The role of nitric oxide in the regulation of glomerular haemodynamics in humans

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Abstract

Background. According to experimental data, the afferent glomerular arteriole is particularly under control of nitric oxide (NO). By use of pharmacological manoeuvres, we examined whether this finding holds true in the human renal circulation in vivo.

Methods. Seventy-seven volunteers (aged 50±9 years) with mild to moderate essential hypertension (n=57) or arterial normotension (n=20) were examined. Basal NO activity in the renal circulation was assessed by the change of renal plasma flow (RPF) through systemic infusion of the NO synthase inhibitor, NG-monomethyl-L-arginine (L-NMMA; 4.25 mg/kg). Hypertensive patients were treated over 8 weeks with either the calcium-channel blocker amlodipine or the AT1-receptor blocker valsartan, primarily dilating the afferent and efferent arteriole, respectively. Subsequently, renal haemodynamics and NO activity in the renal circulation were determined again.

Results. L-NMMA reduced RPF in normotensive (by 57±70 ml/min/1.73 m²; P<0.01) and hypertensive subjects (by 46±56 ml/min/1.73 m²; P<0.001) with no significant difference between the two groups. The decrease of RPF through L-NMMA was closely related with the glomerular filtration rate (GFR; r=0.39, P<0.001). Administration of amlodipine increased GFR by 7.1±12.1 ml/min/1.73 m²; (P<0.01) and in parallel reduced the response of RPF to L-NMMA to 19±48 ml/min/1.73 m²; (P<0.05). In contrast, valsartan maintained GFR and left the response of RPF to L-NMMA unchanged.

Conclusions. NO plays an important role in the regulation of human glomerular haemodynamics, probably with a greater contribution to afferent than to efferent arteriolar tone in man.

Keywords: angiotensin-II receptor blocker; calcium-channel blocker; glomerular haemodynamics; humans; nitric oxide

Introduction

Experimental data from microperfusion studies demonstrate that, compared with the efferent glomerular arteriole, the afferent arteriole is particularly sensitive to the endothelium-derived vasodilator nitric oxide (NO) [1]. However, there are conflicting data over whether the afferent or the efferent arteriole is the major location of endothelium-derived NO synthesis [1,2]. NO plays an important role in the regulation of glomerular haemodynamics [3]. For instance, increased NO synthesis has been associated with glomerular hyperfiltration in early stages of diabetic nephropathy [4].

Despite data from histochemical studies [2], the location of endothelium-derived NO synthesis and release and the major location of action for NO are not yet fully clear in human subjects in vivo. The difficulty to examine the contribution of NO to renal perfusion in humans might account for this lack of data. However, several investigators have already examined the effect of NO synthase inhibition on renal haemodynamics in humans [5–11] and from some of these data a contribution of NO particularly to afferent arteriolar tone was concluded [11]. Such conclusions can be drawn when the effects of NO synthase inhibition on glomerular filtration rate (GFR) are examined, since GFR is determined by the relation between afferent and efferent arteriolar tone. However, the data are not fully consistent and factors such as sodium intake clearly influence the effect of NO synthase inhibition on renal haemodynamics [7]. Interestingly, endogenous NO synthase inhibitors, such as asymmetrical dimethylarginine, have been found to influence renal haemodynamics [12].
Our present study aimed to elucidate more clearly the location of action of NO in the human renal circulation by studying glomerular haemodynamics. We examined the effects of NO synthase inhibition before and after treatment with the calcium-channel blocker amlodipine and the AT1-receptor blocker valsartan, which are known to predominantly dilate the afferent and efferent glomerular arteriole, respectively [13,14].

**Subjects and methods**

**Study population**

Volunteers between 35 and 65 years of age were screened for arterial normotension or mild to moderate hypertension (diastolic blood pressure ≥95 but <115 mmHg, obtained with a standard sphygmomanometer after 5 min rest). All subjects gave their written informed consent prior to all further study-related procedures. The study protocol was approved by the Clinical Investigation Ethics Committee of the University of Erlangen-Nürnberg.

