Attenuated glomerular arginine transport prevents hyperfiltration and induces HIF-1α in the pregnant uremic rat

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Schwartz IF, Grupper A, Soetendorp H, Hillel O, Laron I, Chernichovski T, Ingbir M, Shtabski A, Weinstein T, Chernin G, Shashar M, Hershkoviz R, Schwartz D. Attenuated glomerular arginine transport prevents hyperfiltration and induces HIF-1α in the pregnant uremic rat. Am J Physiol Renal Physiol 303: F396–F404, 2012. First published May 2, 2012; doi:10.1152/ajprenal.00488.2011.—Pregnancy worsens renal function in females with chronic renal failure (CRF) through an unknown mechanism. Reduced nitric oxide (NO) generation induces renal injury. Arginine transport by cationic amino acid transporter-1 (CAT-1), which governs endothelial NO generation, is reduced in both renal failure and pregnancy. We hypothesize that attenuated maternal glomerular arginine transport promotes renal damage in CRF pregnant rats. In uremic rats, pregnancy induced a significant decrease in glomerular arginine transport and cGMP generation (a measure of NO production) compared with CRF or pregnancy alone and these effects were prevented by L-arginine. While CAT-1 abundance was unchanged in all experimental groups, protein kinase C (PKC)-α, phosphorylated PKC-α (CAT-1 inhibitor), and phosphorylated CAT-1 were significantly augmented in CRF pregnant rat, findings that were prevented by arginine. These studies suggest that in CRF rats, pregnancy induces a profound decrease in glomerular arginine transport, through posttranslational regulation of CAT-1 by PKC-α, resulting in attenuated NO generation. These events provoke renal damage manifested by upregulation of renal HIF-1α and loss of the ability to increase glomerular filtration rate during gestation.

end-stage renal disease; renal damage

MATERNAL RENAL FUNCTION may decline as a result of pregnancy among patients with renal disease and this process is determined, in part, by the severity of underlying renal disease. A large number of women with moderate renal insufficiency may have an irreversible decline in glomerular filtration rate that is greater than predicted based on the patient’s previous course. Several small studies suggest that as many as one-third experience an irreversible decline and up to 10% progress to end-stage renal disease (12, 13, 15, 16). The risk of an irreversible decline in renal function may exceed 50% in patients who also have uncontrolled hypertension (16).

Current recommendations suggest that if functional loss is above 50% stated as a serum creatinine above 1.4 mg/dl, pregnancy can adversely influence the mother’s renal prognosis. Therefore, these women are frequently advised to avoid gestation. Endothelial dysfunction is a common denominator of chronic renal failure. Accumulated evidence indicates that endothelial dysfunction is linked to impaired capacity of the constitutive, Ca2+/calmodulin-sensitive nitric oxide synthase (NOS) (endothelial NOS (eNOS)) to generate adequate quantities of NO or decreased local NO bioavailability and this plays a role in inducing renal injury (1, 8, 22, 31). For example, elevated NO production in Wistar-Furth rats was associated with protection from the progression of chronic renal disease after renal mass reduction (8), and kidney damage was accelerated in eNOS knockout mice following renal ablation while it was improved by administration of l-arginine (22).

Previous studies suggest that l-arginine, as the sole precursor for NO generation, governs NOS activity (17, 41). Among several transporters that mediate l-arginine uptake, cationic amino acid transporter-1 (CAT-1) is considered as the predominant arginine supplier for eNOS (21, 30). Several studies have shown that arginine transport is markedly inhibited in uremia (34, 40, 42). We recently found that during normal pregnancy, maternal vasculature is subjected to diminished arginine uptake, implying that both renal failure and pregnancy are independently characterized by diminished arginine uptake velocities (28). Accordingly, the current studies were designed to determine whether the coexistence of pregnancy and renal failure exerts a synergistic deleterious effect on glomerular arginine transport, thus leading to diminished eNOS activity. We also wished to explore whether the aforementioned events worsen renal damage in females with renal failure during gestation.

