Role of nitric oxide-related mechanisms in renal function in ageing rats

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Abstract

Background. The impaired renal function and vasodilatation that accompany age need to be re-addressed based upon the new knowledge concerning vascular nitric oxide (NO)-dependent systems. The present study examined the effects of age on the NO-related renal response.

Methods. The study was performed in euvolæmic, conscious Wistar rats, aged 5 and 18 months. Renal function and haemodynamic measurements with fluorescent microspheres were employed to assess differences between groups.

Results. A first set of experiments showed that ageing rats had a reduced natriuretic and diuretic response to acetylcholine, whereas the response to sodium nitroprusside was preserved. In the same regard, a reduction of the renal functional effects of l-arginine (l-Arg) and l-glycine (l-Gly) was found in the older rats. In the ageing rats, these responses were accompanied by an enhanced effect of the l-Arg competitive analogue, NwNLA, which provoked a marked reduction of renal function. This effect of NwNLA was blocked by the simultaneous administration of a small dose of l-Arg in the ageing but not in the young rats. Systemic haemodynamic studies revealed that in ageing rats, NwNLA reduced renal blood flow and increased renal vascular resistances in a significantly higher proportion than in younger animals. However, flow to other organs, namely, brain, spleen or liver, was affected in a similar manner in both young and old rats. Ultrastructural alterations were found in endothelial cells, which might constitute the anatomical basis for the observed functional derangements.

Conclusions. The present experiments reveal that ageing is accompanied by significant differences in NO-related responses in the kidney which do not appear to affect blood flow to other organs. The response to l-Arg and l-Arg competitive analogues supports the existence of a marked dependency on NO-related mechanisms in the ageing rats, but not of a decreased baseline activity of the NO-dependent pathways.

Key words: ageing; l-arginine; l-glycine; renal function

Introduction

Several alterations in renal function and vascular regulation have been reported in ageing humans and experimental animals [1,2]. Basically, ageing is associated with a progressive decline in glomerular filtration rate (GFR) and renal blood flow (RBF), and augmented vascular resistances [1,2]. The impaired vasodilatation that accompanies age needs to be re-addressed based upon the new knowledge concerning vascular nitric oxide (NO)-dependent systems [3–6]. Hollemberg et al. [7] suggested that ageing may preferentially affect acetylcholine-induced vasodilation. Using the same line of reasoning, other studies explored the possibility of an altered modulatory function of the endothelium on the vascular tone [8–10]. Lee et al. have suggested that the production of endothelium-derived relaxing factor decreases with advancing age [11]. In a study by Baylis et al. [12], the finding of a reduced renal blood flow response to glycin in ageing rats further suggested the existence of an impaired vasodilation to physiological stimuli. More recently, Reckelhoff et al. [13] and Tank et al. [14] have found an exaggerated vasoconstrictive effect to the l-arginine (l-Arg) competitive antagonist, L-NAME, in the renal vasculature of ageing Sprague–Dawley rats.

The above-mentioned studies did not, however, answer the question about the actual mechanisms involved in the age-related defects in NO-related pathways. Furthermore, it is not known whether the age-related changes occur only or predominantly at the renal level or constitute a more generalized disturbance affecting several vascular territories. The present study was designed to examine the role of NO in the renal response in ageing and, more particularly, the extent and organ specificity of the age-related changes. For this purpose, we used an approach which combined...
renal functional tests and microsphere haemodynamic techniques.

Subjects and methods

Chemicals and solutions

Acetylcholine chloride, l-Arg and NwNLA were purchased from Sigma (St. Louis, MO). Sodium nitroprusside was purchased from Merck (Darmstadt, Germany). L-Glycine (l-Gly) was from Merck-Sharp & Dohme (Germany) and [14C]juglone was from Amersham (Buckinghamshire, UK). Fluorescent microspheres (Fluospheres®) were purchased from Molecular Probes (Eugene, OR).

