1-L-Arginine improves endothelial function, independently of arginine uptake, in aortas from chronic renal failure female rats

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1-Arginine improves endothelial function, independently of arginine uptake, in aortas from chronic renal failure female rats. Am J Physiol Renal Physiol 306: F449–F456, 2014. First published December 11, 2013; doi:10.1152/ajprenal.00457.2013.—Endothelial cell dysfunction (ECD) is a common feature of chronic renal failure (CRF). Defective nitric oxide (NO) generation due to decreased endothelial nitric oxide synthase (eNOS) activity is a crucial parameter characterizing ECD. Decreased activity of cationic amino acid transporter-1 (CAT-1), the selective arginine transporter of eNOS, has been shown to inhibit eNOS in uremia. Recently, we failed to demonstrate a decrease in glomerular arginine transport in uremic female rats (Schwartz IF, Grupper A, Soetendorp H, Hillel O, Laron IF). Instead, arginine uptake is markedly inhibited in aortas from CRF females subjected to 5/6 nephrectomy. The contractile and structural changes in uremic female rats have been shown to result in a profound beneficial effect on ECD, independently of arginine uptake, in aortas from chronic renal failure female rats.

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using female Wistar rats weighing 180 – 200 g. Subsequently, rats were segregated into three groups as follows:

Group 1: control; sham-operated rats.

Group 2: CRF; rats underwent a two-stage 5⁄6 nephrectomy (interval of 1 wk). Following the operation, animals were allowed to recover and had free access to standard rat chow and tap water.

Group 3: CRF + L-arginine; 3% in the drinking water, starting from day 1 following the second surgery.

Group 4: sham-operated rats were given L-arginine as in group 3.

All experiments were performed 8 wk following the second operation.

Additional animals were utilized for creatinine clearance (Cr Cl) measurements, which were taken before death, in all experimental groups as previously described (8). Rats were euthanized using CO2.

Preparation of vessels. Following euthanasia, the thoracic aorta was removed immediately for in vitro relaxation studies. The aorta was placed in a modified Krebs-Henseleit (KH) buffer solution consisting of (in mmol/l) 118 NaCl, 4.7 KCl, 4.7, 2 CaCl2 2, 1.2 MgSO4*7H2O, 1.2 KH2PO4, 11.1 glucose, and 25 NaHCO3. Aortas were cleaned to remove fat adhering to the adventitia. The aortas were then sectioned into two transverse rings of 3 mm in length for each rat.

Organ bath technique. Aortic rings were carefully suspended on wire hooks in a 100-ml jacket glass bath, containing modified KH, with temperature maintained at 37°C. The perfusate was bubbled continuously with 95% O2 and 5% CO2. The upper hook was connected to a force transducer (Singer Instrument & Control), and the lower hook was fixed on a Stalin leg. The amount of force was recorded on a Beckman Dynograph Recorder R611. Two organ bath arrangements were run simultaneously.

Tension registration. After the rings were equilibrated without tension on the wire hooks for 1 h, a normalizing procedure was performed. The resting tension applied to each ring was equivalent to that required to stretch the ring to 90% of its internal circumference when distended with a transmural pressure of 100 mmHg. In preliminary experiments, we found that a 1.2-g prestretch for 30 min provides optimal conditions for subsequent induction of maximal contraction. After the normalizing procedure, a steady level of active contraction was established by adding KCl (60 mmol/l) for 15 min. This concentration provided 80% of the maximal contraction of this agent on the thoracic aorta segment.

Concentration-response curves. All concentration-response curves were obtained by the cumulative addition of compounds, allowing force to reach steady state before the addition of the next concentration. Following contraction with KCl, the rings were washed once with fresh modified KH solution and allowed to equilibrate for 20 min. Subsequently, the aortic rings were contracted to a stable steady state with 0.1 μmol/l phenylephrine (PE), which resulted in 70–90% of maximal contraction that remained stable for at least 60 min, which is well under the time required for the experiments. Endothelium-dependent vasorelaxation was tested by adding increasing concentrations of acetylcholine (ACh; 10^-8–10^-2 M) at 5-min intervals. The data are presented as mean percent decrease in tension from the level of maximal induced tone by 0.1 μmol/l PE. A concentration causing 50% of maximal response (EC50) was used as the measure of sensitivity. Eight animals were utilized for each experimental group.

