks before study, and 11 patients had never been treated. Subgroup analysis revealed no significant differences between responses in never-treated and previously treated patients (and no obvious trend). Another possibility relates to the observation that atherosclerosis is strongly associated with endothelial dysfunction that is not restricted to anatomically involved vessels [36], and extends to forearm resistance vasculature in patients with coronary or femoral atherosclerosis [37]. Essential hypertension is a powerful risk factor for atherosclerosis, which is commonly clinically undetected. Consequently, it is difficult to determine whether endothelial dys-

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