Participants underwent a thorough clinical and laboratory examination. Exclusion criteria were the presence of any form of secondary hypertension, any irreversible end-organ damage due to arterial hypertension, any significant disease other than mild essential hypertension, diabetes mellitus, cigarette smoking within a period of 1 year prior to the study, hypercholesterolaemia (LDL cholesterol >160 mg/dl) or current cholesterol-lowering therapy and serum creatinine >50% above the upper limit. Twenty normotensive and 57 hypertensive subjects finally were included and the baseline characteristics of the participants are given in Table 1.

**Study design**

In hypertensive subjects, antihypertensive therapy was discontinued for 4 weeks. In the last 2 weeks of wash-out, a placebo was administered once daily. Normotensive subjects entered the study protocol without further preparation. Renal haemodynamics at baseline and in response to NO synthase inhibition (see below) were determined in all study participants.

Hypertensive subjects were then assigned to either treatment with valsartan 80 mg once daily or amlodipine 5 mg once daily in a randomized and double-blind fashion. Valsartan and matching amlodipine were provided by Novartis Pharma (Nürnberg, Germany) and did not differ in form or taste. Participants were asked to take the study medication at a fixed time in the morning and not to change their usual diet during the study. After 4 weeks of treatment, blood pressure was measured and the dose of active treatment had to be doubled if diastolic blood pressure was still >90 mmHg. According to this criterion, amlodipine dose had to be increased in 19 patients and valsartan dose had to be increased in 16 patients. After another 4 weeks of treatment (i.e. 8 weeks of total double-blind treatment), a second determination of renal haemodynamics at baseline and in response to NO synthase inhibition was performed.

**Determination of renal haemodynamics**

Renal plasma flow (RPF) and GFR were determined by constant input clearance technique with para-aminohippurate (Nephrotest; Merck, Sharp & Dohme, Hertfordshire, UK) and inulin (Inutest; Fresenius, Linz, Austria), respectively, as described in detail previously [15]. In brief, following administration of a loading dose, a steady state between infusion and renal excretion of the tracer substances was reached after 120 min. Blood samples for the determination of para-aminohippurate and inulin to assess baseline RPF and GFR were drawn at this time. All participants drank 10 ml/kg mineral water during the clearance studies. Blood pressure was measured with an oscillometric device (Dinamap 1846 SX; Criticon, Norderstedt, Germany) in parallel with blood sampling. GFR and RPF were indexed on body surface area. Filtration fraction was calculated by dividing GFR by RPF.

Blood samples were centrifuged immediately at 4°C and were stored at −21°C until measurement. Measurement of para-aminohippurate and inulin was performed after completion of the study. The laboratory personnel was unaware of the individual study participant’s blood pressure status and the investigators were still blinded with respect to assignment to amlodipine or valsartan in hypertensive subjects. Details concerning the measurement of inulin and para-aminohippurate have been published previously [15]. Each blood sample was measured in duplicate with a coefficient of variation of <5%. The effects of valsartan and amlodipine on glomerular haemodynamics in hypertensive study participants have been reported in greater detail previously [16].

Table 1. Baseline characteristics of the study cohort

<table>
<thead>
<tr>
<th></th>
<th>Hypertensives</th>
<th>Normotensives</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>51 ± 9</td>
<td>49 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83 ± 14</td>
<td>66 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 ± 0.09</td>
<td>1.69 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>27.9 ± 4.1</td>
<td>23.0 ± 3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.95 ± 0.19</td>
<td>1.75 ± 0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>163 ± 14</td>
<td>123 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>104 ± 8</td>
<td>83 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RPF (ml/min/1.73m²)</td>
<td>451 ± 96</td>
<td>445 ± 72</td>
<td>NS</td>
</tr>
<tr>
<td>GFR (ml/min/1.73m²)</td>
<td>104 ± 17</td>
<td>107 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>23.7 ± 3.4</td>
<td>24.3 ± 2.9</td>
<td>NS</td>
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Inhibition of NO synthesis

Following determination of renal haemodynamics under baseline conditions, N\textsuperscript{(L)-monomethyl-L-arginine (L-NMMA; Clinalfa, Läuflingen, Switzerland) was infused to inhibit endothelial NO synthase (3 mg/kg over 5 min, followed by constant infusion over 25 min with a rate of 3 mg/h; i.e. a total dose of 4.25 mg/kg). After L-NMMA infusion, another blood sample was drawn to measure para-aminohippurate and inulin and, thus, to assess RPF and GFR after NO synthase inhibition. This approach has been evaluated in our laboratory and has been found to specifically reflect the basal contribution of NO to renal haemodynamics [17].

