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Basal and stimulated nitric oxide in control of kidney function in the aging rat

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Abstract

To investigate the activity of nitric oxide (NO) in control of renal hemodynamics during aging, studies were conducted on conscious Sprague-Dawley rats aged 3–5 mo (young, Y) and 18–22 mo (old, O). Blood pressure (BP) and renal vascular resistance (RVR) were higher in O vs. Y in control, and acute systemic NO synthesis inhibition (NOSI) increased BP and RVR, with an enhanced renal vasoconstrictor response in O. Infusion of the NO substrate L-arginine produced similar, selective renal vasodilation in both groups. The endothelium-dependent vasodilator acetylcholine caused similar falls in BP and RVR, whereas sodium nitroprusside produced an exaggerated depressor response in O vs. Y without falls in RVR in either age group. Urinary excretion of the stable NO oxidation products (NO_x) decreased with age, suggesting a decline in the overall somatic NO production. In conclusion, basal tonically produced NO has a more pronounced role in maintenance of renal perfusion in aging, whereas L-arginine- and agonist-stimulated renal vasodilation is not impaired with age. NO production from some source may be reduced with aging, as indicated by falls in 24-h NO_x excretion, although the similarity in pressor response and enhanced renal vasoconstrictor response to NOSI suggests that the role of NO in control of total peripheral and renal vascular resistance is maintained.

Keywords

renal vascular resistance; glomerular filtration rate; L-arginine; acetylcholine; nitric oxide synthesis inhibition

Endothelial factors play important roles in control of vascular tone and renal function. With aging, there is a widespread reduction in vasodilatory endothelial eicosanoids such as prostacyclin (PGI₂), whereas the vasoconstrictor eicosanoids such as thromboxane (TXA₂) remain unchanged or increase (21). The PGI₂-to-TXA₂ ratio is reduced in inner and outer medulla of old rat kidneys (26), an alteration that is vasoconstrictive and proaggregatory. In young adults, renal hemodynamics are not controlled by eicosanoids, but, with advancing age, renal hemodynamics become dependent on vasodilatory prostaglandins while at the same time the renal vasoconstrictor impact of TXA₂ may also increase (17). The nitric oxide (NO) system is an endothelial vasodilator system that, unlike the eicosanoids, plays a very important physiological role in control of systemic and renal hemodynamics in normal young adult humans and animals (15,23). NO is tonically released by vascular endothelium and maintains blood pressure (BP) at the normal low value (23) both by actively vasodilating and by opposing

vasoconstrictor influences. The renal microcirculation of normal mammals is also under tonic vasodilatory control by NO (5). There is some in vitro evidence from animal studies suggesting that, during aging, the peripheral vasculature has a diminished ability to produce NO (12,33). Furthermore, in man, endothelium-dependent renal vasodilation induced by acetylcholine (ACh) becomes impaired with age (16), which may reflect a reduction in agonist-stimulated NO release (23).

In the present studies, we conducted a series of experiments to investigate the role of NO in control of renal hemodynamics in young and old rats. Studies were conducted in the conscious, chronically catheterized rat to investigate the following: 1) effects of acute inhibition of endogenous, tonic NO synthesis (NOSI), 2) responses to NO synthase substrate and agonist-mediated increases in NO synthesis, and 3) the response to the NO donor sodium nitroprusside (SNP) on arterial BP and renal hemodynamics.

METHODS

Studies were conducted on male Sprague-Dawley rats obtained from Harlan Sprague Dawley (Indianapolis, IN), $n = 30$ young adults at 3–5 mo of age (Y) and $n = 37$ old rats, aged between 17 and 22 mo (O). While at Harlan, rats were maintained on a standard rat chow (Teklad LM-485 mouse/rat diet; Harlan Teklad, Madison, WI) containing 20% protein and 0.31% Na^+ , with free access to tap water, and aging rats were maintained under barrier conditions. Rats were shipped to West Virginia University at least 1 wk before surgery and were housed in positive-pressure laminar flow hoods in a private room with free access to food (Prolab RMH 3000, 22.5% protein, 0.44% Na^+ ; Agway, Syracuse, NY) and drinking water. Some rats (10 Y and 14 O) were placed in metabolic cages for 24-h collection of urine, and, after volume measurement, aliquots were stored at -20°C until later analysis.

