Downregulation of nitric oxide synthase in chronic renal insufficiency: role of excess PTH

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Vaziri, N. D., Z. Ni, X. Q. Wang, F. Oveis, and X. J. Zhou. Downregulation of nitric oxide synthase in chronic renal insufficiency: role of excess PTH. Am. J. Physiol. 274 (Renal Physiol. 43): F642–F649, 1998.—The available data on the effect of chronic renal failure (CRF) on nitric oxide (NO) metabolism are limited and contradictory. We studied rats with CRF 6 wk after a five-sixths nephrectomy and compared the results with those in the sham-operated controls, felodipine-treated CRF, and parathyroidectomized (CRF-PTX) animals. CRF was produced by surgical resection of the upper and lower thirds of the left kidney, followed by contralateral nephrectomy. We chose this model, as opposed to that produced by renal artery branch ligation, because the latter causes exuberant hypertension (HTN), which independently affects NO metabolism. The CRF group exhibited a mild HTN coupled with elevated basal platelet cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)i]), blunted hypertensive response to L-arginine, decreased hypertensive response to NO synthase (NOS) inhibitor, N\(^{\text{G}}\)-monomethyl-L-arginine, and normal hypertensive response to NO donor, sodium nitropusside. This was associated with a significant reduction in urinary excretion of stable NO metabolites (NOX) and depressed NOS activity, as well as endothelial and inducible NO synthase (eNOS and iNOS, respectively) protein contents of thoracic aorta and the remnant kidney in the CRF animals. Calcium channel blockade and PTX lowered blood pressure, increased urinary NOX, and enhanced vascular NOS activity, as well as eNOS and iNOS protein expressions in the tested tissues. Thus CRF animals exhibited significant reductions in vascular NO activity and eNOS and iNOS expressions. These abnormalities were reversed by calcium channel blockade and PTX, suggesting the possible causal role of CRF-induced dysregulation of [Ca\(^{2+}\)i].

uremia; hypertension; blood pressure; endothelial-derived relaxing factor; parathyroid hormone; calcium channel blockers; anemia

Nitric oxide (NO), once known as a toxic environmental pollutant, is now recognized as an important endogenous biological modulator with diverse physiological actions. NO modulates vascular resistance, tissue perfusion, blood pressure, and platelet function and serves as a neurotransmitter, a host defense agent, an anti-proliferative factor, and an oxygen free radical scavenger, while being a free radical itself (17, 20).

In vascular smooth muscle, NO stimulates production of cGMP, which promotes vasodilation by lowering intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)i]) and reducing sensitivity of the contractile proteins to Ca\(^{2+}\) (11, 13). NO is synthesized from conversion of L-arginine to L-citrulline by a family of NO synthases (NOS), which include constitutive and inducible isoforms (21). Basal release of NO by endothelial cells contributes to maintenance of normal blood pressure in the resting state. Conversely, relative NO deficiency leads to a rise in arterial blood pressure (15). It has been demonstrated that L-arginine: NO pathway is impaired in animal models and patients with advanced hypertension (HTN) (6, 14, 15, 27).