L-Arginine uptake by aortic rings. Uptake of radiolabeled L-arginine in the rat aorta was measured according to previously described methods (22). Immediately after euthanasia, the aorta was carefully excised from the left renal artery to the aortic valve ring and placed in ice-cold HEPES. The vessels were dissected free from adherent connective tissue and cut into rings (length 3–4 mm). Each segment was cut in half longitudinally. To determine arginine transport, aortic segments of each experimental group were incubated and shaken for 10 min in HEPES buffer at pH 7.4, 37°C. L-[H3]arginine and L-arginine, in a final concentration of 1 mM, were added to a total volume of 2 ml for an additional 1 min. The duration of 1 min was chosen since it was within the linear portion of uptake curves (data not shown). Transport was terminated by rapidly washing the aortic rings with ice-cold PBS buffer (4 times, 3 ml/tube). The rings were then dried and solubilized in 1 ml of 0.5% SDS in 0.5 N NaOH. Seven hundred microliters of the lysate were used to monitor radioactivity by liquid scintillation spectrometry (Betamatic, Kontron). The remaining 300 μl were used for protein content determination by the Lowry method (Lowry assay Kit, Sigma). To correct for nonspecific uptake or cell membrane binding, additional studies were performed in which aortic segments were incubated with 10 mM unlabeled arginine in HEPES buffer, and the associated radioactivity was subtracted from each data point. Results are expressed as means ± SE of 8 different animals.

Assessment of cGMP generation. cGMP generation was determined by ELISA. Freshly harvested aortic rings were incubated in

![Fig. 1. Defective contractile response to KCl (60 mmol/l) in aortic rings from uricemic female rats. CTL, controls; CRF, chronic renal failure; ARG, arginine. *P < 0.05 vs. control; #P < 0.05 vs. CRF; n = 8 rats/group.](image-url)

![Fig. 2. Aortic rings contraction by phenylephrine (0.1 μmol/l) in all experimental groups; n = 8 rats/group.](image-url)
HEPES buffer with or without N\textsuperscript{G}-nitro-l-arginine methyl ester (L-NAME), in which a phosphodiesterase inhibitor (3-isobutyl-1-methyl-xanthine 1 mM) was included to inhibit cGMP degradation. The suspension was then aliquotted (50 μl/tube). Each aliquot was shaken at 37°C for 10 min, after which they were subjected to carbamyl choline (100 mM, Sigma), a selective eNOS agonist for an additional 2 min. Subsequently, the samples were rapidly frozen and then homogenized in TCA (5% at 4°C). The precipitate was removed by centrifugation (3,000 rpm, 10 min), and TCA was ether extracted. Residual ether was removed by heating the samples for 5 min at 70°C. cGMP was measured by ELISA (R&D Systems). The difference between cGMP generation with and without L-NAME was used as an index of eNOS activity. Each experiment was repeated six times. To examine a possible role for neuronal NOS (nNOS) in the aortic response to carbamyl choline, we repeated the above experiments following pretreatment with 7-nitroindazole (7NI), a selective nNOS inhibitor. 7NI was dissolved in DMSO and peanut oil (25/75%), and was administered (25 mg/kg body wt ip) 30 min before the experiments. This dose has been previously shown to exert an antinociceptive activity and affect on the tubuloglomerular feedback response, which did not affect systemic blood pressure, suggesting selectivity to nNOS (16, 26). However, it did not affect the sham animals. The dose has been previously shown to inhibit mononuclear anti-β-actin antibodies as an internal control. The reactive bands corresponding to CAT-1, eNOS, and p-eNOS were detected by ECL (Kodak X-OMAT AR film) and quantified by densitometry (n = 4 different experiments). Determination of AGEs. Serum concentrations of AGEs were determined by ELISA (OxiSelect advanced glycation end product ELISA kit, Cell Biolabs) according to the manufacturer’s instructions. In brief, AGE-BSA standards and serum samples in a final protein concentration of 10 μg/ml were adsorbed onto a 96-well plate for 2 h at 37°C. The AGE protein adducts were probed with an anti-AGE and analyzed on a 7.5% SDS-PAGE gel and transferred onto Hybond ECL nitrocellulose membranes (Amersham). Following blocking, membranes were incubated with polyclonal rabbit anti-rat CAT-1 antibodies, 1:500 (synthesized by Dr. O. Lettnor, Weizmann Institute, Rehovot, Israel), monoclonal mouse anti-rat eNOS, and phosphorylated (SER 1177) eNOS (Santa Cruz Biotechnology) for 1 h at room temperature, washed, and incubated with secondary horseradish per-oxidase (HRP)-conjugated goat anti-rabbit antibody (1:10,000) in PBS-T for 1 h. Membranes were subsequently washed three times, for 5 min each, in PBS-T. Membranes were then stripped and reprobed with monoclonal anti-β-actin antibodies as an internal control. The reactive bands corresponding to CAT-1, eNOS, and p-eNOS were detected by ECL (Kodak X-OMAT AR film) and quantified by densitometry (n = 4 different experiments).

