Loss of nitric oxide and endothelial-derived hyperpolarizing factor–mediated responses in aging

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Loss of nitric oxide and endothelial derived hyperpolarizing factor mediated responses in aging.

Background. Aging has considerable structural and functional effects on the vascular system of the kidney. One such effect is an alteration in vascular tone which potentially will initiate renal damage. Vascular tone is determined by the balance between vasoconstrictors and vasodilators. Therefore, we hypothesized that aging attenuates vasodilatory responses in the kidney. These changes may be mediated by a loss of nitric oxide and endothelial-derived hyperpolarizing factor (EDHF).

Methods. The systemic and renal responses of nitric oxide and EDHF were investigated in aging (18 months old) and young (3 months old) Sprague-Dawley rats.

Results. We demonstrated a general loss of vasodilatory responses in the aging kidney. In addition, nitric oxide levels were reduced in the serum and kidney cortex of aging versus young animals, although this was not accompanied with a loss of endothelial nitric oxide synthase (eNOS) protein in the kidney cortex. Aging animals also exhibited a loss in EDHF-mediated vasodilation following stimulation with either acetylcholine or bradykinin in the isolated perfused kidney.

Conclusion. These findings indicate that not only a defect in the nitric oxide pathway, but also a loss of EDHF-mediated responses may be responsible for impaired vasodilation in the aging kidney. This may result in enhanced vasoconstrictive responses in aging which potentially will cause renal damage and ultimately a loss in glomerular filtration rate (GFR).

Aging is a natural process which has considerable structural and functional effects on the kidney. In aging, renal blood flow is reduced [1, 2] with a parallel decline in glomerular filtration rate (GFR) from the age of 30 onwards in the adult human [3]. In addition, creatinine clearance is decreased by 30% to 40% in individuals over the age of 80 years and there is an increased prevalence of microalbuminuria [4]. Histologically, both in aging human and rat renal biopsies focal and segmental glomerulosclerosis develop which is associated with mesangial matrix expansion and increased basement membrane thickening [4]. In the tubulointerstitial areas, many tubules become dilated and atrophic accompanied by an increased degree of interstitial fibrosis and an influx of infiltrating mononuclear cells [4]. The majority of preglomerular arterioles become thicker [5] and in aging rats a reduction in glomerular and peritubular capillary number [6, 7] has also been observed.

It has been hypothesized that one of the key initiating events leading to renal damage in aging and other progressive renal diseases is an alteration in vascular tone [8, 9]. The endothelium plays a key role in this process by releasing a variety of relaxing and contracting factors that regulate the underlying vascular smooth muscle [10]. In aging humans and animals vasodilatory responses induced by acetylcholine are attenuated [11–13], while the responsiveness to vasoconstrictors such as angiotensin II is enhanced [14, 15]. This may result in enhanced vasoconstrictive responses in aging which potentially will cause renal damage and ultimately a loss in GFR [8, 9].

Two of the most important mediators of vasodilation are nitric oxide and endothelium-derived hyperpolarizing factor (EDHF). Nitric oxide is formed from the precursor, L-arginine, through the activity of constitutive and inducible nitric oxide synthases (iNOS). Total body nitric oxide production is reduced in aging rats as measured by the excretion of nitrates and nitrites [16–18] which also coincides with the progression of renal injury and decreases in renal plasma flow [16, 17, 19]. In addition, elevated systemic levels of the endogenous competitive inhibitor of NOS, N\(^G\)-N\(^G\)-asymmetric dimethyl-L-arginine (ADMA) are observed in both aging rats and humans [18, 19]. Decreased expression of endothelial NOS
(eNOS) and neuronal NOS (nNOS) have been found in the aging male kidney [7, 20]. The currently unidentified EDHF initiates vasodilation by the activation of calcium-induced potassium channels in vascular smooth muscle cells [21]. In aging, impairment of EDHF-mediated blood vessel relaxation has been shown in the superior mesenteric artery of rats [22] and human gastroepiploic distal arteries [23]. In addition, studies by Büssemaker et al [24] show that renal arterial vessels from old spontaneous hypertensive rats, but not Wistar-Kyoto rats exhibit a total loss of EDHF-mediated responses.

This study investigated vasodilatory responses in the aging Sprague-Dawley rat kidney. We hypothesized that aging would lead to impaired vasodilation mediated by a loss of nitric oxide and EDHF. Our results demonstrate that vasodilation is attenuated in the aging kidney and that alterations in both nitric oxide and EDHF may contribute to this diminished response.