The effect of chronic renal failure (CRF) on NO metabolism is incompletely understood. Both increased and decreased activities of L-arginine: NO pathway have been suggested in rats and humans with CRF (1, 2, 7, 16, 24, 29, 30). In this regard, several studies have pointed to the possible reduction of NO production in CRF. For instance, renal insufficiency has been shown to cause accumulation of endogenous NOS inhibitors (methylated L-arginine derivatives and guanidino compounds), which can potentially depress NO production in CRF (16, 30). In addition, rats with renal mass reduction show reduced urinary excretion of NO metabolites (total nitrates + nitrites) and diminished histo-chemically detectable NOS in the remnant kidney, suggesting reduced renal NO production (2). In fact, chronic administration of an NO donor has been shown to retard progressive deterioration of renal function and structure in experimental CRF (7). Although the above observations point to impaired local or systemic NO production in CRF, a number of other studies have offered direct or indirect evidence for increased vascular NO production in CRF (2, 24, 29). First, pharmacological inhibition of NOS has been shown to improve platelet dysfunction in patients and animals with CRF (24, 29). These observations suggest that uremic platelet dysfunction may be due to increased systemic vascular NO production (24, 29). Finally, Aiello et al. (2) recently showed that reduced urinary nitrate/nitrite excretion and decreased NOS appearance in the remnant kidney of rats with renal mass reduction is coupled with increased plasma nitrate/nitrite concentration and histo-chemically detectable NOS in the aorta. The authors concluded that, in this model of CRF, renal NO production is reduced, but systemic vascular NO production is increased (2). They attributed the latter finding to the known stimulatory action of high blood pressure on vascular NOS activity, citing several in vivo and in vitro studies (4, 22, 25, 28). It should be noted that the CRF model employed by this group was produced by ligation of the upper and lower branches of the renal artery, leading to partial infarction of one kidney, followed by contralateral nephrectomy (2). This model of renal mass reduction is associated with an exuberant HTN, which can independently stimulate L-arginine: NO pathway. In the present study, we accomplished renal mass reduction by surgical resection of the upper and lower thirds of one kidney, followed by contralateral nephrectomy, which produces CRF with...
only a mild HTN. This was intended to demonstrate the effect of CRF, per se, on L-arginine:NO pathway by minimizing that of severe HTN. The results showed that, in contrast to the severely hypertensive renal infarction model, the mildly hypertensive surgical-resection model of CRF is associated with significant downregulation of vascular NOS activity and tissue NOS isoform protein expressions. The study further suggests that the observed downregulation of vascular NOS in this model of CRF is related to secondary hyperparathyroidism.

**MATERIALS AND METHODS**

**Animal Models**

Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with an average body weight of 300 g were used. The animals were fed a standard laboratory diet (Purina Rat Chow; Purina Mills, Brentwood, MO) and water ad libitum. Animals were randomly assigned to the CRF and sham-operated normal control groups. Animals assigned to the CRF group underwent five-sixths nephrectomy as follows. Under general anesthesia (pentobarbital sodium, 50 mg/kg ip), the left jugular vein and carotid artery were cannulated with polyethylene tubing (PE-50). A tracheal cannula was also inserted. Rats were then placed on heating pads. Arterial blood pressure was monitored directly via the arterial catheter, which was connected to a Gould P-50 pressure transducer and recorded on a Dyonograph R511A recorder (Sensor Medics, Anaheim, CA). Once animals were stable, baseline blood pressure was recorded for 5 min to determine the baseline value. Subsequently, NO precursor, L-arginine (100 mg/kg; Sigma Chemical, St. Louis, MO), or N\(^\circ\)-monomethyl-L-arginine (L-NMMA, 10 mg/kg; Sigma Chemical), a competitive inhibitor of NOS, were given as bolus intravenous injections. In addition, the hypotensive response to intravenous bolus injection of NO donor, sodium nitroprusside (SNP, 0.5 µg/kg; Sigma Chemical), was determined. Response to each drug was calculated as peak change in blood pressure from baseline. Mean arterial pressure was calculated as the sum of diastolic blood pressure and one-third of the pulse pressure. Each drug was injected at least twice, and the average of the values obtained was used.

**Measurements of NO\(_2\)/NO\(_3\)**

The concentration of total nitrate and nitrite (NOX) in the test samples was determined by a modification of the procedure described previously (5), using the purge system of a Sievers Instruments model 270B Nitric Oxide Analyzer (Sievers Instruments, Boulder, CO). Briefly, urine samples were diluted ten times in distilled water prior to analysis. A saturated solution of VCl\(_3\) in 1 M HCl was prepared and filtered prior to use. Five milliliters of this reagent were added to the purge vessel and purged with nitrogen gas for 5–10 min prior to use. The purge vessel was equipped with a cold water condenser and a water jacket to permit heating of the reagent to 95°C, using a circulating water bath. The hydrochloric acid vapors were removed by a gas bubbler containing ~15 ml of 1 M NaOH. The flow rate into the chemiluminescence detector was controlled, using a needle valve adjusted to yield a cell pressure of ~7 Torr. The flow rate of nitrogen into the purge vessel was adjusted to prevent vacuum distillation of the reagent.