**Fig. 3.** Impaired acetylcholine-induced reduction in contraction evoked by phenylephrine in aortic rings from uremic female rats is restored by arginine. *P < 0.05 vs. control. #P < 0.05 vs. CRF; n = 8 rats/group.

Protein quantification by Western blotting. Aortic CAT-1, eNOS, and phosphorylated eNOS protein expression were determined by immunoblotting. Aortic rings were placed in ice-cold PBS lysis buffer (pH 7.4), containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 4.5 μM leupeptin, and 5 μM aprotinin, ICN Biomedicals), 0.01% Triton X-100, and 0.1% SDS, then mechanically homogenized and left on ice for 45 min. Phosphatase inhibitors, sodium fluoride, and sodium orthovanadate (1 mM, Santa Cruz Biotechnology, Santa Cruz, CA) were added to measure phosphorylated proteins. Homogenates were subsequently centrifuged (13,000 rpm for 10 min at 4°C). Cell lysates were stored in aliquots in −70°C. A membrane fraction was obtained by adding an equal volume of lysis buffer supplemented with Tween 20 (0.25%). The protein content of each sample was determined by the method of Lowry. Equal amounts of protein (30 μg) were prepared in sample buffer (2% SDS, 0.01% bromophenol blue, 25% glycerol, 0.06 25 M Tris-HCl, pH 6.8, 5% mercaptoethanol) and analyzed on a 7.5% SDS-PAGE gel and transferred onto Hybond ECL nitrocellulose membranes (Amersham). Following blocking, membranes were incubated with polyclonal rabbit anti-rat CAT-1 antibodies, 1:500 (synthesized by Dr. O. Lettnor, Weizmann Institute, Rehovot, Israel), monoclonal mouse anti-rat eNOS, and phosphorylated (SER 1177) eNOS (Santa Cruz Biotechnology) for 1 h at room temperature, washed, and incubated with secondary horseradish per-oxidase (HRP)-conjugated goat anti-rabbit antibody (1:10,000) in PBS-T for 1 h. Membranes were subsequently washed three times, for 5 min each, in PBS-T. Membranes were then stripped and reprobed with monoclonal anti-β-actin antibodies as an internal control. The reactive bands corresponding to CAT-1, eNOS, and p-eNOS were detected by ECL (Kodak X-OMAT AR film) and quantified by densitometry (n = 4 different experiments). Determination of AGEs. Serum concentrations of AGEs were determined by ELISA (OxiSelect advanced glycation end product ELISA kit, Cell Biolabs) according to the manufacturer’s instructions. In brief, AGE-BSA standards and serum samples in a final protein concentration of 10 μg/ml were adsorbed onto a 96-well plate for 2 h at 37°C. The AGE protein adducts were probed with an anti-AGE and analyzed on a 7.5% SDS-PAGE gel and transferred onto Hybond ECL nitrocellulose membranes (Amersham). Following blocking, membranes were incubated with polyclonal rabbit anti-rat CAT-1 antibodies, 1:500 (synthesized by Dr. O. Lettnor, Weizmann Institute, Rehovot, Israel), monoclonal mouse anti-rat eNOS, and phosphorylated (SER 1177) eNOS (Santa Cruz Biotechnology) for 1 h at room temperature, washed, and incubated with secondary horseradish per-oxidase (HRP)-conjugated goat anti-rabbit antibody (1:10,000) in PBS-T for 1 h. Membranes were subsequently washed three times, for 5 min each, in PBS-T. Membranes were then stripped and reprobed with monoclonal anti-β-actin antibodies as an internal control. The reactive bands corresponding to CAT-1, eNOS, and p-eNOS were detected by ECL (Kodak X-OMAT AR film) and quantified by densitometry (n = 4 different experiments). Determination of AGEs. Serum concentrations of AGEs were determined by ELISA (OxiSelect advanced glycation end product ELISA kit, Cell Biolabs) according to the manufacturer’s instructions. In brief, AGE-BSA standards and serum samples in a final protein concentration of 10 μg/ml were adsorbed onto a 96-well plate for 2 h at 37°C. The AGE protein adducts were probed with an anti-AGE
polyclonal antibody, followed by an HRP-conjugated secondary antibody. The quantity of AGE adduct in our samples was determined by comparing the absorbance at 450 nm with that of a known AGE-BSA standard curve \( \frac{n}{H11005} \) animals.