METHODS

Materials. All standard reagents were obtained from Sigma unless indicated otherwise. H3 l-arginine was supplied by Perkin Elmar Life and Analytical Sciences (Boston, MA).

All animal experiments described in this study were conducted in accord with the protocol approved by the institutional committee on ethics in animal experiments. Studies were performed using female Wistar rats at 12–14 wk of age. Subsequently, rats were segregated into seven groups, respectively: group 1: control, sham-operated rats; group 2: chronic renal failure (CRF): rats underwent a two-stage 5/6 nephrectomy (interval of 1 wk). Following the operation, animals were allowed to recover and have free access to a standard rat chow and tap water. All experiments were performed 6 wk following the second operation. Group 3: pregnancy (P): rats destined to become pregnant were housed with a male breeder, in a climate-controlled
room with a 12:12-h light-dark schedule. Day 1 of pregnancy was
documented by the presence of spermatozoa in the vaginal lavage.
Pregnant rats at 14–18 days of gestation were used for the experi-
mental procedures. Group 4: CRF + P: rats destined to become preg-
nant were housed with a male breeder, 4 wk following the second
stage nephrectomy. Experimental procedures were performed at
14–18 days of gestation as in group 3. Group 5: CRF and arginine
(CRF + ARG): l-arginine 3% in the drinking water was administered
to CRF rats following the second surgery. Group 6: P + ARG: l-argi-
nine as in group 5 was administered to pregnant rats from day 1
of gestation. Group 7: CRF + P + ARG: l-arginine was given to rats
prepared as in group 4.
Renal plasma flow (RBF) and glomerular filtration rate (GFR) were
measured by sodium p-aminohippurate (PAH) and inulin, respec-
tively, in all experimental groups, before death, as previously de-
scribed (32).
Additional animals were given intraperitoneal injections of α-to-
copherol, a PKC inhibitor (90 mg/kg body wt every other day), or
Castor oil as a vehicle, starting from day 1 of pregnancy or for a
Corresponding to CAT-1, PKC-
ylated goat anti-rabbit antibody (1:10,000) in PBS-T for 1 h. Mem-
be computed by the method of Lowry. Equal amounts of protein (30 μg) were
preparations to be applied to 2% SDS, 0.01% bromophenol blue, 25% glycerol, 0.0625 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 being blocked,
membranes were incubated with polyclonal rabbit anti-rat CAT-1
antibodies, 1:500 (synthesized by Dr. O. Leitner, Weizmann Institute,
Rehovot, Israel), monoclonal mouse anti-rat PKC-α, phosphorylated
PKC-α (p-PKC-α), eNOS, phosphorylated (SER1177) eNOS, and
HIF-1α (from Santa Cruz Biotechnology) for 1 h at room temperature,
and incubated with secondary horseradish peroxidase-conju-
gated goat anti-rabbit antibody (1:10,000) in PBS-T for 1 h. Mem-
branes 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, PKC-α, p-PKC-α, eNOS, p-eNOS, and
HIF-1α were detected by enhanced chemiluminescence (Kodak X-
GRT) and film and quantified by densitometry (n = 4 different
experiments).
Immunoprecipitation studies. Aliquots of glomerular tissue cell
lysates (1 ml) from the different experimental groups were used for
immunoprecipitation. Each tissue lysate sample was incubated with
20 μl of anti-CAT-1 antibodies for 2 h, at 4°C. Optimal antibody
concentration was determined by titration. This was followed by
addition of 20 μl of protein A-agarose (Santa Cruz Biotechnology)
and incubation overnight at 4°C on a rotating device. Pellets were
collected by centrifugation (3,000 rpm) for 30 s, 4°C. The superna-
tants were discarded and each pellet was subsequently washed three
times with PBS. After the final wash, the pellets were resuspended in
40 μl of 2X electrophoresis sample buffer, boiled for 3 min, and
subjected to immunoblotting with antibodies against either CAT-1 or
the phosphorylated tyrosine residue of CAT-1 (Santa Cruz Biotech-
nology). Representative results of three separate experiments are
shown. To estimate the phosphorylation of CAT-1 in the different
groups, the density of bands for CAT-1 and its phosphorylated form
on a film were analyzed as above. Results are adjusted for CAT-1
levels and expressed in arbitrary units (means ± SE, n = 3 rats).
Plasma arginine, asymmetric dimethyl arginine, and symmetric dimethyl
arginine measurement by high-performance liquid chromatography. Levels of
l-arginine, asymmetric dimethyl arginine (ADMA), and symmetric
dimethyl arginine (SDMA) in plasma samples were determined by
means of high-performance liquid chromatography as previously
described with modifications (20). Samples were collected into EDTA
tubes, centrifuged at 4°C, and stored at −80°C. Before being ana-
yzed, samples (500 μl) were combined with homoarginine (250 μl)
repeated the above experiments following pretreatment with 7-nitro-
indazole (7NI), a selective nNOS inhibitor. 7NI was dissolved in
DMSO and peanut oil (25/75%) and was administered (25 mg/kg ip
body wt) 30 min before the experiments. This dose has been previ-
ously shown to exert an antinoceptive activity and affect tubuloglo-
merular feedback response, both effects related to nNOS activity. Yet
it did not affect systemic blood pressure, suggesting selectivity to
nNOS (24, 38).