Animals, instrumentation and experimental procedures

The studies were performed in two types of euvoelaemic, conscious male Wistar rats: ageing (18 month old) and young (5 month old). All the experiments were carried out following the international recommendations for animal studies. Before the surgical procedures, the rats were main- tained overnight without food and with water ad libitum. The animals were anaesthetized with sodium pentobarbital and prepared as previously described [15–19], with catheters inserted in the left femoral vein and artery and in the bladder. At the end of surgery, the animals were placed in cages with food and water ad libitum. During the experiments, the temperature was held constant at 24°C in the animal housing.

Mean arterial pressure (MAP) was monitored continuously with a pressure transducer (Statham Instruments, Hato Rey, Puerto Rico). All the animals were studied after full recovery with a pressure transducer (Statham Instruments, Hato Rey, Puerto Rico). All the animals were studied after full recovery.

Study I. Intravenous acetylcholine (10 μg min kg body weight, 15 min), sodium nitroprusside (200 μg min kg bw, 15 min) were administered and the diuretic and natriuretic effects were examined, according to a previously defined model [17,19].

Study II. Intravenous l-Arg competitive antagonist, NwNLA was administered. First, a dose–response curve of the arterial pressure-increasing effect was constructed by administrating a successive and cumulative bolus (0.2–0.5 mg/kg bw) of NwNLA, beginning at 0.2 mg/kg bw. Values were analysed by the Enzfitter program which provided the DE50 and the DE90 values. Based on these results, a dose of 7.5 mg/kg was chosen for the experiments.

Study III. l-Arg (5 mg/kg) or l-Gly (5 mg/kg) were adminis- tered in the presence or absence of NwNLA (7.5 mg/kg). The different amino acids were given by i.v. bolus (0.4 ml, 30 s). The i.v. bolus of NwNLA (7.5 mg/kg bw) was adminis- tered 10 min before the bolus of amino acids. Control experiments were performed following the same protocol, but using only physiological saline solution (PSS).

Study IV. Haemodynamic measurements by fluorescent microspheres were carried out in young and ageing animals before and after administrating a bolus of NwNLA (7.5 mg/kg).

Study V. Experiments similar to studies I and IV were done in animals after 24–30 h of the surgical procedure. All the infusions were freshly prepared in sterile PSS (in mM: 140 NaCl, 4.6 KCl, 2.0 CaCl2, 1 MgCl2, 10.0 d-glucose, 10 HEPES, pH 7.4), immediately before use. The temperature and pH of the solutions were held at 37°C and pH 7.4; no additional fluids were given throughout the experiment to avoid plasma volume expansion.

Measurement of organ blood flow by fluorescent microspheres

Surgical preparation. The rats were prepared as described elsewhere [15,16]. The other aspects of the procedure were similar to those described above. A set of experiments were done at 4–5 h after the surgical procedure, in animals with a status similar to those described above. To address further the validity of the experiments at 4–5 h, additional studies were conducted in similarly operated animals at 24–30 h after the cannulation procedure. At that time, the animals had uniform MAP and heart rate, and no behavioural alterations were evident.

Experimental procedure. During the experiment, MAP was recorded by a pressure transducer connected to a polygraph (Polygraph 2006). Once the blood pressure stabilized, the fluorescent-labelled microspheres (3 x 109) were injected via the right carotid artery. The fluorescent-labelled microspheres were vortexed and sonicated immediately before injection. The injections (500 μl) were performed over 10 s and were followed by saline flushes (500 μl). Starting 10 s after the injection of microspheres, blood was sampled for 60 s from the femoral catheter. The sample’s volume was replaced by an equal amount of isogenic donor rat blood. Five minutes after the first injection, NwNLA (7.5 mg/kg) was injected via the femoral artery. When MAP reached its maximum level, a second microsphere injection of a different fluores- cence spectrum was done. After completion of the injections, the animals were killed by the administration of potassium chloride, and the liver, spleen, brain and kidney were removed. The fluorescence of each tissue and the reference blood samples were determined as described below.