Statistics

All statistical analysis was carried out using SPSS software (release 10.0; SPSS Inc., Chicago, IL). Paired and unpaired Student’s t-tests were used where appropriate. Where indicated, a multiple stepwise linear regression analysis with significance levels of 0.05 (entry of a variable) and 0.10 (removal of a variable) at each forward step was calculated. A two-tailed P-value of <0.05 was considered to be significant. All values are expressed as means±SD.

Results

Response of renal haemodynamics to NO synthase inhibition

NO synthase inhibition with L-NMMA induced a decrease of RPF, an increase of GFR and an increase of filtration fraction. There was no significant difference in the effect of L-NMMA between normotensive and hypertensive subjects (Table 2). There were also small but statistically significant effects of L-NMMA on systemic haemodynamic parameters, i.e. an increase of mean arterial pressure and a decrease of heart rate in each group (Table 2).

Relation between the contribution of NO to renal perfusion and GFR

There was a close relation between the response of RPF to NO synthase inhibition with L-NMMA infusion and GFR. In a multiple stepwise regression analysis, the response of RPF to NO synthase inhibition with L-NMMA was a major factor to explain GFR (β = 0.39, P < 0.001), whereas mean arterial pressure, age and sex did not enter the final model. Accordingly, a split-half analysis performed according to the mean response of RPF to L-NMMA in all subjects (which was 49 ± 59 ml/min/1.73 m\textsuperscript{2}) revealed a greater GFR in subjects with greater response of RPF to NO synthase inhibition with L-NMMA as compared with the other group: GFR was 110 ± 16 vs 100 ± 15 ml/min/1.73 m\textsuperscript{2} (P < 0.05).

Effects of amlodipine and valsartan on the contribution of NO to renal perfusion

Both amlodipine and valsartan significantly lowered blood pressure from 160±14/105±9 to 143±13/94±7 mmHg and from 165±14/102±8 to 153±17/95±8 mmHg, respectively (all P < 0.001). There was no significant difference between the blood pressure-lowering effect of both drugs (P = 0.19 for systolic and P = 0.13 for diastolic blood pressure), although the numerical reduction in blood pressure was greater in the amlodipine than in the valsartan group.

RPF was maintained with both valsartan and amlodipine treatment. GFR increased with amlodipine treatment. In parallel, the response of RPF to NO synthase inhibition with L-NMMA was reduced from 51±55 to 19±48 ml/min/1.73 m\textsuperscript{2} (P < 0.05) in patients treated with amlodipine. In contrast, valsartan maintained both GFR and the response of RPF to NO synthase inhibition with L-NMMA (Table 3, Figure 1).

Discussion

NO plays an important role in the regulation of glomerular haemodynamics [3]. Data from microperfusion experiments demonstrate a considerable contribution of NO to the tone of the afferent glomerular arteriole, but a lesser contribution to the tone of the efferent arteriole [1]. Nevertheless, there are conflicting data whether the afferent or the efferent glomerular arteriole is the main location for endothelium-derived NO synthesis [1,2]. In human subjects, there are no direct functional data about the sensitivity of afferent