**METHODS**

Reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

**Animals**

Studies were conducted in “old” (18 months old) ($N = 7$) and “young” (3 months old) ($N = 7$) adult male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA). All animals were fed a standard laboratory diet and water ad libitum. For determination of protein and creatinine in the urine, animals were placed in individual metabolic cages for 24-hour urine collection. During this time, food was withheld from all animals. Protein concentration was determined using a commercially available kit (Bio-Rad Laboratories, Hercules, CA, USA). Creatinine was measured by reacting urine samples with alkaline picrate to form a complex whose absorbance could be measured at 510 nm as according to manufacturer’s instructions (Diagnostic Chemicals Limited, Oxford, CT, USA). Animals were sacrificed and kidneys fixed in either methyl Carnoy’s solution or periodate-lysine paraformaldehyde (PLP) for immunohistochemistry or snap-frozen for protein analysis. For samples snap-frozen the kidneys were separated into cortex and medulla tissue. Before sacrifice, serum and plasma was obtained. All animal procedures were approved by the Animal Care Committee of Baylor College of Medicine (Houston, TX, USA).

**Measurement of nitrites and nitrates**

Serum and tissue from the kidney cortex and medulla of young and old animals were analyzed for nitrites and nitrates (NO$\div$) levels. Kidney cortex tissue was first homogenized in cell lysis buffer (Cell Signaling Technology, Beverly, MA, USA) and then centrifuged at 13,000 × g for 30 minutes. The supernatant was removed and the protein content was measured with a commercially available kit (Bio-Rad Laboratories). NO$\div$ content was determined by chemiluminescence using a Sievers Instruments Nitric Oxide Analyzer (Boulder, CO, USA) as previously described [25]. In brief, samples were injected into a purge vessel containing vanadium which converted nitrates and nitrates into nitric oxide. The nitric oxide was then propelled by the inert gas nitrogen into a reaction chamber where nitric oxide was oxidized into NO$_2$ by ozone. The chemiluminescence associated with this reaction was read in millivolts and recorded as a deflection on a data recorder. The area under the curve reflected the NO$\div$ content in the samples. This was read initially as a value in luminescence units, which when divided by the slope of a standard curve gave the precise NO$\div$ content. Every sample was measured at least twice. For serum and tissue levels of NO$\div$ the measurements were converted to μmol/L of NO$\div$/L and μmol/L of NO$\div$/mg of protein, respectively.

**Immunohistochemistry**

Five micrometer frozen sections were cut from the PLP-fixed tissue. Sections were thawed at room temperature for 2 hours and blocked with 10% fetal calf serum (FCS), 0.2% bovine serum albumin (BSA), and 0.1% Tween-20 in phosphate-buffered saline (PBS) (pH 7.4) for 2 hours at room temperature. Sections were reacted overnight at 4°C with mouse antihuman eNOS (1:2000) (BD Biosciences, Pharmingen, San Diego, CA, USA) which cross reacts with rat eNOS according to manufacturer’s data sheet. Endogenous peroxidase was then quenched with 0.3% H$_2$O$_2$ in methanol for 30 minutes. Bound primary antibodies were detected with an EnVision kit (Dako, Carpinteria, CA, USA). Brown color was generated by using a diaminobenzidine (DAB) substrate and nuclei counterstained with hematoxylin. To determine numbers of macrophages, ED-1 staining was performed. Five microgram sections were cut from organs fixed in methyl Carnoy’s solution. Sections were rehydrated and treated with H$_2$O$_2$ and blocking solution. Sections were reacted overnight with mouse anti-macrophage ED-1 antibody (1:50) (Serotec, Raleigh, NC, USA) and bound primary antibodies detected as described above. Some sections were also stained with periodic acid-Schiff (PAS) reagent to evaluate histology. Negative controls consisted of omission of the primary antibody or substitution of the primary antibody with the appropriate preimmune serum. Staining was evaluated in a blinded manner ($N = 5$ for young and aging animals).