Tissue digestion, microsphere recovery and calibration. The tissue pieces were individually and completely digested in
0.5 ml of 4 M KOH within 24 h; at this time, they were individually put into 2 ml of xyol, vortexed and centrifuged (2000 r.p.m.); the supernatant was transferred to individual glass tubes. A sample of dye/solvent from each organ was then pipetted in a glass cuvette and the fluorescence intensity was determined with a Perkin Elmer LS-50 luminiscence spectrophotometer (Beckman, Buckinghamshire, UK). The excitation and emission wavelengths were set manually at the beginning of each study. We used excitation/emission wavelengths of 565/596 and 360/420 nm for red and blue microspheres, respectively, according to previously performed scans of the wavelength intensities of the xyol-dissolved dyes. Organ blood flow was calculated by the formula: organ blood flow (ml/min) = sample fluorescence × reference blood flow/reference blood fluorescence. Blood flow rate was matched by wet organ weight. The values of MAP matched by organ blood flow were employed for the calculation of vascular resistances. Previous calibration studies showed that the blood flow rates obtained by the present method are similar to those obtained by radio-labelled microspheres (Tan et al., unpublished data) and to those previously described by other authors [22,23].

**Measurement of renal function**

Measurements of renal function were done as described [15–18], with a loading dose of metoxi-[14C]IN (3 μCi/ml) and [3H]PAH (13 μCi/ml) and an infusion (1.2 ml/h) of metoxi-[14C]IN (0.62 μCi/ml) and [3H]PAH (2.5 μCi/ml) in isotonic saline. After 45 min equilibration, two 30 min urine collections were completed, with blood sampling (150 μl) at the beginning and end of each clearance period. Packed red cell volume was determined by the microcapillary method. 3H and 14C activities were measured using a two-channel liquid scintillation counter which corrects for the interference between isotopes. CIN and CP AH were used for calculating GFR and RPF, respectively, by standard formulae. In all the experiments, urine and plasma electrolytes were measured by an automatic analyser (Astra IV Beckman, Fullerton, USA).

**Pathology studies**

Kidney samples were obtained for optical (OM) and electronic microscopy (EM). The EM samples were fixed in 2.5% glutaraldehyde and double fixed in 4% osmium tetroxide, both in phosphate buffer. The fixed samples were embedded in araldyte and contrasted with uranyl acetate and lead citrate. The samples for optical microscopy were fixed in 15% formaldehyde, and the 3–4 μm sections were stained with haematoxylin–eosin and Masson trichrome. For morphometric studies, the samples of OM and the negative films of EM were digitalized and processed by the image analyser MICROM (Hardware IMCO 10, Kontron Bildanalyse, Software Microm IP, Microm Espana). For qualitative purposes, the percentage of sclerosed glomeruli was determined by the Masson-stained preparations, by counting the number of obsolete glomeruli within a total of 200 glomeruli in each sample.

**Statistical analysis**

Values are shown as mean ± SEM. Unless otherwise mentioned, values correspond to a minimum of six experiments. Changes in variables within the same group were analysed by one-way analysis of variance and subsequently Scheffe’s and Fisher’s test. Differences between groups were analysed using the unpaired Student’s t-test; the Mann–Whitney test was used in the case of groups of <6 animals. A value of P < 0.05 was considered significant.

**Results**

**Baseline and renal pathology data**

Experimental data obtained at baseline in both groups are depicted in Table 1.

The percentage of sclerosed glomeruli was 2.5±0.4 and 4.8±0.4 in young and old rats, respectively (P NS between groups). Further findings included hypercellularity and enlargement of Bowman’s space, which were observed by OM in the glomeruli of the ageing rats. EM revealed that ageing animals had widening of the glomerular basal membrane, with focal loss or fusion of the podocytes and increase of the mesangial matrix, with a fibrillar, basal membrane-like material. Cytoplasmic degenerative changes with oedema, vacuolization and increased number of microvilli were observed in visceral epithelial and endothelial cells.

**Effects of acetylcholine and sodium nitroprusside**

We assessed the diuretic and natriuretic effects of acetylcholine and sodium nitroprusside as a screening measurement of the NO-related response. The diuretic and natriuretic effects of acetylcholine were decreased in the ageing group (n = 6; P < 0.01 compared with the young, Table 2), without significantly affecting the sodium nitroprusside-induced diuresis and natriuresis.