Table 2. Response of renal haemodynamics to NO synthase inhibition

<table>
<thead>
<tr>
<th></th>
<th>Hypertensives (n = 57)</th>
<th>Normotensives (n = 20)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>L-NMMA</td>
</tr>
<tr>
<td>RPF (ml/min/1.73 m\textsuperscript{2})</td>
<td>451 ± 96</td>
<td>405 ± 72\textsuperscript{a}</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m\textsuperscript{2})</td>
<td>104 ± 17</td>
<td>108 ± 16\textsuperscript{a}</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>23.7 ± 3.4</td>
<td>27.1 ± 3.0\textsuperscript{a}</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>118 ± 13</td>
<td>125 ± 14\textsuperscript{a}</td>
</tr>
<tr>
<td>Heart rate (min\textsuperscript{-1})</td>
<td>64 ± 8</td>
<td>59 ± 7\textsuperscript{a}</td>
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\textsuperscript{a,b}The effect of L-NMMA on the respective haemodynamic parameter is significant from baseline (\textsuperscript{a}P < 0.001, \textsuperscript{b}P < 0.01). There was no significant difference in the effects of L-NMMA between hypertensive and normotensive study participants.
and efferent glomerular arterioles to NO or about
the contribution of NO to basal perfusion of those
arterioles.

The examination of endothelium-dependent vasodi-
lation of the human renal vasculature is difficult.
Attempts to use infusions of endothelium-dependent
vasodilators, such as acetylcholine, or inhibitors of NO
synthase, such as L-NMMA, into the renal artery are
highly invasive and will be restricted to certain patients
who undergo renal artery angiography for diagnostic
reasons [18]. Therefore, measurements of changes in
renal haemodynamics in response to the intravenous
administration of L-arginine, the precursor of NO
synthesis, and L-NMMA have been used instead.
Limitations of intravenous infusion of L-NMMA
comprise the question of adequate dosage and the
simultaneous systemic haemodynamic effects [17].
Nevertheless, this technique has been employed suc-
cessfully by us and others as a measure to analyse the
contribution of NO to basal renal perfusion [5–11].

GFR is determined by the relation between afferent
and efferent arteriolar tone. Vasodilatation of the
afferent arteriole will lead to an increase in GFR as
long as efferent arteriolar tone remains unchanged, or
at least almost unchanged. NO is a potent vasodilator,
therefore the greater the contribution of NO to afferent
arteriolar tone, the greater the GFR will be. In humans,
it is not possible to measure the sensitivity of afferent
and efferent arterioles to NO directly and in vivo.
However, data about a decrease in GFR in response to
NO synthase inhibition can be interpreted as indirect
evidence of a greater NO sensitivity of the afferent as
compared with the efferent arteriole [11].
Unfortunately, these data are not fully convincing
due to the small number of volunteers examined.
Moreover, the combined effect of L-NMMA on renal
perfusion (i.e. a reduction of RPF) will influence the
effect of L-NMMA on GFR, which is also dependent
on RPF. Consequently, not all investigators found a
decrease of GFR in response to NO synthase inhibition
[5,6,10].

We have chosen an alternative approach to examine
the effects of NO on the afferent and efferent
glomerular arterioles in our present study. We mea-
ured the response of RPF to L-NMMA infusion and,
thus, assessed the combined effect of afferent and

Table 3. Effect of treatment on renal haemodynamics in patients with essential hypertension

<table>
<thead>
<tr>
<th></th>
<th>Valsartan (n = 28)</th>
<th>Amlodipine (n = 29)</th>
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<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>RPF (ml/min/1.73 m²)</td>
<td>441 ± 106</td>
<td>452 ± 102</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>102 ± 15</td>
<td>102 ± 16</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>24 ± 4.1</td>
<td>23 ± 3.6</td>
</tr>
</tbody>
</table>

Effect of L-NMMA on

<table>
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<tr>
<th></th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P-value</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF (ml/min/1.73 m²)</td>
<td>−41 ± 57</td>
<td>−33 ± 47</td>
<td>NS</td>
<td>−51 ± 55</td>
<td>−19 ± 48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>+3.1 ± 4.4</td>
<td>+3.2 ± 6.1</td>
<td>NS</td>
<td>+4.1 ± 6.1</td>
<td>+5.5 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>+2.9 ± 2.1</td>
<td>+2.3 ± 1.8</td>
<td>NS</td>
<td>+3.9 ± 2.2</td>
<td>+2.4 ± 2.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Fig. 1. Changes in RPF through L-NMMA before (open bars) and after (shaded bars) treatment with valsartan (left) and amlodipine (right).
efferent arteriolar vasoconstriction as a decrease of RPF. Then, we examined the relation between the response of RPF to L-NMMA and GFR to draw conclusions about the main location of action for NO. This procedure is certainly also an indirect one. However, due to the large number of study participants, and due to the more consistent effects of L-NMMA on RPF reported in the literature as compared with the effects on GFR [5–11], this procedure should lead to more reliable and valid conclusions. In fact, our finding of a clear-cut and independent relationship between GFR and the contribution of NO to renal perfusion showed the validity of our approach.