Protein quantification by Western blotting. Glomerular CAT-1, eNOS, phosphorylated-eNOS, PKC-α, phosphorylated PKC-α, and
hypoxia inducible factor-1α (HIF-1α) protein expression were deter-
mined by immunoblotting. Glomeruli were placed in ice-cold PBS
lysis buffer (pH 7.4), containing protease inhibitors (1 mM phenyl-
methylsulfonyl fluoride, 4.5 μM leupeptin, and 5 μM aprotinin; ICN
Biomedicals, 0.01% Triton X-100 and 0.1% SDS), and then
mechanically homogenized and left on ice for 45 min. Phosphatase
inhibitors, sodium fluoride and sodium orthovanadate (1 mM, Santa Cruz Bio-
technology), were added to measure phosphorylated proteins. Homog-
enates 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
by Tween-20 (0.25%). Protein content of each sample was determined
using CO2.

Isolation of glomeruli. Kidneys from all experimental groups were
harvested, decapsulated, bisected, and the cortex was carefully dis-
sected free. Glomeruli were prepared using a sieving technique as
previously described (33). Briefly, cortices were minced to a fine paste
with a razor blade and gently pressed through a 106-μm stainless steel
sieve. The resulting material was suspended in HEPES buffer (5 mM
KCl, 0.9 mM CaCl2, 1 mM MgCl2, 5.6 mM d-glucose, 25 mM
HEPES, 140 mM NaCl), at 4°C, pH 7.4. The suspension was forced
through a 20-gauge needle to decapsulate the glomeruli and then
passed through a 75-μm sieve. Glomeruli that were trapped on the
sieve were washed and pelleted by centrifugation at 1,000 rpm for 1
min. This was repeated three times. This fraction consisted of more
than 95% glomeruli, the majority of which were decapsulated. These
isolated glomeruli were used for arginine uptake and cGMP measure-
ments as well as protein extraction.