### Table 1. Baseline renal and systemic parameters in young (n=26) and ageing (n=20) conscious rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young</th>
<th>Ageing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>333.3 ± 9.4</td>
<td>421.2 ± 11.1*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>101.7 ± 1.5</td>
<td>107.1 ± 4.3</td>
</tr>
<tr>
<td>GFR (ml/min/100 g bw)</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.06</td>
</tr>
<tr>
<td>RPF (ml/min/100 g bw)</td>
<td>1.8 ± 0.2</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>VRV (ml/Hg/ml/min)</td>
<td>16.9 ± 2.1</td>
<td>18.2 ± 2.0</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>53 ± 9.2</td>
<td>60.2 ± 11.8</td>
</tr>
</tbody>
</table>

Values correspond to mean ± SEM.

*Significant differences (P < 0.05) with respect to ageing animals.

### Table 2. Values of diuresis and natriuresis after infusions of acetylcholine (ACh) and sodium nitroprusside (SNP) in conscious rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ACh (n=6 each)</th>
<th>SNP (n=5 each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>Ageing</td>
<td>Young</td>
</tr>
<tr>
<td>ΔUV, ml/h</td>
<td>0.89 ± 0.02</td>
<td>0.27 ± 0.08*</td>
</tr>
<tr>
<td>ANa,V, μEq/h</td>
<td>399 ± 25.2</td>
<td>135 ± 10*</td>
</tr>
</tbody>
</table>

Dose of ACh (10 μg/kg bw/min); dose of SNP (200 μg/kg bw/min). Data are expressed as Δ increase in urinary volume (UV) and urinary Na × volume (Na,V). Values are mean ± SEM.

*Significant differences (P < 0.05) between young and ageing animals.
shown below, the changes found were consistent with microspheres. Those detected in the experiments using fluorescent competitive antagonist, NwNLA.

The administration of 7.5 mg/kg of NwNLA to ageing rats (n = 6), reduced both GFR and RPF (Figure 1) and increased renal vascular resistances to a significantly higher degree than in the young rats (n = 8, P < 0.001, P < 0.01 and P < 0.01, respectively). As shown below, the changes found were consistent with those detected in the experiments using fluorescent microspheres.

In addition to the results obtained on renal functional parameters, we found that in the ageing rats, the threshold dose, DE$_{50}$ and DE$_{max}$ for the pressor effect of NwNLA were smaller (n = 5, P < 0.05; Table 3). These results using NwNLA alone are meaningful for understanding the consequences of NO synthesis inhibition by NwNLA on the renal effects of the administration of amino acids, which are shown below.

**Figure 1.** Effects of NwNLA (7.5 mg/kg) on conscious young and ageing rats. The values of GFR and RPF correspond to mean ± SEM of two baseline periods of 30 min each, and two periods post-treatment of 30 min each with NwNLA (7.5 mg/kg bw) in young (n = 6) and ageing (n = 6) rats. #P < 0.05, ##P < 0.02 compared with basal value. Changes are expressed as the percentage increase or decrease with respect to the baseline.

Values of threshold, EC$_{50}$ and DE$_{max}$ for the pressor effect of NwNLA are shown in Table 3. These values were lower in the ageing rats compared to the young rats (P < 0.05). The decrease in MAP with sodium nitroprusside was 54.2 ± 3.6 and 53.5 ± 8.8 mmHg in young and ageing rats, respectively.

**Table 3.** Values of threshold, EC$_{50}$ and maximal dose for the arterial pressure-increasing response to an i.v. bolus of NwNLA, in control and ageing rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>Ageing (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold</td>
<td>1.4 ± 0.05</td>
<td>0.7 ± 0.05*</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>6.9 ± 0.5</td>
<td>5.01 ± 0.4*</td>
</tr>
<tr>
<td>Maximal</td>
<td>17.1 ± 0.05</td>
<td>15.1 ± 0.05*</td>
</tr>
</tbody>
</table>

Doses are shown in mg/kg bw. Values are mean ± SEM.