Samples were injected into the purge vessel to react with the VCl\(_3\)/HCl reagent, which converted nitrate, nitrite, and S-nitroso compounds to NO. The NO produced was stripped from the reaction chamber (by purging with nitrogen and vacuum) and detected by ozone-induced chemiluminescence in the chemiluminescence detector. The signal generated (NO peak and peak area) was recorded and processed by a Hewlett-Packard model 3390 integrator. In a typical assay, 5 µl of the test sample was injected to the purge vessel, and all samples were run in triplicate.

**Tissue Preparation**

Thoracic aorta and remnant kidneys were used for determination of NOS. Rats were killed by decapitation, and thoracic aorta and remnant kidneys were immediately excised, cleaned with PBS, frozen in liquid nitrogen, and stored at ~75°C. Homogenates (25% wt/vol) were prepared in 10 mM HEPES buffer, pH 7.4, containing 320 mM sucrose, 1 mM EDTA, 1 mM dithiothreitol, 10 µM leupeptin, and 2 µM aprotonin at 0–4°C, with the aid of a tissue grinder fitted with a motor-driven ground-glass pestle. Homogenates were centrifuged at 12,000 g for 5 min at 4°C to remove tissue debris.
without precipitating plasma membrane fragments. The supernatant was used for determination of NOS activity.

For Western blot analysis, tissue was homogenized in five volumes of lysis buffer (1% SDS, 1.0 mM sodium vanadate, 10 mM Tris-HCl, pH 7.4) containing 10 µM pepstatin, 13 µM leupeptin, and 1 mM phenylmethylsulfonfyl fluoride. The homogenate was centrifuged at 12,000 g for 5 min at 15°C to remove tissue debris without precipitating plasma membrane fragments (8, 18). Protein concentration was determined by using a Bio-Rad kit (Bio-Rad, Hercules, CA).

Western Blot Analysis

These measurements were carried out to determine the endothelial and inducible NOS (eNOS and iNOS, respectively) protein mass, as previously described (8, 18). Anti-eNOS monoclonal antibody, anti-Mac NOS-I, human endothelial positive control, and mouse macrophage positive control were supplied by Transduction Laboratories (Lexington, KY). Peroxidase-conjugated goat anti-mouse IgG antibody was purchased from Pierce (Rockford, IL). Briefly, tissue preparations (50 µg of protein) were size fractionated on 4–12% Tris-glycine gel (Novex, San Diego, CA) at 120 V for 3 h. After electrophoresis, proteins were transferred onto Hybond-ECL enhanced chemiluminescence membrane (Amersham Life Sciences, Arlington Heights, IL) at 500 mA for 120 min, using the Novex transfer system. The membrane was prehybridized in 25 ml of Super Block blocking buffer (Pierce) for 1 h and then hybridized for an additional 1-h period in the same buffer containing 10 µl of the given anti-NOS monoclonal antibody (1:2,500). The membrane was then washed for 60 min in a shaking bath, with the wash buffer (Tris-buffered saline containing 0.1% Tween 20) changed every 10 min prior to 1 h of incubation in Super Block blocking buffer plus goat anti-mouse IgG-horseradish peroxidase antibody, which was visualized with enhanced chemiluminescence membrane (Amersham Life Sciences, Arlington Heights, IL). NOS activity was determined from the production of [3H]citrulline per minute per milligram of protein.