In preliminary surgery, catheters were placed in the left femoral artery and vein and in the urinary bladder, primed, and plugged, and, after recovery, rats were returned to individual cages. This surgery was conducted while rats were under general anesthesia with the short-acting barbiturate Brevital (Eli Lilly, Indianapolis, IN), using full sterile technique; full details have been published previously (5,6,24). This preparatory surgery was carried out on 20 Y and 27 O. Rats were handled and trained to accustom them to the activity in the laboratory, and a period of 7–9 days elapsed between surgery and the first acute experiment to measure renal function. Usually, more than one study was conducted on the same animal, and at least 2 days of rest were allowed between experiments.

On the day of renal function measurements, rats were placed in a restraining cage, the bladder pin was removed for collection of urine, and the arterial catheter was connected to a pressure transducer and blood pressure recorder for direct measurement of arterial BP and occasional sampling of blood. A continual intravenous infusion of [^3H]inulin ($2\text{--}5\ \mu\text{Ci/ml}$) and *p*-aminohippurate (PAH, 1%) was given in 0.9% NaCl at $5\ \mu\text{l} \cdot \text{min}^{-1} \cdot 100\ \text{g rat body wt}^{-1}$. After 80 min of equilibration, two 20-min control urine collections were made with midpoint arterial blood samples ($\sim 150\ \mu\text{l}$). The blood was centrifuged, plasma was removed for analysis (see below), and red blood cells were reconstituted with sterile, isotonic NaCl and restored to the rat.

After completion of control measurements, one of the following four series of experiments was conducted in groups of both Y and O. In *series 1*, two groups of O were studied: at 18 mo of age, when declines in renal function are not yet evident, and at 20.5 mo of age, when there is substantial functional evidence of age-dependent deterioration. Rats received a 10 mg/kg intravenous bolus of the NO synthesis inhibitor N^G -nitro-L-arginine methyl ester (L-NAME, Sigma), a dose that produces the maximum rise in arterial BP in this preparation, as previously

reported by us for the young adult male (5). Pilot studies confirmed that a maximal sustained rise in arterial BP was also achieved in the old rats with this dose. Two further 20-min clearance measurements were made 5 min after administration of L-NAME when the increase in arterial BP had stabilized. *Series 2* rats received the NO synthase substrate L-arginine, (L-Arg; 300 mg/kg iv bolus, 50 mg · kg⁻¹ · min⁻¹) for 50 min. L-Arg was added to the inulin and PAH solution used in control in a concentration of 1 g/ml, and two further 20-min clearance measurements were made at 10–50 min into the L-Arg infusion. This L-Arg infusion protocol produces a selective renal vasodilation in young rats (5). In *series 2*, O were aged 19.3 mo. In *series 3*, in which O were aged 20.1 mo, the infusate solution given in control was changed to one containing identical inulin and PAH concentrations but also containing ACh (0.2 mg/ml), so that rats received an intravenous infusion of ACh in a dose of 10 µg · kg⁻¹ · min⁻¹. ACh stimulates endothelial NO release, leading to vasodilation (23). Two 20-min clearance measurements were begun 10 min after ACh infusion was started, when a stable depressor response (5–10% below baseline) was achieved. In *series 4* rats, the infusate solution was changed to one containing identical inulin and PAH concentrations as in control but also containing SNP (20 mg/ml), an endothelium-independent vasodilator that functions as an NO donor (20). This delivered a dose of 10 µg · kg⁻¹ · min⁻¹, which elicits a fall in mean arterial BP of 10–25% below control. Here, old rats were 20.2 mo of age.

At the end of each experiment, after restoration of red blood cells, the vascular and bladder catheters were primed and plugged and the rats were returned to their home cages. When all renal function experiments had been conducted on a given animal (usually 1–3 experiments), the rat was killed with an overdose of intravenous barbiturate and the vascular and bladder catheters and kidneys were examined to ensure that the rat was free of any infection. The left kidney was removed, weighed, and placed in 10% buffered Formalin. After fixation, blocks of kidney tissue were dehydrated and embedded in paraffin, and 3-µm sections were cut and stained with periodic acid Schiff with Harris hematoxylin counter-stain. The tissues were coded and examined by light microscopy, on a blinded basis, for the degree of glomerular sclerosis. Injury was assessed using a 0–4+ scale on at least 100 cortical glomeruli/section; each section was scored two to three times and averaged, and the overall average was used.