*Significant differences (P < 0.05) with respect to young animals.

**Effects of the different amino acids on renal function**

**GFR and RPF.** As shown in Table 4, the administration of l-Arg (5 mg/kg, n = 7) or l-Gly (5 mg/kg, n = 6) to young rats increased both GFR and RPF significantly, with a decrease in renal vascular resistance (RVR). The effect of l-Arg on these parameters was dose-dependent, i.e. with a dose of 2.5 and 0.5 mg/kg bw, GFR increased by 16.5 ± 5.2% and 7.2 ± 0.4%, respectively compared with the 30.5 ± 0.8% observed with the dose of 5 mg/kg (Table 5). A similar degree of increase was observed in RPF (data not shown). On the contrary, a blunted effect of either l-Arg (5 mg/kg, n = 5) or l-Gly (5 mg/kg, n = 3) was observed in ageing rats (Table 4, P < 0.05 compared with the young rats).

**Table 4.** Effects of the different amino acids on renal function (mean ± SEM)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>GFR (%)</th>
<th>RPF (%)</th>
</tr>
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<tbody>
<tr>
<td>l-Arg</td>
<td>30.5 ± 0.8</td>
<td>7.2 ± 0.4</td>
</tr>
<tr>
<td>l-Gly</td>
<td>16.5 ± 5.2</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

**Effects of NwNLA on the amino acid-induced renal functional changes**

**GFR and RPF.** This group of data should be interpreted in the light of the aforementioned results concerning the effect of NwNLA alone. In the young group, the pre-treatment with NwNLA (7.5 mg/kg bw) inhibited the GFR and RPF responses elicited by l-Arg (n = 4) and l-Gly (n = 4, P < 0.05 compared with l-Arg or l-Gly alone). This effect is illustrated in Table 5. As can be seen, in the presence of NwNLA there was a decrease in both GFR and RPF, which was not reversed by the administration of either l-Arg or l-Gly, i.e. in presence of NwNLA, l-Arg or l-Gly failed to increase GFR and RPF.

As depicted in Table 6, the administration of l-Arg or l-Gly reversed in part, albeit significantly, the profound decrease in GFR and RPF observed in the
natriuresis (89.7 ± inhibition, $P_{\text{e}}$ NwNLA). In the same manner, a marked inhibitory with respect to the response in the absence of $V$ and natriuretic e 97.8 l (7.5 mg. In the young group, NwNLA Diuresis and natriuresis Arg or l with the absence of a NwNLA-reversing e presence of NwNLA alone. This finding contrasted by fluorescent microspheres are depicted in Figures 2 Values of GFR and RFP were expressed as mean ± SEM of two clearance periods of 30 min each in basal condition and after the administration of l-Arg (5 mg/kg bw) or l-Gly (5 mg/kg bw) and the NO antagonist, NwNLA (7.5 mg/kg bw, 10 min before the amino acids). *$P<0.05$.  

**Table 6.** Effects of NwNLA on changes in renal function induced by l-Arg (n=4) and l-Gly (n=4) in conscious ageing rats  

<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min)</th>
<th>RPF (ml/min)</th>
<th>RVR (mmHg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Arg + NwNLA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.0 ± 0.5</td>
<td>4.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Post-bolus</td>
<td>1.7 ± 0.3</td>
<td>2.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>l-Gly + NwNLA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.7 ± 0.9</td>
<td>2.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Post-bolus</td>
<td>1 ± 0.4</td>
<td>1.2 ± 0.5</td>
<td></td>
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</tbody>
</table>

Values of GFR and RPF are expressed as mean ± SEM of two clearance periods of 30 min each, both in basal condition and post-administration of l-Arg (5 mg/kg bw) or l-Gly (5 mg/kg bw) and the NO antagonist NwNLA (7.5 mg/kg bw). presence of NwNLA alone. This finding contrasted with the absence of a NwNLA-reversing effect of l-Arg or l-Gly in young animals (see above). **Diuresis and natriuresis.** In the young group, NwNLA (7.5 mg/kg) almost totally inhibited the diuretic and natriuretic effects of l-Arg (96.7 ± 2.3% and 97.8 ± 1.9% degree of inhibition, respectively, $P<0.01$, with respect to the response in the absence of NwNLA). In the same manner, a marked inhibitory effect was observed on the l-Gly-induced diuresis and natriuresis (89.7 ± 4.0% and 92.1 ± 5.2% degree of inhibition, $P<0.01$).