l-Arginine uptake by isolated glomeruli. Uptake of radiolabeled
l-arginine in the rat glomeruli was measured as previously described
(33). In brief, isolated glomeruli of each experimental group were
incubated and shaken for 10 min in HEPES buffer at pH 7.4, 37°C
l-[3H]l-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. Transport was
terminated by rapidly washing the specimen with ice-cold PBS buffer
(4 times, 3 ml/tube). The tissue was then dried and solubilized in 1 ml
of 0.5% SDS in 0.5 N NaOH. Seven hundred microliters of the lysate
(4 times, 3 ml/tube). The tissue was then dried and solubilized in 1 ml
of 0.5% SDS in 0.5 N NaOH. Seven hundred microliters of the lysate
was removed by centrifugation (3,000 rpm) for 10 min after which they were subjected to Carbamyl
Choline (100 mM, from Sigma), a selective eNOS agonist for an
additional 2 min. Subsequently, the samples were rapidly frozen and
then homogenized in 5% trichloroacetate (TCA) at 4°C. The precipi-
tate 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 (5). Each experiment
was repeated six times. To examine a possible role of neuronal NOS
(nNOS) in the glomerular response to carbamyl choline (CCCh), we
solution (internal standard) and diluted by adding borate buffer (2 ml, 50 mM), pH 8.5. ADMA, SDMA, 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. Arginine, ADMA, and SDMA 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 µl H₂O, and centrifuged in spin-x tubes with 0.22-µm filter. Standard solutions were prepared by mixing stock solutions of arginine, ADMA, and SDMA combined with homoarginine. Final concentrations of standard solutions were 12.5 µM arginine, 12.5 µM ADMA, and 12.5 µM SDMA. Samples and standards were derivatized in auto-sampler, using freshly prepared orthophthalaldehyde solution. Separation was carried out using column Lichrocart 250-4.6 purosden star RP 18 E (5 µm) 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, Jasco Fluorometer was used. It was set at 340-nm excitation and 455-nm emission. Data were acquired onto EZ chrom data software and peak heights were determined.

Renal histology. Kidneys were fixed in 10% buffered formalin and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin and evaluated by light microscopy. Morphologic changes in the glomeruli or tubulointerstitial area were scored on a scale of 0 to 5: 0, none of the glomeruli or no tubulointerstitial area was affected by morphologic changes; 1, <20%; 2, <40%; 3, <60%; 4, <80%; 5, full. In the analysis of glomeruli, scores were assigned for intraglomerular cell proliferation, glomerular thrombosis, and extracapillary cellular proliferation (crescent formation), and the total score was the sum of the three scores. In the analysis of interstitium, scores were assigned for infiltration of mononuclear cells, tubular damage, and interstitial fibrosis, and the total score was the sum of the three scores. Fifty glomeruli per rat were assessed for glomerular damage and at least 20 separate ×200 fields per rat were assessed for tubulointerstitial damage.

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

RESULTS

To explore a possible effect of gestation on CAT-1 system in uremic rats, glomerular arginine uptake was measured during pregnancy. Glomerular arginine transport in virgin uremic rats was unchanged from controls. Pregnancy resulted in a significant decrease in arginine transport. In pregnant uremic rats, arginine transport was significantly lower, even compared with pregnant healthy animals. The administration of l-arginine completely prevented the decrease in arginine transport observed in pregnant and in uremic pregnant animals, while it had no effect in CRF rats (Fig. 1A). Serum arginine levels significantly decreased in the pregnant and pregnant uremic rats. The administration of l-arginine resulted in a marked increase in serum arginine concentrations compared with the corresponding untreated experimental groups (Fig. 1B).

The abundance of glomerular eNOS and phosphorylated eNOS significantly decreased during pregnancy. While eNOS protein content remained unchanged in all other experimental groups, p-eNOS decreased in pregnant rats treated with arginine and increased in uremic rats both treated and untreated (Fig. 2).

Next, we measured glomerular cGMP generation, following stimulation with CCh, a selective constitutive NOS agonist, with and without the coadministration of l-NAME (NOS inhibitor). We used the difference between these values as an index of constitutive NOS activity (Fig. 3). eNOS activity was unchanged in uremic virgin and nonuremic pregnant dams, but it was significantly decreased in CRF pregnant rats. l-Arginine administration completely abolished the decrease in cGMP levels exhibited in CRF pregnant rats while it had no effect in pregnant and uremic virgin rats. When the aforementioned studies were repeated following the systemic administration of 7-NI (nNOS antagonist), no differences were found between animals pretreated with 7NI and vehicle in all experimental groups, suggesting that the decrease in glomerular NO generation derives predominantly from eNOS inhibition (Fig. 3C).