On the 24-h urine samples, the total protein concentration was measured by Bradford assay (8) and nitrate + nitrite (NO_x) concentration was measured by the Griess assay after use of the nitrate reductase enzyme, as described by us previously (30). The NO₂ content of the urine is routinely < 1% of the total NO_x, demonstrating that the urine samples are not contaminated with bacteria (25). In the acute studies, the volume of urine samples was measured gravimetrically, and then the urine was analyzed for [³H]inulin activity, PAH, Na⁺, and K⁺ concentrations and blood samples were analyzed for hematocrit, plasma [³H]inulin activity, and PAH, Na⁺, and K⁺ concentrations. Activity of [³H]inulin was measured in 10-µl samples of urine and plasma in a scintillation counter. PAH concentration was measured calorimetrically, and Na⁺ and K⁺ concentrations were measured with a flame photometer. These measurements allowed calculation of glomerular filtration rate (GFR), renal plasma flow (RPF), filtration fraction (FF), renal vascular resistance (RVR), urine flow (V), urinary excretion of Na⁺ and K⁺ (U_{Na} V and U_K V, respectively), and the fractional excretion of Na⁺ (FE_{Na}). Details of these analyses and calculations have been published previously (5,6, 24,30).

Statistical analyses of functional data was by paired *t*-test within each group, and unpaired *t*-test on percent changes from control was used to compare the response between Y and O to a given maneuver. Statistical evaluation of histological data was by Wilcoxon rank sum analysis. Statistical significance is defined when *P* < 0.05. Data are expressed as means ± SE. All animal procedures were approved by the West Virginia University Animal Care and Use Committee.

RESULTS

In all studies, we made initial observations in the control, baseline state, in a total of 38 experiments in 27 O (age range, 18–21.5 mo, mean = 19.4 ± 0.2 mo) and 33 experiments in 20 Y (age range, 3–5 mo, mean = 4.2 ± 0.3). Body weight was higher in O vs. Y (535 ± 7 vs. 381 ± 8 g; $P < 0.001$). Overall, BP and RVR were higher in O vs. Y (130 ± 2 vs. 117 ± 1 mmHg, $P < 0.001$, and 43 ± 3 vs. 23 ± 1 mmHg $\cdot$ ml $\cdot$ min $^{-1}$ $\cdot$ 100 g body wt, $P < 0.005$, respectively). GFR and RPF were reduced in the old animals (0.52 ± 0.02 vs. 0.72 ± 0.02 ml $\cdot$ min $^{-1}$ $\cdot$ 100 g body wt $^{-1}$, $P < 0.001$, and 2.0 ± 0.1 vs. 2.9 ± 0.1 ml $\cdot$ min $^{-1}$ $\cdot$ 100 g body wt $^{-1}$, $P < 0.001$). Of importance, RVR and BP are altering rapidly over the ages of 18 to 21 mo in male Sprague-Dawley rats, as shown in Fig. 1. BP and RVR are essentially unchanged vs. Y at 18 mo and markedly increased by 20 mo of age. This impacts on the baseline values of BP and RVR in individual groups of O (see below). V, U_{Na} V, and U_K V are similar in O vs. Y rats, when factored for body weight (5.8 ± 0.3 vs. 4.6 ± 0.3 μ l $\cdot$ min $^{-1}$ $\cdot$ 100 g body wt $^{-1}$, 0.48 ± 0.03 vs. 0.46 ± 0.03 μ eq $\cdot$ min $^{-1}$ $\cdot$ 100 g body wt $^{-1}$, and 0.67 ± 0.02 vs. 0.63 ± 0.02 μ eq $\cdot$ min $^{-1}$ $\cdot$ 100 g body wt $^{-1}$, respectively).