Plasma l-arginine and ADMA measurements by HPLC. Levels of l-arginine and ADMA in plasma samples were determined by HPLC as previously described with modifications (14). Samples were collected into EDTA tubes, centrifuged at 4°C, and stored at \(-80°C\). Before analysis, samples (500 \( H9262 \) l) were combined with homoarginine solution (250 \( H9262 \) l; internal standard) and diluted by adding borate buffer (2 ml, 50 mM, pH 8.5). ADMA and arginine were extracted from plasma samples using Supelco LC SCX cation exchange columns at room temperature. The columns were activated with methanol and equilibrated with borate buffer. ADMA and arginine were eluted with aqueous solution containing 10% NH4OH and 50% methanol into glass tubes. Eluates were evaporated under air at 60°C, dissolved in 500 \( H9262 \) lH2O, and centrifuged in spin-x tubes with 0.22-\( m \) filters. Standard solutions were prepared by mixing stock solutions of arginine, ADMA, and SDMA combined with homoarginine. Final concentrations of standard solutions were 12.5 \( H9262 \) m arginine and 12.5 \( H9262 \) m ADMA. Samples and standards were derivatized in an auto-sampler, using freshly prepared orthophthaldialdehyde (OPA) solution. Separation was carried out using column Lichrocart 250–4.6 µm (5 \( H9262 \) m) and guard 4–4 of the same material. Mobile phase included 25 mM acetate buffer, pH 6.8, with methanol (ratio 62:38); flow 1 ml/min. For detection, a Jasco Fluorometer was used. It was set at 340-nm excitation and 455-nm emission. Data were

![Fig. 5. A: representative Western blot analysis showing regulation of eNOS protein level in freshly harvested aortic rings. B: densitometric analysis of aortic eNOS content from the various experimental groups. Each bar represents the mean of the relative density units ± SE from 4 different experiments. C: representative Western blot analysis showing regulation of aortic phosphorylated (SER1177) eNOS protein level. D: densitometric analysis of aortic phosphorylated eNOS content in freshly harvested aortic rings from the various experimental groups. Each bar represents the mean ± SE from 4 different rats. Data from control are normalized to 1. *P < 0.05 vs. CTL.](image-url)

![Fig. 6. Aortic arginine transport remains unchanged in female uremic rats. Uptake of radiolabeled arginine ([³H]l-arginine) by freshly harvested aortic rings. Data are presented as means ± SE; \( n = 8 \) rats/group.](image-url)