**Western blotting**

Fifty micrograms of protein samples prepared as described above were denatured at 100°C for 5 minutes and separated on sodium dodecyl sulfate (SDS)-8%
polyacrylamide electrophoresis gels. Proteins were transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) by electroblotting (Bio-Rad Laboratories). Blots were blocked for 1 hour with 5% (wt/vol) fat-free milk powder, 0.1% BSA, and 0.1% Tween-20 in PBS, and incubated with mouse antihuman eNOS (1:1000) antibody at 4°C overnight. Blots were washed in PBS with 0.2% Tween-20 and once in blocking solution. They were incubated for 30 minutes with secondary antibodies and bands detected by chemiluminescence (Amersham Pharmacia Biotech). Proteins were sized with Rainbow markers (Amersham Pharmacia Biotech). As a positive control, protein samples from human umbilical vein endothelial cells were used. Blots were stripped and then reprobed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5000) (Abcam, Cambridge, MA, USA) as a housekeeping gene. The intensities of these bands were measured by densitometry and analyzed as the eNOS/GAPDH ratio between aging and young samples (N = 4 in each group).

### Isolated perfused kidney

Isolated perfused kidneys were prepared from aging and young animals as previously described [26, 27]. Briefly, after anesthesia with peritobarbital sodium (50 mg/kg intraperitoneally), the left kidney was exposed by midline ventral laparotomy. The left renal artery was cannulated through the abdominal aorta and the kidney was perfused by means of a peristaltic pump (model 7515-10) (Cole Parmer Instrument Company, Vernon Hills, IL, USA) with warmed (37°C) oxygenated (95% O₂ and 5% CO₂) Krebs-Henseleit perfusion buffer composed of 118 mmol/L NaCl, 25 mmol/L NaHCO₃, 1.9 mmol/L CaCl₂, 1.19 mmol/L MgSO₄, 4.75 mmol/L KCl, 1.19 mmol/L KH₂PO₄, and 11.1 mmol/L dextrose (pH 7.2). The inferior vena cava was cut to allow the flow of perfusate and the kidney was removed from the surrounding fat and placed in an organ chamber prewarmed at 37°C. Perfusion pressure was monitored constantly by means of a transducer (Transbridge, model TBM-4) (World Precision Instrument, Sarasota, FL, USA) which is connected to a computer by a signal manifold (models DI 720 and DI 205) (DataQ Instruments, Akron, OH, USA). Renal vascular tone was elevated by addition of phenylephrine (150 μg/L) to the Krebs buffer. Acetylcholine and bradykinin (50 to 500 ng) were injected randomly into the perfusate line proximal to the kidney and changes in perfusion pressure used as an index of renal vascular resistance with a decrease in perfusion pressure indicating vasodilation. To assess the role of different vasodilator mediators, some experiments were performed with the cyclooxygenase inhibitor, indomethacin (10 μmol/L) and a NOS inhibitor, L-arginine analogue N-nitro-L-arginine methyl ester (L-NAME) (100 μmol/L) added to the perfusion buffer. In addition, to analyze the contribution of potassium channels and epoxyeicosatrienoic acid (EET) to the EDHF response, some experiments were performed either in the presence of both apamin (250 nmol/L) and charybotoxin (10 nmol/L) or 6-(2-propargyloxyphenyl) hexanoic acid (PPOH) (30 μmol/L) in the perfusion buffer, respectively. All responses were measured as the differences between the basal preconstricted pressure and the peak vasodilatory response.

### Statistics

Data between aging and young animals were compared using the nonparametric Mann-Whitney U test, with differences being considered significant when P < 0.05. All values provided are means ± SEM.

### RESULTS

#### General measurements

Studies were performed in both aging (18 months old) and young (3 months old) rats. Body weight was significantly elevated in aging animals (649 ± 42 g) when compared to young animals (237 ± 5 g) (N = 7 in each group) (P < 0.001). The ratio of 24-hour urine protein/urine creatinine was increased in the aging rats (0.93 ± 0.08 in aging animals versus 0.733 ± 0.05 in young animals) (P < 0.01).

#### Microscopic findings in young and aging rats

Sections from aging and young rats were evaluated by PAS staining to determine morphology (N = 5 from both aging and young animals). The PAS-stained sections of kidneys from the young rats showed no abnormalities in any renal compartment (Fig. 1A). The kidney from the aging rats showed focal cortical areas with atrophic tubules, thickened tubular basement membrane, interstitial fibrosis, and interstitial mononuclear inflammatory cell infiltrates. Focal tubular dilatation with or without casts were also seen. It should be noted that the chronic injury involved a small portion (<5%) of the cortex with the remaining areas displaying no significant changes. The glomeruli within or adjacent to these areas showed global or segmental collapse and thickening of the glomerular basement membrane (Fig. 1B). Immunostaining for ED-1, a macrophage marker, showed that kidneys from young animals contained sparse macrophages (Fig. 1C). In contrast, ED-1–positive cells were prominent in the areas of chronic tubulointerstitial injury in aging animals (Fig. 1D).
Long et al: Nitric oxide and EDHF-mediated responses in aging