In *series 1* experiments, two groups of rats were studied at 18.0 ± 0.2 mo and at 20.5 ± 0.1 mo. In the 18-mo-old rats, BP was similar in Y and O rats in the baseline state, whereas by 20.5 mo significant age-dependent increases in BP and RVR and falls in GFR were evident (Table 1). All animals received a similar dose of the NO synthesis inhibitor L-NAME, which produced a maximal, sustained rise in BP in both Y and O. The magnitude of the pressor response was similar in Y, 18 mo O, and 20.5 mo O rats, but, in contrast, an enhanced renal vasoconstrictor response was seen in both groups of O vs. Y rats during acute NOSI (Fig. 2; Table 1). The falls in RPF and GFR and increase in FF due to NOSI were similar in O vs. Y. A natriuresis, increase in FE_{Na} , and diuresis with no change in U_K V were seen in the young rats with NOSI, as reported previously (5). In O, the response was more variable and there was no significant change in V, U_{Na} V, or FE_{Na} , although a fall occurred in U_K V (Table 1).

In *series 2* rats, the average age of O was 19.3 ± 0.24 mo. All rats received a continuous infusion of the NO substrate L-Arg in a dose previously shown to produce a selective renal vasodilation in the young rat (5). L-Arg had no effect on BP in either group and produced a similar renal vasodilation and increase in RPF with little impact on GFR in either age group (Table 1; Fig. 2). In this series, the RVR was elevated in old rats, whereas BP was numerically but not statistically different between the two age groups. L-Arg had a profound and similar diuretic, natriuretic, and kaliuretic action in Y and O, at least partly because of a nonspecific osmotic diuretic action of the L-Arg (12). (Table 1).

In *series 3*, the average age of O was 20.1 ± 0.1 mo. In this series, all rats received a continual infusion of ACh in a similar dose that evoked a small fall in BP in both age groups (Table 1; Fig. 3). Equivalent falls in RVR (expressed as percent of control) and increases in RPF were also seen in Y and O without a change in GFR, due to offsetting changes in other determinants of GFR, as reported earlier (3). In *series 3*, both RVR and BP were higher in the baseline state in O vs. Y ($P < 0.05$). ACh had no effect on V or U_K V but led to similar increases in U_{Na} V and FE_{Na} in Y and O (Table 1).

In *series 4*, the average age of O was 20.2 ± 0.2 mo. In this series, RVR and BP were higher in O vs. Y in the baseline state (both $P < 0.01$). All rats received an infusion of SNP in an equivalent dose, and a greater depressor response (when expressed as percent fall in BP) was seen in O vs. Y. There was no change in RVR in either Y or O with SNP. Similar falls in RPF and GFR occurred in both age groups, presumably due to auto-regulatory failure secondary to the reduction in BP (Table 1; Fig. 2). Reductions were also seen in V, U_K V, and U_{Na} V, although there was no effect on FE_{Na} , (Table 1).

Histological data are shown in Table 2 for 7 Y and 13 O on which functional data were also provided. Clearly O had substantial focal and segmental glomerular sclerosis as well as an increased incidence of more severe and global glomerular damage (three and four times). Overall, O had only 56% normal, undamaged glomeruli, whereas in young animals 93% were intact. The O on which histology were done averaged 19.3 ± 0.4 mo of age.

In addition, we measured the 24-h urinary excretion of NO_x (U_{NO_x} V) in 10 Y (age 4.4 ± 0.2 mo, body wt 416 ± 10 g) and 14 O (age 18.9 ± 0.3 mo, body wt 570 ± 13 g) rats. As shown in Fig. 3, the U_{NO_x} V, factored for body weight, is significantly higher in Y vs. O, suggesting a generalized decrease in NO synthesis with age. Rats were not pair fed in this study, and thus dietary NO_x intake was not rigidly controlled. However, pilot studies indicated that young and old rats ingest a constant food (and thus NO_x) intake per one hundred grams of body weight; therefore the fall in U_{NO_x} V factored for body weight reflects an overall decline in NO production. This decline in U_{NO_x} V preceded a significant rise in BP, because it was evident in 19-mo-old rats (Fig. 1).