![Fig. 7. Aortic cationic amino acid transporter-1 (CAT-1) protein does not change in uremic rats. A: representative Western blot analysis showing regulation of CAT-1 protein level in freshly harvested aortas. B: densitometric analysis of aortic CAT-1 content from the various experimental groups. Each bar represents the mean of the relative density units ± SE from 4 different experiments.](image-url)

![Fig. 8. Arginine decreases serum levels of advanced glycation end products (AGEs) in control and CRF rats. Serum levels of AGEs in the various experimental groups are shown. *P < 0.05 vs. control. #P < 0.05 vs. CRF; \( n = 5 \) rats/group.](image-url)
acquired onto EZ chrom data software, and peak heights were determined (n = 5 rats).

Transmission electron microscopy. Ultrastructural specimens were fixed in glutaraldehyde fixative (2.5% glutaraldehyde, 0.1 M sodium cacodylate buffer, pH 7.4) at 4°C. Tissue samples were then washed in 0.1 M sodium cacodylate buffer and postfixed with 1% osmium tetroxide and lead citrate. Subsequently, specimens were washed in dd H2O and dehydrated through increasing concentrations of ethanol acetone and embedded in eponate 12 resin (TED Pella). Ultrathin sections (0.8 μm) were stained with uranyl acetate, before viewing in a transmission electron microscope (Jem100B, Tokyo, Japan); n = 5 rats/group.

Statistical analysis. Data are presented as means ± SE. One-way ANOVA was conducted for comparison between groups. Post hoc analysis using a least significant difference algorithm was performed to allocate the source of significance.

RESULTS

Body weight obtained at the conclusion of the study was lower in all CRF rats than in the control animals. Cr Cl was significantly decreased in the CRF group compared with controls. The administration of l-arginine to CRF animals had no effect on Cr Cl but prevented the decrease in body weight (Table 1).

Contractile response to potassium and PE. Contractile response to KCl (60 mmol/l) was significantly reduced in CRF animals compared with healthy rats. The administration of arginine abolished this decline (Fig. 1). Aortic ring contraction by PE (0.1 μmol/l) did not differ in all experimental groups (Fig. 2).

Relaxation response to ACh. Next, we studied ACh-induced reduction in contraction evoked by 0.1 μmol/l PE in the aortic rings. We found that in the higher range of ACh concentrations (10−5–10−2 M), vasodilation was significantly impaired in the aortas from CRF dames compared with the control group. This finding was less apparent in lower ACh concentrations (10−8–10−6 M). Treatment with l-arginine normalized the capacity of aortic vasodilation in CRF rats and augmented aortic relaxation in healthy animals. Furthermore, sensitivity to ACh was significantly lower in CRF rats compared with the control group but was completely restored by arginine administration (Fig. 3).

Interestingly, in CRF animals we have observed an initial “paradoxical” contraction (5–10% of initial preconstruction value) when exposed to ACh, a finding which is considered indicative of ECD. This was not observed in either controls or arginine-treated CRF animals.

Aortic l-arginine-NO axis. We measured aortic cGMP generation following stimulation with carbamyl choline, a selective eNOS agonist, with and without the coadministration of l-NAME (NOS inhibitor). We used the difference between these values as an index of eNOS activity (Fig. 4). eNOS activity was significantly decreased in CRF rats, and l-arginine administration completely abrogated the decrease in cGMP levels exhibited in CRF. When the aforementioned studies were repeated following the systemic administration of 7-NI (nNOS antagonist), no differences were found between animals pretreated with 7NI or vehicle in all experimental groups, suggesting that the decrease in aortic NO generation derives predominantly from eNOS inhibition (Fig. 4B). The abundance of aortic eNOS and phosphorylated eNOS significantly decreased in CRF, a phenomenon which was completely prevented by l-arginine therapy (Fig. 5). Arginine transport in both CRF and arginine-treated CRF animals was unchanged compared with sham-operated rats (Fig. 6). CAT-1 protein abundance did not differ in all experimental groups (Fig. 7).