The finding that GFR was greater in subjects with a greater response of RPF to L-NMMA points towards the afferent arteriole as the main location of action for NO. It is an interesting finding that GFR is clearly dependent on NO, but, as shown in the regression analysis, other factors, including blood pressure, play only a minor role in the determination of GFR. This is even more interesting in light of our previous finding that the contribution of NO to basal renal perfusion is no different between normotensive and hypertensive subjects [6], a finding which has been confirmed in the present study.

It was the other goal of our study to examine the main location of synthesis and/or release of endothelium-derived NO in the human renal vasculature. We hypothesized that, like in other vascular beds, NO release is dependent on the amount of shear stress in the renal circulation. Therefore, NO-independent vasodilatation of the afferent or efferent arteriole should reduce shear stress and, thus, reduce NO synthesis and/or release. We used the calcium-channel blocker amlodipine and the AT1-receptor blocker valsartan to predominantly dilate the afferent and efferent glomerular arteriole, respectively [13,14]. Our data show that the contribution of NO to renal perfusion is reduced following amlodipine but not following valsartan treatment. These results suggest that the afferent arteriole contributes more to NO synthesis and/or release, since a reduction of shear stress in the afferent but not in the efferent arteriole changed the contribution of NO to renal perfusion.

Clearly, our study has several limitations, some of which are due to the general difficulty to examine the endothelium-dependent vasodilatation of the renal circulation in humans, some of which are due to our specific study design. First, we have certainly not only assessed the contribution of endothelium-derived NO to glomerular arteriolar tone, but also that of NO synthesized by inducible and neuronal NO synthase isoforms, which contribute considerably to the amount of NO in the renal circulation [3]. This might influence our data on the location of action for NO, but should not influence the second part of the study, i.e. the results before and after amlodipine and valsartan treatment within the same subject. It is a straightforward explanation of our findings that following NO-independent vasodilatation of the afferent arteriole by amlodipine, the synthesis of NO must be reduced to maintain vascular tone. This is particularly true when the afferent arteriole is the main location of NO synthesis.

Second, one might speculate that the effect of amlodipine on afferent arteriolar tone was greater than that of valsartan on efferent arteriolar tone, since amlodipine caused a marked increase in GFR whereas valsartan did not reduce GFR significantly. Thereby, the reduction of shear stress might have been greater in the afferent than in the efferent arteriole. This would, in fact, explain why the contribution of NO to renal perfusion was unchanged following valsartan treatment. However, this interpretation is in contrast to previous findings; valsartan might even increase NO synthesis in the renal circulation, for instance by stimulation of AT2-receptor through endogenous angiotensin II. We have found such effects in a previous study with the AT1-receptor blocker eprosartan [10]. Thus, our finding that valsartan maintains the contribution of NO to renal perfusion can be interpreted as evidence for the minor role of the efferent arteriole for NO synthesis and/or release, but can also demonstrate an increased NO synthesis through AT2-receptor stimulation which counterbalances a reduced NO release due to reduced shear stress. Of note, other investigators have also found that AT1-receptor blockade does not affect the response of RPF to NO synthase inhibition [8,9].

One should be cautious to transfer data from this functional study to clinical situations or to use them to explain recent findings from large-scale clinical trials comparing the renal outcome of patients treated with calcium-channel blockers and AT1-receptor blockers [19,20]. However, it is more and more clear that NO is a major player in the regulation of renal haemodynamics, which might have serious consequences in patients with hypertension and other diseases [21].

In conclusion, our data demonstrate the important role of NO in the regulation of glomerular haemodynamics. NO appears to contribute more to the afferent than to the efferent arteriolar tone and the afferent arteriole appears to be the main location of endothelium-derived NO synthesis in humans.

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Conflict of interest statement. R.H. is an employee of Novartis Pharma GmbH.

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