Fig. 1. Histologic observations in the aging rat kidney. (A) The periodic acid-Schiff (PAS)-stained sections of kidneys from the young rats showed no abnormalities in any renal compartment. (B) The kidney from the aging rats showed focal cortical areas with atrophic tubules (*), thickened tubular basement membranes (*), interstitial fibrosis and interstitial mononuclear inflammatory cell infiltrates. A glomerulus within the area of chronic tubulointerstitial injury showed global collapse and thickening of the glomerular basement membrane. (C) Kidneys from young animals contained sparse macrophages. (D) Macrophages were more prominent in the areas of chronic tubulointerstitial injury in aging animals.

Attenuated vasodilation in aging kidneys induced by acetylcholine

We used the isolated perfused kidney preparation to determine the renal vasodilatory response to acetylcholine. In both young and aging rat kidneys there was a dose-dependent increase in vasodilation induced by acetylcholine (N = 5 for each group). Renal vasodilation tended to be reduced in the aging animals at all doses, although this was only significant with 50 ng of acetylcholine (P < 0.05) (Fig. 2). This data provided a rationale for further studies investigating the biochemical mediators of vasodilation in aging animals.

Nitric oxide measurement in serum and tissue samples

Aging animals had a significantly reduced level of NO÷ in the serum when compared to young animals (16.72 ± 0.3 μmol/L versus 26.6 ± 0.90 μmol/L in young animals) (P < 0.05) (Fig. 3). Levels of NO÷ in the aging animal cortex (7.77 ± 0.56 μmol/L of NO÷/mg of protein) were also significantly reduced when compared to young animals (19.18 ± 4.55 μmol/L of NO÷/mg of protein) (P < 0.05) (Fig. 4).

eNOS expression in the aging kidney cortex

In young animals, positive staining for eNOS was observed in the glomeruli and in some peritubular capillaries (Fig. 5A). Compared to young rats, the kidneys in aging rats displayed a similar expression of eNOS in glomeruli but increased expression of eNOS in peritubular capillaries (Fig. 5B). Staining was not observed using control nonimmune sera (data not shown). To confirm the immunostaining findings, Western blotting for eNOS was performed. Protein levels of eNOS in aging animals were shown to be up-regulated in the kidney cortex compared to young rats (P < 0.05) (Fig. 6) as assessed by densitometric analysis.
EDHF-mediated responses in the aging rat

The contribution of EDHF to vasodilation in aging was determined using acetylcholine and bradykinin as agonists in the isolated perfused kidney in the presence of cyclooxygenase and nitric oxide inhibitors. Both acetylcholine and bradykinin induced a dose-dependent increase in vasodilation in both young and aging rats ($N = 12$ for both young and aging animals). There was a significant inhibition ($P < 0.05$) in vasodilation induced by acetylcholine and bradykinin in the aging animals indicating an impairment of the EDHF pathway (Fig. 7). The attenuation of vasodilation in the aging animals tended to be more marked for responses induced by acetylcholine than those with bradykinin.

In young animals, the nonselective EET inhibitor, PPOH significantly impaired vasodilatory responses ($P < 0.05$) induced by acetylcholine at all doses (Fig. 8A) ($N = 7$ for young animals with/without PPOH). In aging rats, although there was a tendency for acetylcholine responses to be impaired with PPOH (Fig. 8B) ($N = 3$ for aging animals with/without PPOH) this was not significant and in some experiments PPOH did not reduce acetylcholine dilation at all. Vasodilation induced by bradykinin was attenuated by PPOH in young animals (Fig. 9A) ($N = 7$ for young animals with/without PPOH). In contrast, the vasodilatory responses induced by bradykinin in aging animals were not altered in the presence of PPOH (Fig. 9B) ($N = 7$ for aging animals with/without PPOH).

Finally, in both young and aging animals, the potassium channel blockers, apamin and charybdotoxin, significantly inhibited acetylcholine-induced EDHF-mediated responses at all doses of agonist used (Fig. 10) ($P < 0.05$) ($N = 5$ in all groups). In addition, EDHF-mediated vasodilation in young rats induced by bradykinin was significantly inhibited at all doses (Fig. 11A) ($P < 0.05$) ($N = 7$ for young animals with/without apamin and charybdotoxin). In contrast, the bradykinin responses in aging animals showed no inhibition in the presence of potassium channel blockers (Fig. 11B) ($N = 7$ for aging animals with/without apamin and charybdotoxin).