DISCUSSION

The present study demonstrates that in the normal, conscious state the role of tonically produced NO is more pronounced in the maintenance of renal perfusion in the old vs. young male rat. NO is a physiologically important vasodilator that has major roles in maintaining a normal low BP and adequate renal perfusion (5,15,23). In addition, NO opposes the actions of vasoconstrictors such as angiotensin II (ANG II), and, when ANG II is influencing renal vascular tone, NO becomes critical in maintaining renal perfusion and GFR (6). We have previously reported functional evidence that, despite the falls in circulating plasma renin activity with age, intrarenal ANG II is controlling RVR in the normal unstressed state, unlike the young rat (1). Thus the enhanced renal vascular dependence on endogenous NO in aging seen here may reflect a physiological adaptation to increased vasoconstrictor stimuli. In the rats in the present study, the enhanced renal vascular dependence on NO was seen at 18 mo of age, before age-dependent increases had occurred in BP or RVR, and persisted at 20.5 mo when age-dependent renal dysfunction was evident. Several recent reports by others have also suggested that NO becomes increasingly important in control of renal vascular tone in aging because both acute and chronic NO inhibition in the rat cause exaggerated renal vasoconstriction in old animals (28,31).

The functional response to NO inhibition provides an index of the level of activity of tonic, shear-stimulated endothelial NO, and this is a physiologically relevant measure. Many workers have also assessed the ability of the NO precursor L-Arg or various agonists to stimulate NO-dependent vasodilation in aging, and in this study we have also evaluated the renal vasodilatory actions of L-Arg and ACh. L-Arg is the native NO substrate, but NO generation by the constitutive endothelial NO synthase is not thought to be substrate limited (22). The selective renal vasodilatory action of L-Arg may reflect a selective intrarenal arginine deficiency and substrate limitation or, alternatively, may be due to competitive inhibition of the high intrarenal level of endogenous substituted L-Arg derivatives that function as NOSI (34). Thus, although L-Arg-induced renal vasodilation is most likely NO dependent, the precise mechanism remains unclear. Irrespective of mechanism, the present study shows that the renal vasodilatory response to acute L-Arg infusion is similar in young and old rats. In contrast, the renal vasodilatory response to acute infusion of the amino acid glycine and to chronic high-protein feeding (both thought to be mediated in part by NO) are blunted with advancing age in the rat (4,10). We have also previously reported that, with chronic NO inhibition-induced hypertension, the renal vasodilatory response to L-Arg is maintained, whereas that to glycine is blunted (24). Collectively, these findings support the suggestion that the renal vasodilatory response to glycine or mixed amino acids is determined by other factors in addition to NO

(9). Based on the present study, alterations in these alternative (non-NO) factors may be responsible for the age-dependent blunting of glycine and protein feeding-induced renal vasodilation seen in the rat. Studies in humans have indicated that the renal vasodilation in response to amino acid infusion is not attenuated by aging (13); perhaps in humans this renal vasodilatory action is more NO dependent.

Endothelium-dependent vasodilation can also be triggered by the agonist ACh. ACh has a widespread vasodilatory action and produces relaxation of vascular smooth muscle by release of endothelial NO and vasodilatory prostanoids and by unidentified hyperpolarizing factors (23,29). Because the interactions between these various vasodilatory pathways is complex and may vary between vascular beds and species, the response to ACh does not exclusively monitor activity of agonist-stimulated NO, but rather indicates overall endothelial vasodilatory responses to ACh. In the present study we administered a dose of ACh that had similar, mild depressor actions in young and old rats. This dose produced similar renal vasodilatory responses in both young and old rats. Because the ACh-dependent fall in RVR can be completely inhibited with NOSI in the rat (32), these data suggest that agonist-stimulated NO is unaffected by advancing age in the renal vasculature. These observations are at variance with earlier reports in humans by Hollenberg et al. (16), which showed that renal blood flow (measured by xenon washout) became refractory to the actions of ACh in old subjects. The reason for this difference is unclear. It may reflect a species difference in the effect of aging on ACh-induced NO release and/or response in the kidney. Alternatively, humans may have a greater non-NO-dependent component to the ACh-induced renal vasodilation, which is blunted with advancing age. Finally, there may be age-dependent alterations in the renal vascular endothelial ACh receptor in humans that do not occur in the rat.