Age. Plasma levels of AGEs were significantly elevated in CRF rats compared with control animals. Arginine treatment completely prevented the increase in AGEs product levels in CRF. Furthermore, arginine significantly decreased AGEs in healthy rats as well (Fig. 8).

Serum ADMA and arginine levels. To determine whether the effect of CRF on endothelial-dependent vasodilatation in female rats is associated with elevated levels of endogenous NOS inhibitors, ADMA levels were measured. Indeed, we have found a significant increase in serum ADMA concentration in CRF animals compared with control, along with an increase in the ADMA/l-arginine ratio. Arginine levels were unchanged between these groups, and arginine treatment in CRF rats restored the ADMA/l-arginine ratio (Fig. 9).

Pathology. The aortas of normal rats demonstrated preserved endothelia, with a continuous layer of endothelial cells attached to the underlying connective tissue and connected to each other by junctions. The endothelial cells showed normal fine ultrastructure with intact organelles (Fig. 10A).

Examination of aortas of rats with CRF revealed extensive loss of endothelial cells, with focal denudation of internal elastic lamina. The contacts between many of the endothelial cells were loosened or lost, and the cellular changes included blebbing, swelling of cytoplasm, and cytoplasmic organelles with vacuolization (Fig. 10B).

The administration of arginine to rats with CRF enabled the preservation of a continuous aortic endothelial monolayer, with considerably decreased endothelial cell damage compared with the untreated CRF rats (Fig. 10C).

Fig. 9. Serum levels of asymmetric dimethyl arginine (ADMA; A) and l-arginine from the various experimental groups (B). C: ratio of serum concentration of ADMA (nmol/l) to l-arginine (μmol/l). Values are means ± SE. *P < 0.05 vs. controls. #P < 0.05 vs. CRF; n = 5 rats/group.
DISCUSSION

The main novel finding demonstrated in the present study is that while uremic female rats exhibit a whole spectrum of structural and functional characteristics of endothelial dysfunction, including decreased eNOS activity, the delivery of arginine, the sole substrate for NO synthesis, remains intact. Unexpectedly, when arginine was given to CRF dames, all the phenomena associated with dysfunction of the endothelium were abolished. These findings are in accord with those reported by Yammamizu et al. (34), who have also shown, in male Sprague-Dawley rats with CRF, that the supplementation of L-arginine has a beneficial effect on endothelial function.

Fig. 10. Effect of arginine treatment on ultrastructural changes in endothelial monolayer in aortas of CRF rats. Shown are the following: normal aorta intima (A; magnification ×5,000) with well-preserved endothelial cells (B; magnification ×25,000) of untreated rats; damaged aorta intima (C; magnification ×5,000) with marked degenerative changes and extensive loss of endothelial cells (D; magnification ×25,000) of CRF rats; and continuous endothelial layer with focal damage (E; magnification ×5,000) and considerable regenerative changes in endothelial cells (F; magnification ×25,000) of CRF arginine-treated rats; n = 5 rats/group.
Several studies provided evidence supporting an important role for decreased arginine transport in the pathogenesis of ECD, including uremia. In vitro studies performed by Baylis and colleagues (31, 33) suggested that arginine uptake is decreased in renal failure. Moreover, they have shown that plasma from uremic patients has the capacity to inhibit arginine uptake by cultured endothelial cells, an effect attributed to urea uptake by endothelial cells via urea transporters type B (31, 33). We have shown in 56% nephrectomized male rats that arginine transport is attenuated by two different mechanisms, namely, a decrease in CAT-1 translation and upregulation of PKCα (a CAT-1 inhibitor) (9, 22). Interestingly, in contrast to the above publications, in the current experiments arginine transport remained intact in the female CRF animals.