Platelet Cytosolic Calcium Measurements

At the end of the 6-wk observation period, a subgroup of rats was killed, and platelets were isolated using a procedure described previously (10). The isolated platelets were suspended in HEPES-buffered saline (HBS) with the following composition (in mmol/l): 145 NaCl, 5 KCl, 0.8 Na2HPO4, 0.2 KH2PO4, 1 MgCl2, 10 glucose, and 10 HEPES, pH 7.4. This resulted in a platelet suspension containing 2 × 108 cells/ml. [Ca2+]i was determined with the fluorescent calcium indicator, fura 2-AM. The suspended platelets were loaded with 4 mol fura 2-AM in the presence of 0.02% Pluronic F-127 to facilitate entry of the indicator into the cells. In addition, 2 mM probenecid was added to minimize leakage of fura 2 out of the platelets (10). Cells were incubated for 60 min at 37°C, then centrifuged at 400 g for 20 min. The supernatant was decanted, and an equal volume of HBS added. The cells were incubated at 37°C for 1 h to allow complete hydrolysis of the AM group. The dye-loaded cells were suspended in HBS containing 0.5 mol CaCl2 and kept under constant magnetic stirring in a thermostatically controlled cuvette of a spectrofluorometer (DMX 1000; SLM Instruments, Urbana, IL). Alternating excitation wavelengths of 340 and 380 nm were used, with an emission wavelength of 510 nm. Ratios of fluorescence (R = 340/380 nm) were measured every second, automatically corrected for autofluorescence, and plotted graphically for each sample analyzed. Values of autofluorescence were <5% of the fluorescence of the dye-loaded cells and were measured for every experiment.

[Ca2+]i was calculated as described earlier (33), using the following formula: 

$$[Ca^{2+}]_i = (K_b)(R - R_{min})/(R_{max} - R)$$

where B is the ratio of 380 nm fluorescence in the absence and presence of saturating concentration of calcium, Kb is the dissociation constant for fura 2 (assumed to be 225 nM), and R is the ratio of fluorescence, as defined above. The cells were lysed with Triton (0.05%) to obtain R_{max} in the presence of 2 mmol EGTA, and R_{min} was obtained by the addition of 10 mmol EGTA and sufficient NaOH to raise the pH to 8.5.

Data Presentation and Analysis

Data are presented as means ± SE. ANOVA and Duncan multiple range test were used as appropriate. P ≤ 0.05 was considered statistically significant.

RESULTS

General

As shown in Table 1 and Fig. 1, the body weight and hematocrit obtained at the conclusion of the study were significantly lower in the CRF, CRF-FEL, and CRF-PTX groups, compared with the normal control group, despite comparable values at the time of randomization. As expected, the CRF groups showed a significant rise in serum creatinine and a significant decline in creatinine clearance. In addition, the CRF group exhibited a significant increase in arterial blood pressure, compared with both the baseline value and those found in the normal control, CRF-FEL, and CRF-PTX groups.
The CRF-PTX group showed marked hypocalcemia (5.46 ± 0.5 mg/dl) prior to initiation of calcium supplementation. The associated hypocalcemia was corrected by addition of calcium gluconate to the drinking water, yielding a serum calcium concentration comparable to values found in the other groups (CRF-PTX, 9.6 ± 0.31; CRF, 9.0 ± 0.35; and normal control group, 9.7 ± 0.14 mg/dl).

Effect of CRF

Blood pressure response to L-arginine, L-NMMA, and SNP. In both the normal and CRF animals, blood pressure declined within a few seconds after bolus injection of L-arginine and returned to baseline promptly thereafter (Fig. 2). The magnitude of the L-arginine-induced fall in arterial blood pressure was ~1.5-fold greater in the control group than that observed in the CRF group (P < 0.05). L-NMMA administration led to a rise in blood pressure lasting 10–20 min in both groups. Maximal pressor response to L-NMMA was significantly lower (P < 0.01) in the CRF group compared with the normal controls. However, the magnitude of hypotensive response to NO donor, SNP, in the CRF group was similar to that found in the control group.