We have also used the endothelium-independent NO donor SNP to investigate responsivity to administered NO in aging. SNP is a vasodilator and also has powerful venodilator actions that account for much of the fall in BP seen with this agent (20). In the present study, the depressor response to the dose of SNP was similar in young and old rats, but SNP did not produce a renal vasodilation in either age group. Thus in vivo studies with SNP are not helpful in assessing renal vascular responsiveness to NO. The more informative functional measurements with acute NOSI are consistent in suggesting that activity of the intrarenal vascular NO system is intact or possibly enhanced in the aging rat.

In the present study, aging leads to increased BP and RVR and falls in RPF and GFR, as well as increasing glomerular injury, as reported earlier (2,4,19). Age-dependent glomerular damage occurs quite rapidly in the Sprague-Dawley rat, with substantial injury being present at 18–20 mo. The cause of age-dependent glomerulopathy is not known, and, although we have recently excluded glomerular hypertension and hypertrophy as primary causal factors (2), glomerular hypertension probably contributes to the rapid development of glomerular damage in aging rats of the Sprague-Dawley strain. Growth and extracellular matrix production by glomerular mesangial cells apparently play a crucial role in the development of all forms of glomerular injury, and alterations in activity of the various vasoactive systems influence mesangial extracellular matrix production. NO may inhibit hypertrophic growth and extracellular matrix formation of contractile cells (14,18), and studies by us have demonstrated that chronic NO deficiency leads to glomerular injury (7). However, functional data in the present study suggest that renal NO is not deficient in the aging kidney; thus others factors must be responsible for development of glomerular damage.

In contrast to the observations in the old rat kidney, there is in vitro evidence that, overall, the peripheral vasculature has a diminished ability to produce NO with advancing age (12,33) and that the vascular response to administered nitrodilators is impaired in some blood vessels with aging (11). Of importance, however, these in vitro observations are usually conducted on

conduit vessels, which do not contribute to total peripheral vascular resistance. Reckelhoff and colleagues (27) have reported an overall reduction in NO production in the old rat, as measured by 24-h urine NO_X output, which may be related to decreased arginine availability. The in vivo activity of NO cannot be directly measured because NO is very unstable, rapidly undergoing oxidation initially to nitrite (NO₂) and ultimately to the stable end product nitrate (NO₃) (30). Oxidation of NO is normally near complete in vivo; thus NO₂ is present only in trace amounts in normal urine, and increased quantities of NO₂ reflect urinary tract infections (25). The NO_X in the urine comes both from endogenous biosynthesis of NO throughout the body and from dietary NO₂ and NO₃ intake. If NO_X intake is controlled, alterations in 24-h U_{NO_X} V can give a qualitative indication of alterations in endogenous NO synthesis; however, it is important to note that this assay gives no indication of the origin of the NO. In the present study and in earlier work by Reckelhoff and colleagues (27), falls in 24-h U_{NO_X} V precede increases in BP, and because we have separate evidence that renal NO production is not diminished, the fall in U_{NO_X} V probably reflects a reduction in NO synthesis from extrarenal sites. Because the pressor response to acute NOSI is also undiminished in aging, it is possible that the age-dependent reduction suggested by falls in U_{NO_X} V occurs in nonresistance vessel locations. It is also possible that alterations in vascular NO production that have substantial effects on vascular tone and thus BP cannot be detected by this relatively insensitive method.

In aggregate, the present studies show that, in the normal, unstressed conscious rat, neither basal nor agonist-stimulated renal vascular NO production is impaired by aging and that kidney perfusion is more dependent on NO in old than in young kidneys.

Perspectives

The age-dependent alterations in the kidney include renal vasoconstriction and development of glomerular damage, but the present studies suggest that these are not the result of local intrarenal NO deficiency. As with the prostaglandins, renal perfusion becomes more dependent on NO with aging, perhaps reflecting a compensatory, general upregulation of renal endothelial vasodilatory factors in response to increased constrictor tone. The use of 24-h U_{NO_X} V is becoming more common as an in vivo method of assessing the level of NO production, but these studies highlight potential problems in interpretation of these measurements. Despite falls in base-line U_{NO_X} V acute NOSI produces a similar pressor response in O vs. Y, thus U_{NO_X} V does not correlate with increased BP in O in this study. Because NO is made in most cell types within the body, U_{NO_X} V presumably reflects the overall somatic production rate of NO and, in aging, the reduction in NO measurable by this method is not necessarily involved in BP regulation.