DISCUSSION

Aging has considerable effects on renal structure and function in both humans and mammals. While not all humans show marked structural changes with aging, in most subjects focal and/or global glomerular sclerosis develops that is characterized by capillary loss, mesangial expansion, and glomerular basement membrane thickening [28]. Tubulointerstitial fibrosis also occurs, with tubular atrophy, thickening of tubular basement membranes, and focal areas of interstitial fibrosis and inflammation [28]. The intima of renal arteries becomes thicker [5], alongside a reduction in glomerular and capillary endothelial cell number [6, 7]. We and others have hypothesized that one of the key initiating events leading to renal damage in aging may be an alteration in vascular structure and function [8, 9]. As demonstrated in this study and others [11–13], the vasodilatory responses induced by acetylcholine are attenuated in aging animals and humans.

One important mechanism by which acetylcholine induces vasodilation is by the stimulation of endothelial cells to release nitric oxide. Nitric oxide induces vascular smooth muscle relaxation primarily by the activation of soluble guanylate cyclase, leading to an increase in cyclic guanosine monophosphate (cGMP), which causes a decrease in intracellular calcium ions [29]. The observation in this study that serum and renal cortical nitrites/nitrates (which represent the stable end products of nitric oxide metabolism) were reduced in aging animals is consistent with the reduced 3-nitrotyrosine content that was reported previously.
with other studies that have reported a loss of nitric oxide systemically in aging animals [16–18]. The mechanism for the low systemic nitric oxide levels is complex and may be due to both decreased synthesis and/or increased degradation [17, 30], although these changes may not translate to the adult human [31]. A low level of renal nitric oxide would be expected to result in a reduction in renal blood flow and an increase in the renal vascular resistance [32]. In addition, aging renal blood vessels tend to be more sensitive to blocking nitric oxide. Vasoconstriction induced by administration of the nitric oxide inhibitor, L-NAME, is enhanced in aging renal blood vessels [16, 33]. This suggests that maintaining nitric oxide responses may be more functionally important in aging renal vessels, perhaps by compensating for the enhanced vasoconstriction induced by angiotensin II [14, 15] to maintain vascular tone.

A potential mechanism to explain the nitric oxide reduction in the aging kidney is a loss in NOS enzymes. The main vascular isoform of constitutive NOS is eNOS and has been implicated in vasodilation induced by acetylcholine [34–36]. Therefore, we measured protein levels of eNOS in the aging kidney. Interestingly, we demonstrated elevated expression of eNOS by immunohistochemistry and Western blotting in 18-month-old versus 3-month-old animals. The role of eNOS protein in the aging rat kidney is not clear. A study by Adler et al [37] in Fischer 344 male rats showed similar levels of eNOS protein are present in the renal cortical tissues of young and aging rats, while others [7, 20] found that eNOS protein is diminished in Sprague-Dawley aging animals. It is possible that the variation in these studies and our data could be due to rat species or age differences. The animals in our experiments were 18-month-old rats, whereas a reduction in eNOS expression was observed in studies using older (22 to 24 months old) animals [7, 20]. Intriguingly, we observed a decrease in eNOS mRNA in our animals as determined by real-time polymerase chain reaction (PCR) [Long DA, Price KL, Johnson RJ, personal observation].
Fig. 7. Endothelial-derived hyperpolarizing factor (EDHF)–mediated responses in the aging rat. Isolated perfused kidneys were preconstricted with phenylephrine (PE) and acetylcholine (Ach) and bradykinin (BKN) used as agonists to elicit vasodilation. Indomethacin (INDO) and L-arginine analogue N-nitro-L-arginine methyl ester (L-NAME) were added to the perfusate buffer to block the contribution of the cyclooxygenase and nitric oxide pathways, respectively, and therefore assess the role of EDHF (N = 12 for both young and aging animals at each dose). Acetylcholine (A) and bradykinin (B) induced a dose-dependent increase in vasodilation in both young and aging rats. The vasodilation induced by acetylcholine and bradykinin was impaired in aging rats. *P < 0.05 between young and aging animals.