In contrast to the GFR and RPF changes described above, NwNLA treatment inhibited the diuretic and natriuretic effects of the amino acids in an almost complete fashion (95.4 ± 1.6% and 96.1 ± 2.3% degree of inhibition respectively, $P<0.01$ with respect to the response without NwNLA); this degree of inhibition was not statistically different from that observed in the young animals.

**Organ blood flow studies**

Data on RBF, RVR and organ blood flow as measured by fluorescent microspheres. *$P<0.02$ between young ($n=6$) and ageing ($n=7$) rats. Blood flow was significantly less in the NwNLA period with respect to the baseline for both ageing and young groups.
compared with rats studied at 4–5 h after surgery). Our interpretation of the findings concerning the
ving direct MAP measurements or plethysmographic dependency on NO to maintain a stable renal function.

Furthermore, the marked e
formed at 24–30 h. These results, if extrapolated to presence of increased nitrite production in arteries
the present study suggest, however, that considering GFR and RPF and those on natriuresis and diuresis

acetylcholine to stimulate NO production fully is i.e. GFR and RPF being more related to haemo-

Table 7. Effect of NwNLA (7.5 mg/kg bw) on organ blood flow (ml/g), measured by fluorescent microspheres

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-t-NwNLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Brain</td>
<td>10.2 ± 1.6</td>
<td>10.6 ± 1.4</td>
</tr>
<tr>
<td>Liver</td>
<td>0.95 ± 0.4</td>
<td>0.81 ± 0.2</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.43 ± 1.8</td>
<td>3.95 ± 1.1</td>
</tr>
</tbody>
</table>

*P<0.05 compared with baseline.

provoked a marked reduction in organ blood flow, with the exception of the hepatic arterial flow, which remained unaltered. The most relevant finding was that in the ageing rats, the reduction in renal flow was significantly more pronounced than in the young animals, whereas no age-related differences were evident in the percentage reduction in flow to the other organs. No differences were evident between the results shown in Figures 1 and 2 and Table 7 and those obtained in the experiments done at 24 h after surgery. In the latter experiments, RBF (baseline/post-NwNLA) was 10.3 ± 1.1/4.4 ± 0.8 ml/min and 8.3 ± 2.1/1.5 ± 0.6/ml/min in young and ageing rats, respectively, (n = 3 young and n = 3 ageing rats, P NS compared with rats studied at 4–5 h after surgery).

Discussion

The present study provides new evidence indicating that NO-dependent mechanisms are significantly altered in ageing. A first set of experiments was done using the administration of acetylcholine and sodium nitroprusside as a screening procedure to determine endothelial dysfunction. Our findings support the existence of an alteration of the endothelium-dependent mechanisms, with preserved endothelium-independent mechanisms. This is consistent with previous results of Koga et al. [24] and Hynes et al. [25] using blood vessels. Furthermore, the present results add new information showing a dissociation between acetylcholine and sodium nitroprusside in renal excretory parameters in ageing animals. The validity of the short-term experiments was confirmed by the studies performed at 24–30 h. These results, if extrapolated to human subjects, open up the practical possibility that a simple test of urinary Na and volume excretion might efficiently substitute for more sophisticated tests involving direct MAP measurements or plethysmographic techniques. The findings in the other experiments of the present study suggest, however, that considering the decreased response to acetylcholine as synonymous of decreased NO production might well be an over-simplified interpretation of the data. The ability of acetylcholine to stimulate NO production fully is dependent of a complete receptor-related complex signal transduction mechanism; therefore, more than one step of this mechanisms may be affected by ageing; moreover, it is mandatory to keep in mind the possibility that mechanisms unrelated to NO production might be relevant in the response to acetylcholine [26].