To determine whether gestation-induced changes in arginine uptake in CRF rats are associated with parallel directional changes in CAT-1, glomerular CAT-1 protein levels were evaluated. CAT-1 protein was identified as ~90 kDa. We found that CAT-1 abundance did not differ in all experimental groups (Fig. 4). Therefore, we searched for a posttranslational effect of pregnancy on CAT-1, in CRF, by looking at PKC-α. The abundance of the membrane-bound fraction of both PKC-α and its activated form, phosphorylated PKC-α, was
significantly augmented in CRF, pregnant, and pregnant CRF animals, a phenomenon that was prevented by the administration of L-arginine (Fig. 5, A–D). Immunoprecipitation studies for phosphorylated CAT-1 demonstrated a significant increase in its signal in CRF, pregnancy, and CRF/pregnancy and a significant decrease in the arginine-treated experimental groups (Fig. 5, E–F).

The administration of α-tocopherol resulted in a significant increase in arginine transport both in pregnant and CRF pregnant rats compared with vehicle-treated animals (Fig. 6A).

Ex vivo incubation of glomeruli with PMA, a potent stimulant of PKC, significantly attenuated the effect of tocopherol on arginine uptake in pregnant and in pregnant uremic rats (Fig. 6B).

Serum levels of the two endogenous N-dimethylated L-arginine derivatives, L-ADMA and L-SDMA, significantly increased in uremia, an effect which was blunted in pregnancy. Arginine administration had no effect on serum concentrations of neither ADMA nor SDMA (Fig. 7).

Fig. 2. A: representative Western blot analysis showing regulation of endothelial nitric oxide synthase (eNOS) protein level in freshly harvested glomeruli. B: densitometric analysis of glomerular 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 glomerular phosphorylated (SER1177) eNOS protein level. D: relative integrated optical density (IOD) ratios of phosphorylated eNOS to eNOS in freshly harvested glomeruli from the various experimental groups. Each bar represents means ± SE from 3 different rats. Data from control are normalized to 1. *P < 0.05 vs. CTL.

Fig. 3. Glomerular eNOS activity is reduced in pregnant uremic rats and is normalized by treatment with L-arginine in the drinking water. A: cGMP generation by freshly harvested glomeruli following stimulation with carbamyl choline (CCh; 100 mM), a constitutive NOS agonist, with and without coincubation with the NOS inhibitor nitro-L-arginine-methylester-HCl (L-NAME; 0.5 mM). Results shown are means ± SE of duplicate determinations (n = 6 rats per group). B: absolute increase in cGMP generation mediated by NO synthesis [CCh - (CCh + L-NAME)] (NO-mediated CCh responsiveness). *P < 0.05 vs. CTL, #P < 0.05 vs. CRF + P. C: glomerular NO generation is not affected by systemic neuronal NOS blockade. cGMP generation by freshly harvested glomeruli following stimulation with CCh (100 mM). A comparison between animals pretreated with 7-nitroindazole (7NI), a selective nNOS inhibitor (25 mg/kg ip body wt), 30 min before the experiments vs. a vehicle.
The last set of experiments was designed to explore whether the effects of pregnancy on the L-arginine-NO system, in uremic rats, may represent a bridging pathophysiological mechanism between the accelerated decline in renal function in CRF and gestation. Pregnancy resulted in a significant increase in both inulin and PAH clearances (2.6 ± 0.1 and 7.1 ± 0.42 ml/min), while CRF induced a significant decrease compared with controls (0.9 ± 0.08 and 2.25 ± 0.28 vs. 1.9 ± 0.21 and 4.75 ± 0.38 ml/min, respectively, P < 0.01). Renal hemodynamics in uremic pregnant and CRF rats were similar. The administration of L-arginine to pregnant uremic rats markedly attenuated the decrease in inulin and PAH clearances when compared with control or pregnant animals, while it had no effect in all other experimental groups (Fig. 8). Evaluation of renal histology revealed no differences between all experimental groups (data not shown). Renal cortex expression of HIF-1α was studied, as an index of tissue injury. Protein expression of HIF-1α was significantly increased only in the pregnant uremic group, a phenomenon that was prevented by L-arginine administration, while no differences were noted between all other experimental groups (Fig. 9).