Table 1. Body weight, plasma creatinine concentration, plasma creatinine clearance, and hematocrit in CRF and normal control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body Wt, g</th>
<th>Plasma Cr, mg/dl</th>
<th>CCr, ml/min</th>
<th>Hematocrit, %</th>
</tr>
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<tbody>
<tr>
<td>CRF</td>
<td>6</td>
<td>363 ± 4.1</td>
<td>1.29 ± 0.18</td>
<td>0.82 ± 0.15</td>
<td>39.8 ± 0.84</td>
</tr>
<tr>
<td>CRF-FEL</td>
<td>6</td>
<td>365 ± 8.4</td>
<td>1.03 ± 0.08</td>
<td>0.84 ± 0.08</td>
<td>39.4 ± 0.83</td>
</tr>
<tr>
<td>CRF-PTX</td>
<td>9</td>
<td>359 ± 5.2</td>
<td>1.15 ± 0.07</td>
<td>0.87 ± 0.1</td>
<td>38.7 ± 0.79</td>
</tr>
<tr>
<td>NL</td>
<td>6</td>
<td>398 ± 7.2</td>
<td>0.40 ± 0.03*</td>
<td>2.59 ± 0.17*</td>
<td>46.4 ± 1.14*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Cr, plasma creatinine concentration; CCr, creatinine clearance; CRF, chronic renal failure; FEL, felodipine; PTX, parathyrodectomy; NL, normal control. *P < 0.05 vs. other groups.

Fig. 2. Change in mean arterial pressure (ΔMAP) from baseline in response to intravenous bolus injections of L-arginine (L-Arg, 100 µg/kg), N²-monomethyl-L-arginine (L-NMMA, 10 µg/kg), and sodium nitroprusside (SNP, 0.5 µg/kg) in rats with CRF and sham-operated controls. Each column represents mean values obtained in 6 animals. Bars denote SEM. *P < 0.05 vs. control group.

Urinary excretion and plasma concentration of NOX. Data are given in Table 2 demonstrate that the CRF group showed a significant reduction in urinary excretion of total nitrates and nitrites. This was accompanied by normal plasma NOX concentration. These findings point to a possible reduction in total body NO production.

NOS activity and protein mass. The results in Figs. 3–7 indicate that the CRF group showed a significant reduction in aorta NOS activity. The reduction in the thoracic aorta NOS activity in the CRF group was accompanied by parallel reductions in the aorta eNOS and iNOS protein contents. The concomitant reductions in vascular tissue NOS activity with those of eNOS and iNOS proteins point to the quantitative deficiency, as opposed to the mere functional impairment of NOS in this model of CRF. As with the aorta, eNOS and iNOS protein abundance was significantly reduced in the remnant kidneys of the CRF animals.

Platelet cytosolic [Ca²⁺]. Compared with the normal control group, resting platelet [Ca²⁺] was significantly elevated (P < 0.05) in the CRF animals. In contrast, thrombin-stimulated rise in [Ca²⁺] was significantly attenuated in the CRF group (Fig. 8). Thus the magnitude of change from basal to peak value was greatly diminished in CRF animals.

Table 2. Urinary NOX excretion and plasma concentration of NOX in the NL control group and CRF animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urinary NOX, µmol/mg creatinine</th>
<th>Plasma NOX, µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL</td>
<td>0.479 ± 0.08</td>
<td>24.6 ± 1.90</td>
</tr>
<tr>
<td>CRF</td>
<td>0.270 ± 0.05*</td>
<td>23.5 ± 4.50</td>
</tr>
<tr>
<td>CRF-FEL</td>
<td>0.895 ± 0.02*†</td>
<td>56.0 ± 3.95*</td>
</tr>
<tr>
<td>CRF-PTX</td>
<td>0.667 ± 0.39*†</td>
<td>28.4 ± 1.40</td>
</tr>
</tbody>
</table>

Values are means ± SE; N = 6/group. NOX, total nitrates and nitrites. *P < 0.05 vs. control group. †P < 0.05 vs. CRF group.
Effect of Calcium Channel Blockade