Fig. 8. Effect of 6-(2-propargyloxyphenyl) hexanoic acid (PPOH) on acetylcholine (ACh)-induced endothelial-derived hyperpolarizing factor (EDHF)–mediated responses in young and aging animals. To determine the contribution of epoxyeicosatrienoic acid (EET), acetylcholine-induced EDHF-mediated vasodilation was measured in young and aging animals in the presence or absence of PPOH. (A) In young animals, PPOH was able to significantly impair vasodilatory responses by acetylcholine at all doses (N = 7 for animals with/without PPOH). (B) A similar trend was observed in aging rats but there was no significant difference between the animals with or without PPOH (N = 3 in each group). *P < 0.05 between vasodilatory responses in the presence or absence of PPOH. Abbreviations are: PE, phenylephrine; INDO, indomethacin; L-NAME, L-arginine analogue N-nitro-L-arginine methyl ester.

It is possible, that with aging there is an initial loss of capillaries [6, 7, 38], resulting in tissue hypoxia that stimulates eNOS expression [39], but that over time there is a loss of eNOS, first at the mRNA level and then at the protein level. Therefore, the decrease in eNOS mRNA observed in our animals may translate into less protein 6 months later. The alternative possible mechanism to explain this phenomenon is a change in the stability of mRNA in aging, altered rates of translation or protein degradation [40]. There is considerable interest in this area with regard to changes in these processes in aging and this would be an interesting area for future study.

Our observation that eNOS protein levels are elevated, but serum nitric oxide is reduced is of interest. The regulation of nitric oxide production by eNOS is complex, but the cofactor tetrahydrobiopterin (BH4) plays a key role [41]. In settings of oxidative stress BH4 can become oxidized leading to the production of superoxide rather than nitric oxide [42, 43], a phenomenon known as eNOS uncoupling [44, 45]. As aging is associated with increased oxidative stress [46] we could hypothesize that BH4 becomes oxidized in aging, leading to the production of superoxide and therefore a loss of nitric oxide, while eNOS levels are maintained.
The most novel finding in this study was the demonstration that aging rats have a defect in EDHF-like responses in their renal vasculature. The EDHF-like response was investigated by examining the renal vasodilatory response induced by acetylcholine and bradykinin in the isolated perfused kidney model in animals in which both nitric oxide and prostaglandins were inhibited. Importantly, a marked attenuation in renal vasodilation was observed in aging rats in response to either acetylcholine or bradykinin.

There is currently no consensus on the nature of EDHF. One possibility is that it may be due to several molecules, including EETs, potassium ions, and H$_2$O$_2$ [47]. To investigate the role of EETs, we examined the effect of PPOH, a selective inhibitor of the epoxygenation of arachidonic acid catalyzed by cytochrome P450 enzymes [48]. PPOH was able to attenuate EDHF-like responses induced by bradykinin and acetylcholine in the young rat. However, this was not the case in aging animals, with no significant difference in acetylcholine- or bradykinin-induced EDHF responses with/without PPOH. This data would suggest that there may be different EET pathways involved in EDHF-mediated vasodilation in the aging rat which are not PPOH sensitive.
EDHF is also thought to be mediated by potassium channels. To investigate this possibility, we performed experiments in the presence of the potassium channel blockers, apamin and charybdotoxin. Responses mediated by both bradykinin and acetylcholine were blocked by apamin and charybdotoxin in young animals. Similarly, acetylcholine-induced EDHF vasodilation was also attenuated by potassium channel blockers, but not responses induced by bradykinin. This indicates that the acetylcholine and bradykinin vasodilatory response in the aging rat are likely to be mediated by two different mechanisms, the latter which involves pathways independent of potassium channels.

There are some important considerations when evaluating the data from these experiments. First, the differences in serum and tissue NO•/cGMP may partly reflect dietary intake in aging rats [49]. We cannot exclude in our study the possibility that older rats ate less and hence have lower NO•/cGMP levels. Second, although many studies have previously used the isolated perfused kidney to assess vasodilation [50, 51] there are advantages and disadvantages to this model. An advantage of the isolated perfused kidney is that one can assess vasodilatory responses of the whole kidney in a controlled manner without systemic influences. However, a potential disadvantage is that mediators released from within the kidney, such as from tubules, may contribute to the EDHF response, and this needs to be considered when interpreting the results from these experiments.

CONCLUSION

Our data indicates that there is an impaired renal vasodilatory response with aging, and that this involves both a loss of nitric oxide and EDHF-mediated responses. The EDHF responses in aging are complex and may involve impairment in both EET-dependent and potassium channel–dependent pathways. The loss of EDHF and nitric oxide–mediated responses could result in impaired renal autoregulation, impaired vasodilatory responses, and therefore potentially higher basal vasomotor tone and greater sensitivity to vasoconstrictor agents.

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