DISCUSSION

The present study demonstrates that in uremic rats, pregnancy induces a profound decline in maternal glomerular arginine uptake, thus leading to a significant decrease in local NO generation. Unexpectedly, we also found, in the current experiments, that arginine transport did not decrease in female rats subjected to 5/6 nephrectomy, in contrast to our findings in male rats. These findings are consistent with data published by our group, showing that old female rats, in contrast to old males, were protected from age-dependent inhibition of arginine transport (35), supporting the assumption that females are capable of maintaining adequate arginine transport velocities under conditions that were found to adversely affect the arginine-NO system in males. Somehow, this capacity is lost during gestation. One possible explanation is the concomitant decrease in serum arginine concentrations to levels that are...
substantially lower than the \( K_m \) of CAT-1 that could be the result of increased consumption by the placenta. Yet, we feel that the mechanism responsible for the aforementioned phenomenon remains elusive and deserves further studies.

Interestingly, we found that in healthy pregnant animals, glomerular NO generation did not increase while both eNOS and phosphorylated eNOS protein contents actually decreased; these phenomena challenge the common notion that renal hyperfiltration during pregnancy is a result of augmented endothelial NO synthesis and indirectly support previous studies by Alexander et al. (2) and recent findings published by Baylis and co-workers (37) who showed that eNOS activity and protein expression are decreased during gestation. In fact, one can argue that our findings should lead to a decrease in NO generation during normal pregnancy. The lack of decline in NO generation may stem from the fact that we looked at glomerular endothelial NO generation rather than the renal vasculature endothelium.

The aforementioned data raise two questions: 1) why the decrease in arginine transport impacts NO generation in the pregnant uremic animals and not in healthy pregnant rats and 2) is this decrease in arginine transport and NO generation responsible for the accelerated decline in kidney function provoked by pregnancy in chronic kidney disease (CKD) females. A simple answer for the first question would be that arginine transport was significantly lower in the pregnant uremic rats than in healthy pregnant animals. Another possibility is that other factors that have been shown to inhibit NOS activity in uremia including: ADMA, advanced glycation end products, oxidative stress, and tetrahydrobiopterin deficiency may have prevented the NO system to adapt to the changes in CAT activity (3, 10, 18, 27, 39).

As for the second question, previous studies, using a similar experimental model, failed to demonstrate a deleterious effect of pregnancy on kidney function in uremic rats (7). Also, we could not find any differences between the study groups when looking at renal histology and renal function tests. Nevertheless, CRF and uremic pregnant rats exhibited similar RBF and GFR.

Fig. 6. A: inhibition of PKC-\( \alpha \) restores normal glomerular arginine transport velocities. Effect of \( \alpha \)-tocopherol, a PKC inhibitor (90 mg/kg body wt every other day for 4 wk), or castor oil as a vehicle on arginine transport in freshly harvested glomeruli. Data are presented as means ± SE (n = 5 rats/group). *\( P < 0.05 \) vs. CTL. #\( P < 0.05 \) vs. corresponding untreated animals. B: ex vivo activation of PKC-\( \alpha \) abolishes the effect of tocopherol on glomerular arginine transport in pregnant and pregnant uremic rats. Arginine transport was measured in freshly harvested glomeruli from pregnant and pregnant uremic animals, pretreated with tocopherol (a PKC inhibitor; 90 mg/kg body wt ip) every other day for 2 wk. Glomeruli were incubated with 0.1% DMSO as a vehicle or PMA (a PKC activator) 50 nM (in 0.1% DMSO) for 30 min. Data are presented as means ± SE (n = 5 rats per group). *\( P < 0.05 \) vs. the corresponding vehicle.