Data in Table 2 and Figs. 1 and 3–6 show that calcium channel blockade with felodipine mitigated the CRF-induced HTN and abrogated the CRF-associated elevation of basal \([\text{Ca}^{2+}]_i\). However, calcium channel blockade did not interfere with thrombin-stimulated rise in platelet \([\text{Ca}^{2+}]_i\). Thus the difference between the peak-stimulated and basal values (\(\Delta[\text{Ca}^{2+}]_i\)) was improved with this therapy. Calcium channel blockade resulted in a significant rise in urinary excretion and plasma concentration of NOX to supranormal values. This was accompanied by a complete restoration of renal and vascular tissue eNOS and iNOS proteins, as well as vascular NOS activity in the CRF-FEL group. This phenomenon suggests that the CRF-associated downregulation of vascular eNOS and iNOS expressions may be due to CRF-induced dysregulation of \([\text{Ca}^{2+}]_i\). In addition, the data suggest that the antihypertensive effect of calcium channel blockade in this model may be, in part, mediated by upregulation of vascular NOS expression and NO production.

Effects of Parathyroidectomy

Data in Table 2 and Figs. 3–7 show that, as with calcium channel blockade, PTX increased urinary excretion of NOX and enhanced vascular tissue NOS activity, as well as renal and vascular tissue eNOS and iNOS protein expressions. This was accompanied by a significant amelioration of CRF-associated HTN. Thus the effects of PTX were directionally similar to those of calcium channel blockade.

DISCUSSION

As expected, the CRF animals exhibited a mild but significant rise in arterial blood pressure, coupled with a significant increase in basal \([\text{Ca}^{2+}]_i\). Investigation of the L-arginine:NO pathway in the CRF animals revealed a blunted hypotensive response to the NO precursor, L-arginine, in the face of normal response to NO donor, SNP. These observations suggest the pres-
ence of a possible defect in NO production from l-arginine, as opposed to a resistance to the action of NO. This supposition is further supported by the blunted hypertensive response to NOS inhibitor, l-NMMA, which points to reduced NO dependence/availability in the CRF animals. In addition, the combination of normal response to NO donor and diminished response to NOS inhibitor in this model argues against excess vascular NO production, which would have been marked by reduced sensitivity to exogenous NO and exaggerated response to NOS inhibition, not seen here.

Depressed l-arginine:NO pathway in this model could be due to decreased l-arginine availability. However, the blunted response to l-arginine administration shown here argues against this possibility. Alternatively, accumulation of endogenous NOS inhibitors in uremia can account for the observed depression of l-arginine:NO pathway activity. In fact, increased concentration of methylated arginine and guanidino compounds with NOS inhibitory properties has been previously shown in patients with end-stage renal disease (16, 30). Although this is in part true, competitive inhibition of the enzyme should not lead to the reduction of immunodetectable quantity of the NOS proteins as shown here. In fact, eNOS and iNOS proteins were significantly reduced in the CRF animals, suggesting decreased enzyme availability as opposed to a mere competitive inhibition of the enzyme. Thus the present study provides evidence for acquired NOS deficiency in the vascular and renal tissue of mildly hypertensive rats with CRF produced by five-sixths nephrectomy using surgical resection. Further support for the down-regulation of l-arginine:NO pathway in this model comes from the marked reduction in urinary NOX excretion in the CRF animals. The reduction in urinary NOX excretion in the CRF animals was accompanied by normal plasma NOX concentration, suggesting depressed total body NO generation. This is because, if systemic NO generation were normal, CRF would have raised plasma NOX concentration, as exemplified by various other nitrogenous end products whose plasma concentrations rise in CRF (azotemia). Thus plasma NOX level more likely reflects renal function and volume distribution rather than systemic NO generation.

These findings, in part, differ from those reported by Aiello et al. (2), who used a different CRF model produced by ligation of the upper and lower renal artery branches of one kidney followed by contralateral nephrectomy. Unlike the model employed in the present study, in which CRF is associated with mild HTN, the CRF model of renal infarction is associated with severe exuberant HTN. The authors rightfully attributed the upregulation of vascular NOS in their CRF animals to the well-known stimulatory action of HTN, citing several in vivo and in vitro studies of the direct effects of pressure and flow (4, 22, 25, 28). In contrast to the renal infarction model employed by Aiello et al., the surgical resection model of five-sixths nephrectomy is associated with a relatively mild HTN. Thus the intense stimulation of vascular NOS by severe HTN was
far greater in the CRF animals employed in the latter study than in those employed in the present study. Based on the results of the present study, we believe that CRF, per se, leads to a reversible downregulation of vascular NOS. Consequently, removal of the suppressive factor (e.g., excess PTH) can correct this abnormality. Similarly, the presence of intense stimulatory influences such as severe HTN, volume expansion, and increased shear stress can override the downregulatory action of CRF, leading to a net increase in vascular NOS activity and protein mass. This was clearly exemplified by CRF animals described by Aiello et al. (2).