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Fig. 1.

Mean arterial blood pressure (BP), renal vascular resistance (RVR), and glomerular filtration rate (GFR) in conscious, chronically catheterized young adult rats ($n = 31$) and old rats aged 18 ($n = 7$), 19 ($n = 9$), and 20 ($n = 17$) mo. * Significant change vs. young adult. #Significant change vs. 19 mo.

Fig. 2.

Effect on BP and renal function [RVR, renal plasma flow (RPF), and GFR] in young (Y) and old (O) male Sprague-Dawley rats of the following acute maneuvers: systemic nitric oxide (NO) inhibition N^G -nitro-L-arginine methyl ester (NOSI), infusion of the NO substrate L-arginine (L-Arg), infusion of the endothelium-dependent vasodilator acetylcholine (Ach), and infusion of the NO donor sodium nitroprusside (SNP). All changes are expressed as percent change from control. Numbers in parentheses are group age, to nearest month. * Difference in %change from control between old and young rats. $P < 0.05$.

Fig. 3. Comparison of 24-h urinary excretion of NO oxidation products (U_{NOX} V) when factored for body weight in young and old male Sprague-Dawley rats. * Statistically significant decline with age, $P < 0.05$.

Effect on renal function in Y and O male rats of acute systemic NOS1 with L-NAME alone, infusion of the NOS substrate L-Arg, infusion of the endothelium-dependent vasodilator ACh, and infusion of the endothelium-independent vasodilator SNP

Table 1

L-NAME	BP, mmHg	RVR, $\frac{\text{mmHg} \cdot \text{min}^{-1}}{\text{ml} \cdot 100 \text{ g body wt}^{-1}}$	RPF, $\frac{\text{ml} \cdot \text{min}^{-1}}{100 \text{ g body wt}^{-1}}$	RPF, $\frac{\text{ml} \cdot \text{min}^{-1}}{100 \text{ g body wt}^{-1}}$	FF	V, $\frac{\mu\text{l} \cdot \text{min}^{-1}}{100 \text{ g body wt}^{-1}}$	U _{Na} V, $\frac{\mu\text{eq} \cdot \text{min}^{-1}}{100 \text{ g body wt}^{-1}}$	U _K V, $\frac{\mu\text{eq} \cdot \text{min}^{-1}}{100 \text{ g body wt}^{-1}}$
<i>Series 1: NOS1</i>								
Y								
Control (n = 7)	117±3	22.7±1.7	2.9±0.3	0.73±0.03	0.272±0.028	4±1	0.39±0.07	0.76±0.06
+L-NAME	150±6	45.1±3.8	1.8±0.2	0.60±0.05	0.347±0.033	14±4	1.07±0.37	0.73±0.06
P	<0.001	<0.001	<0.01	<0.05	<0.05	<0.05	=0.05	NS
O, 18 mo								
Control (n = 7)	120±3	27.5±1.8	2.5±0.1	0.66±0.02	0.262±0.011	5±1	0.40±0.06	0.58±0.04
+L-NAME	150±3	72.8±4.8	1.2±0.1	0.48±0.01	0.402±0.020	11±4	0.88±0.33	0.39±0.02
P	<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS	<0.005
O, 20.5 mo								
Control (n = 5)	132±4	43.7±6.8	1.9±0.2	0.41±0.05	0.220±0.006	8±1	0.46±0.07	0.80±0.06
+L-NAME	161±3	116.7±18.4	0.9±0.1	0.28±0.03	0.315±0.016	5±1	0.27±0.07	0.57±0.03
P	<0.001	<0.005	<0.001	<0.005	<0.005	NS	<0.05	<0.005
<i>Series 2: L-Arg</i>								
Y								
Control (n = 9)	118±2	24.3±1.5	2.7±0.2	0.70±0.03	0.265±0.015	4±1	0.45±0.50	0.65±0.07
+L-Arg	119±3	16.7±1.2	4.1±0.3	0.81±0.06	0.203±0.018	62±6	6.47±0.70	2.09±0.13
P	NS	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001
O, 19.3 mo								
Control (n = 9)	125±4	39.0±5.0	2.0±0.2	0.58±0.04	0.318±0.041	5±1	0.46±0.11	0.59±0.04
+L-Arg	132±5	25.3±2.2	3.1±0.2	0.60±0.03	0.202±0.018	69±4	7.56±0.57	2.07±0.07
P	<0.005	<0.05	<0.001	NS	<0.01	<0.001	<0.001	<0.001
<i>Series 3: ACh</i>								