Fig. 7. Asymmetric dimethyl arginine (ADMA) and symmetric dimethyl arginine (SDMA) levels are not increased during pregnancy. Serum levels of ADMA and SDMA from the various experimental groups. Results shown are means ± SE. *\( P < 0.05 \) vs. CTL (n = 6 rats per group). #\( P < 0.05 \) vs. CRF.

Fig. 8. Uremic rats fail to increase renal blood flow (RBF) and glomerular filtration rate (GFR) during pregnancy and regain this ability when L-arginine is administered. RBF indexed by \( p \)-aminohippurate (PAH) clearance and GFR indexed by inulin clearance. *\( P < 0.01 \) vs. CTL (n = 6). #\( P < 0.05 \) vs. pregnancy.
in four different experimental models characterized by diminished arginine transport, namely, hypercholesterolemia, chronic renal failure, aging in the male rat, and most importantly, pregnancy in normal rats, a posttranslational regulation of CAT-1, which was associated with upregulation of PKC-α (14, 28, 34, 36). Therefore, we decided to focus exclusively at the membrane fraction of PKC-α, since this is where the interaction between PKC-α and CAT-1 occurs. Similar with our previous findings, we found in the current set of experiments a significant elevation in glomerular membrane-bound fraction of PKC-α and its activated form phosphorylated PKC-α, that was accompanied by increased phosphorylation of CAT-1. Treatment with α-tocopherol, a PKC inhibitor, prevented the decrease in arginine transport in the uremic pregnant rats, and ex vivo exposure of glomeruli harvested from tocopherol-treated uremic rats to PMA (a PKC stimulant) abolished its beneficial effect. Taken together, these data establish the notion that during gestation in CRF rats, the decrease in arginine transport is primarily derived from PKC-α activation. Although, caution should be carried in analyzing the data of protein phosphorylation assays since they were performed in vitro.

Given the fact that intracellular levels of L-arginine far exceed the $K_m$ of NOS enzyme, it appears unlikely that the administration of substrate affects cellular NO production. Surprisingly, numerous studies, both in animals and in humans, unequivocally demonstrated beneficial effects of arginine on 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 (6, 26). This phenomenon, in which arginine supplementation stimulates NO synthesis despite saturating intracellular concentrations, has been termed “the arginine paradox.” In the current experiments, the effects of L-arginine on arginine transport velocities and NO generation were accompanied by the prevention of increase in PKC-α and p-PKC-α protein content. These findings provide a possible explanation for the arginine paradox. We propose that the beneficial effects of arginine administration on the arginine-NO axis are far beyond a mere substrate supply, but rather via an entirely different mechanism, which involves downregulation of signal transduction pathways that inhibit CAT-1 through PKC-α activation.

Another mechanism that can potentially interfere with L-arginine transport involves the two N-dimethylated L-arginine derivatives: L-ADMA and L-SDMA. CAT proteins transport L-arginine analogs 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 do not only compete with L-arginine for transport but are also capable of driving out intracellular L-arginine (4). During pregnancy, the increase in serum levels of both ADMA and SDMA observed in CRF was prevented, implying that these agents do not contribute to the decrease in arginine transport exhibited in uremic rats during gestation, leaving PKC activation as a sole mechanism proven to modulate CAT-1 during gestation.

To summarize, in CRF rats, a decrease in glomerular arginine transport is aggravated during pregnancy. This leads to attenuated local NO generation with renal injury manifested by
upregulation of renal HIF-1α and loss of ability to augment GFR during pregnancy.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