The CRF animals exhibited a significant elevation of basal [Ca$^{2+}$]$_i$, and a defective rise in stimulated [Ca$^{2+}$]$_i$, consistent with our earlier observations (32). Excess production of PTH has been linked to elevation of [Ca$^{2+}$]$_i$ in a variety of cell systems in CRF. In addition, increased PTH production and the associated elevation of [Ca$^{2+}$]$_i$ in CRF has been found to depress expression of several enzymes, including hepatic lipase and lipoprotein lipase, as well as several hormone receptors. Furthermore, parathyroidectomy has been shown to reverse these abnormalities in patients and animals with CRF (3, 12, 19, 31). We therefore sought to examine the role of secondary hyperparathyroidism in downregulation of renal and vascular NOS expression by including a group of CRF-PTX animals. The CRF-PTX animals showed a significant increase in remnant kidney and vascular tissue eNOS and iNOS protein expressions together with a significant rise in urinary NOX excretion. These results point to the important role of excess PTH in the pathogenesis of CRF-associated depression of vascular NOS.

In an attempt to determine whether the effect of excess PTH on l-arginine:NO pathway in CRF animals is mediated by the change in [Ca$^{2+}$], we tested the effect of pharmacological modification of [Ca$^{2+}$]. To this end, a subgroup of rats was treated with calcium channel blockade, using implanted osmotic pumps loaded with felodipine. The results showed an expected amelioration of CRF-associated hypertension, coupled with the restoration of normal basal [Ca$^{2+}$]. Interestingly, remnant kidney and vascular eNOS and iNOS protein contents and vascular NOS activity were increased significantly and urinary NOX excretion reached supranormal levels by this therapy in the CRF animals. The directional similarities of the effects of felodipine and parathyroid ablation (which both lower resting [Ca$^{2+}$]) on vascular NOS expression and NO production in rats with CRF suggest that these abnormalities may be linked to the CRF-induced dysregulation of [Ca$^{2+}$]. It should be noted that hemodynamic alterations may have also contributed to the observed improvement of NO production and NOS expression with PTX and calcium channel blockade in the CRF animals. Our data further indicate that the antihypertensive action of calcium channel blockade in this model may be, in part, mediated by NO.

Interestingly, calcium channel blockade and to a lesser extent PTX resulted in the rise of urinary excretion and plasma concentration of NOX to supranormal levels. This was accompanied by similar rises in NOS proteins of the tested tissues. The available data do not allow definitive explanation for this exaggerated response. However, lifting of the inhibitory action of abnormal [Ca$^{2+}$] may have unmasked the upregulatory action of the associated anemia (23, 26) in CRF animals. Alternatively, the observed phenomenon may have been mediated by hemodynamic alterations caused by these interventions.

In conclusion, the present study demonstrated marked reductions of eNOS and iNOS proteins in mildly hypertensive CRF rats employed here. These abnormalities were reversible with parathyroidectomy and calcium channel blockade, suggesting the possible causal role of CRF-induced dysregulation of cytosolic Ca$^{2+}$. We wish to note that although CRF, per se, downregulates remnant kidney and vascular NOS, several conditions that frequently accompany CRF, such as severe HTN, volume expansion, inflammatory disorders, immune activation, anemia, and pharmacological agents, can independently affect NOS in the opposite direction. Thus NO production and NOS activity/expression in a given CRF patient or animal model is determined by the collective actions of these influences.
REFERENCES