L-NAME	BP, mmHg	RVR, mmHg· ml ⁻¹ ·min ⁻¹ ·100 g body wt	RPF, ml· min ⁻¹ · 100 g body wt ⁻¹	RPF, ml· min ⁻¹ · 100 g body wt ⁻¹	FF	V, μl· min ⁻¹ · 100 g body wt ⁻¹	U _{Na} V, μeq· min ⁻¹ · 100 g body wt ⁻¹	FE _{Na} , %	U _K V, μeq· min ⁻¹ · 100 g body wt ⁻¹
Y									
Control (n = 9)	117±3	22.8±0.9	3.1±0.2	0.73±0.04	0.238±0.012	5±1	0.52±0.07	0.50±0.06	0.51±0.05
+ACh	109±3	15.7±1.2	3.9±0.2	0.71±0.04	0.189±0.016	4±1	0.72±0.07	0.72±0.06	0.55±0.04
P	<0.01	<0.005	<0.05	NS	<0.01	NS	<0.05	<0.01	NS
O, 20.1 mo									
Control (n = 9)	135±4	44.5±6.8	2.0±0.2	0.53±0.05	0.279±0.021	7±1	0.51±0.03	0.72±0.07	0.59±0.05
+ACh	125±5	31.4±5.6	2.8±0.4	0.52±0.06	0.208±0.032	6±1	0.82±0.09	1.21±0.18	0.56±0.04
P	<0.005	<0.01	<0.01	NS	<0.01	NS	<0.05	<0.01	NS
Series 4: SNP									
Y									
Control (n = 8)	117±3	22.2±1.6	3.0±0.2	0.71±0.04	0.243±0.012	6±1	0.47±0.07	0.48±0.08	0.79±0.05
+SNP	101±3	25.9±2.0	2.3±0.2	0.59±0.04	0.264±0.023	4±1	0.24±0.02	0.29±0.03	0.55±0.07
P	<0.001	NS	<0.05	<0.01	NS	<0.001	<0.05	NS	<0.05
O, 20.2 mo									
Control (n = 8)	138±6	59.5±9.4	1.5±0.2	0.42±0.04	0.293±0.026	6±1	0.53±0.07	0.96±0.18	0.65±0.05
+SNP	108±4	53.5±8.7	1.3±0.2	0.34±0.003	0.279±0.046	3±1	0.28±0.02	0.60±0.06	0.47±0.04
P	<0.001	NS	<0.05	<0.001	NS	<0.001	<0.05	NS	<0.005

Values are means ± SE. BP, blood pressure; RVR, renal vascular resistance; RPF, renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; V, urine flow; U_{Na} V, urinary sodium and potassium excretion; FE_{Na}, fractional excretion of sodium; Y, young (3–5 mo) rats; O, old (18–21 mo) rats; NOS1, nitric oxide (NO) synthesis inhibition; L-Arg, L-arginine; L-NAME, N^G-nitro-L-arginine methyl ester; ACh, acetylcholine; SNP, sodium nitroprusside; NS, not significant. P values denote paired difference from control to experimental within each group. Differences between O and Y are discussed in the text.

Table 2
Comparison of glomerular sclerotic damage between young and old male Sprague-Dawley rats

	0	1	2	3	4
Young	93.3±0.7	5.9±0.5	1.4±0.2	0.3±0.2	0.5±0.3
Old	56.5±4.0	11.0±0.6	12.1±1.4	6.0±0.8	15.2±2.0
P	<0.001	<0.001	<0.001	<0.001	<0.001

Values are means ± SE expressed as % glomeruli in the following injury categories: 0 = no injury; 1 = up to 25% injury; 2 = 25–50% injury; 3 = 50–75% injury; 4 = global sclerosis.