Several studies were done to identify the mechanisms involved in the aforementioned deranged responses. The main new finding was that the disturbances in ageing rats occurred predominantly at the renal level, to a degree not observed in other organs, namely, brain, liver or spleen. As mentioned previously, Reckelhoff et al. [13] and Tank et al. [14] have described a significant difference between young and ageing rats in the intensity of flow reduction response by l-Arg antagonism. Our present data demonstrate that a higher degree of flow reduction in ageing animals is found only in the renal circulation. The stronger effect of NwNLA on the renal circulation of ageing animals highlights the importance of NO as a critical element in renal functional regulation and adaptation.

The reduction in cerebral blood flow by NwNLA found in our studies is consistent with previous reports by other groups [27,28], albeit that it was rather more intense in the present study; this might be related to differences in the experimental model. The absence of change in liver hepatic arterial blood flow produced by NwNLA suggests that the hepatic territory may have a differentiated haemodynamic regulation, which is not significantly regulated by NO.

Our interpretation of the findings concerning the effects of NwNLA on RBF was completed by the analysis of the response to amino acid administration. At a first glance, the absence of an increase in GFR and RPF in ageing rats by l-Arg suggested a lack of capacity of this amino acid to stimulate the normal response to amino acid administration [18,29,30]. This assertion was, however, not valid in every condition, since after the inhibition of NO production by NwNLA, an effect of a small dose of l-Arg on GFR and RPF appeared in the ageing rats which was absent in the younger rats. These results, therefore, showed that the NO synthase activity inhibition by NwNLA was, at least in part, reversed by the 5 mg/kg dose of the amino acids in the older but not the younger animals. The most likely explanation is that older rats have increased amounts or different kinetics of NO synthase than the younger animals. This assertion is in agreement with other results from our laboratory (Cernadas et al., unpublished data) which showed the presence of increased nitrite production in arteries from 18-month-old compared with 5-month-old rats. Furthermore, the marked effect of NwNLA alone in the older animals suggests that they have an increased dependency on NO to maintain a stable renal function. In ageing rats, a dissociation between the effects on GFR and RPF and those on natriuresis and diuresis was also evident, since no response was found to l-Arg after NwNLA administration. This difference can be related to the diverse nature of each type of effect, i.e. GFR and RPF being more related to haemodynamic changes and natriuresis and diuresis more dependent of tubular effects.

The data of Reckelhoff et al. [13] in Sprague–Dawley
rats favour our present interpretation that the NO synthesis in the ageing animals is not blocked and, instead, is of critical importance in preserving renal functionality with ageing. The similarity between the effects of t-Arg and t-Gly suggests that both amino acids act through a related pathway. Even though t-Gly is not directly involved in NO formation, our laboratory has found evidence supporting the fact that NO is involved in the renal effect of t-Gly [18]. Specifically, the inhibition of t-Gly action by NωNLA advocates for the existence of a NO-related effect. Another possible link between the in vivo effects of both t-Arg and t-Gly may involve the stimulation of insulin secretion and, therefore, insulin-related effects, as has been shown recently in human studies [31]. Further experiments are needed to examine this potentially interesting effect in the present model.

The observations by OM ruled out the possibility that the impaired renal responses were due to an actual decrease in the number of functioning nephrons. The small number of sclerosed glomeruli in the present series of ageing rats is coincident with data from other groups [32,33]. On the other hand, the findings by EM support the existence of ultrastructural damage; this may account for the observed differences in functional response. The actual cellular mechanisms leading to the observed structural changes are of great interest and deserve further experimental attention.

In summary, our study showed a differentiated role for NO-dependent pathways in the kidneys of ageing rats. We suggest that ageing animals rely more on NO-related mechanisms than their younger counterparts, and that the kidney is affected selectively by the endothelium-related changes accompanying ageing.

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