These findings resemble data published by our group (22) showing that old female rats, in contrast to old males, were protected from age-dependent inhibition of arginine transport. The hypothesis generated from these findings suggests that females are capable of maintaining adequate arginine transport velocities under conditions which were found to adversely affect the arginine-NO system in males, such as aging and CRF. Both estrogen and progesterone have been shown to increase eNOS activity via two different mechanisms: a rapid pathway involving eNOS phosphorylation through the phosphatidylinositol 3-kinase/Akt pathway and a delayed one through an increase in eNOS mRNA and protein synthesis (5). However, the role of sex hormones with regard to CAT-1 activity remains elusive.

Surprisingly, the administration of L-arginine to our CRF rats prevented the decrease in eNOS activity, enhanced ACh-induced relaxation, and mitigated the deranged endothelial cell ultrastructure, despite the fact that arginine transport velocities were unchanged and arginine treatment did not alter intracellular arginine influx. In other words, arginine improved endothelial function without entering the cells.

An attractive explanation of our findings in the current experiments is reversal of the extracellular ADMA-arginine ratio in L-arginine-treated CRF rats. CAT proteins transport ADMA both into and out of cells, resulting in an exchange of cationic amino acids between both sides of the membrane. Thus these L-arginine analogs not only competed with L-arginine for transport but were also capable of driving out intracellular L-arginine (3). Increased ADMA levels have been documented in animal models of CRF as well as in uremic patients and were shown to produce endothelial dysfunction (29, 36). In the current studies, we hypothesize that excess arginine reversed the extracellular ADMA/L-arginine ratio, resulting in a decrease in intracellular influx of ADMA and attenuation of its competitive inhibition on eNOS activity.

An alternative explanation of our findings may be related to a decrease in AGEs formation by L-arginine. Methylglyoxal (MG), a reactive dicarbonyl molecule, produced during glucose, fatty acid, and amino acid metabolism to varying degrees, is a major precursor of the formation of AGEs (10, 25). A major deleterious effect of high glucose and reactive dicarbonyl metabolic intermediates, such as MG, glyoxal, and 3-deoxyglucosone, is the formation of AGEs, which are strongly implicated in the pathogenesis of conditions such as vascular complications of diabetes, atherosclerosis, aging, and CRF (1, 30). MG has great affinity for L-arginine and is proposed to react rapidly with the guanidino group of L-arginine (12). Arginine has been shown to bind and inactivate MG, thus decreasing the formation of AGEs and improving endothelial function in an eNOS-independent mechanism. Indeed, in the current experiments the administration of L-arginine significantly decreased serum AGEs levels in both CRF and control rats.

The beneficial effects of arginine in various models of ECD have fascinated researchers in this field for more than a decade. Given the fact that intracellular levels of L-arginine far exceed the $K_m$ of the NOS enzyme, it appears unlikely that the administration of a substrate affects cellular NO production. Surprisingly, numerous studies, both in animals and in humans, unequivocally demonstrated beneficial effects of arginine on the vascular response in several conditions, including hypercholesterolemia, hypertension, and diabetes. Moreover, even oral feeding of arginine, which only doubles plasma arginine levels, was found to positively affect atherosclerosis development in cholesterol-fed rabbits and in hypertensive rats (4, 18). This phenomenon, in which arginine supplementation stimulates NO synthesis despite saturating intracellular concentrations, has been termed “the arginine paradox.” The two different mechanisms, demonstrated in the current study, namely, a decreased serum ADMA/arginine ratio and inhibition of AGEs generation, can serve to explain this paradox. Arginine administration also improved ACh-induced vasodilation in aortic rings harvested from control animals while all indices of eNOS activity were unchanged. Several additional mechanisms have been proposed to explain improved vascular function by arginine administration independently of eNOS, including stimulation of histamine release from mast cells, decreased NE activity, increased insulin secretion, and alteration in intracellular pH (13).

In conclusion: while the aortic endothelium in CRF female rats exhibits a full spectrum of endothelial dysfunction characteristics, the arginine transport system is spared. The current studies provide additional data to support the pleiotropic beneficial effects of arginine in diseases characterized by ECD which are far beyond substrate supplementation. We believe that sufficient information has been accumulated to encourage clinical trials which will determine the role of arginine in the management of chronic kidney disease.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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