Endogenous Nitric Oxide Enhances Prostaglandin Production in a Model of Renal Inflammation

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Abstract

The interaction between nitric oxide (NO) and cyclooxygenase (COX) was studied in a rabbit model of renal inflammation, the ureteral obstructed hydronephrotic kidney (HNK). Ex vivo perfusion of the HNK but not the control kidney (e.g., unobstructed contralateral kidney, CLK), led to a time-dependent release of nitrite (NO_2^-), a breakdown product of NO. Stimulation of the HNK with bradykinin (BK) evoked a time-dependent increase in prostaglandin E_2 (PGE_2) production. N^G-monomethyl-L-arginine (L-NMMA), which blocks the activity of both constitutive and inducible nitric oxide synthase (cNOS and iNOS), aminoguanidine, a recently described selective iNOS inhibitor, dexamethasone, or cycloheximide abolished the release of NO_2^- and attenuated the exaggerated BK-induced PGE_2 production. This supports the existance of iNOS and COX-2 in the HNK. In the CLK, BK elicited release of both NO_2^- and PGE_2 but this did not augment with time. L-NMMA but not aminoguanidine, dexamethasone, or cycloheximide attenuated NO_2^- and PGE_2 release indicative of the presence of constitutive but not inducible NOS or COX. The current study suggests that the endogenous release of NO from cNOS in the CLK activates a constitutive COX resulting in optimal PGE_2 release by BK. In addition, in the HNK, NO release from iNOS activates the induced COX resulting in markedly increased release of proinflammatory prostaglandin. The broader implication of this study is that the cyclooxygenase isozymes are potential receptor targets for nitric oxide. (*J. Clin. Invest.* 1994. 93:1940–1947.) Key words: prostaglandin E_2 • nitric oxide • renal inflammation

Introduction

Cyclooxygenase (COX)[1] activity is normally low in the rabbit renal cortex and TX synthase is not found in either the cortex or the medulla of the normal rabbit kidney (1). In rabbits, unilateral ureter obstruction (hydronephrosis) results in the sequential invasion of polymorphonuclear leukocytes and monocytes (2), a proliferation of cortical fibroblast-like interstitial cells (3, 4), and marked changes in arachidonate metabolism (5). Hydronephrosis for 3 d exhibits a dramatic increase in renal cortical microsomal COX activity relative to the control unobstructed contralateral kidney (CLK; 1, 6, 7). Furthermore, agonist stimulation of the ex vivo perfused hydronephrotic kidney (HNK), but not CLK with bradykinin (BK) results in a 5–15-fold increase in the production of the eicosanoids, PGE_2, prostacyclin (PGI_2), and TXA_2 (1, 8–10). Macrophages play a pivotal role in the altered AA metabolism. Thus, in rabbits rendered leukopenic with nitrogen mustard before ureteral obstruction (11), in which inflammatory cell influx was decreased, or in the postobstructed HNK (5) where the invading macrophages were diminished, BK-stimulated eicosanoid production by the perfused kidney was not enhanced.

Besides the well-studied constitutive form of COX, a second isoform (COX-2) has been described which is not constitutively expressed, but rather is induced in macrophages (12–14), fibroblasts (15), or endothelial cells (16) by proinflammatory agents including *Escherichia coli* LPS and IL-1β. Induction of COX and its subsequent activation is the likely mechanism accounting for the exaggerated production of PGE_2 observed in the ex vivo perfused rabbit kidney (9).

We have recently reported that nitric oxide (NO) activates the constitutive and inducible forms of COX (17). NO is derived from the guanidino nitrogen atom of L-arginine (L-Arg) by the enzymatic action of nitric oxide synthase (NOS). Two major forms of NOS have been identified to date. The constitutive enzyme, which is a calcium-calmodulin–dependent isoform (cNOS), is activated by agonists (e.g., BK or AA) in cells such as endothelial cells; cNOS activation releases NO in small quantities (18–20). The inducible, calcium-independent isoform (iNOS) is expressed after induction by various proinflammatory agents such as LPS, IL-1β, and/or tumor necrosis factor in endothelial cells (21), smooth muscle cells (22, 23), and macrophages (24, 25). Besides their capacity to block the induction of COX-2, steroids such as dexamethasone also inhibit the induction of NOS in vitro and in vivo without affecting the activity of the constitutive form of the enzyme. The role of the influence of nitric oxide on COX in hydronephrosis is not known. We hypothesized that if the NOS pathway is expressed in the HNK, then it may account, at least in part, for the exaggerated eicosanoid production observed in this model of renal inflammation. For this purpose, we have measured NO_2^- and PGE_2 release in the venous effluent as markers for NOS and COX activity, respectively. The relative contributions of cNOS and iNOS on COX activity were evaluated by (*a*) inhibition of both NOS isoforms with the L-Arg analogue N^G-monomethyl-L-arginine (L-NMMA), (*b*) inhibition of iNOS activity by aminoguanidine (AG), a recently described

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1. *Abbreviations used in this paper:* AG, aminoguanidine; BK, bradykinin; CHAPS, 3,3-cholamidopropyl dimethylammonio 1-propanesulfonate; CLK, contralateral kidney; COX, cyclooxygenase; GTN, glyceryl trinitrate; HNK, hydronephrotic kidney; L-NMMA, N^G-monomethyl-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; cNOS, constitutive NOS; iNOS, inducible NOS; PLA_2, phospholipase A_2; SNP, sodium nitroprusside.

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selective iNOS inhibitor (26–28), and (*c*) inhibition of the induction of iNOS by cycloheximide or dexamethasone.

Methods

Ureteral obstruction. Male New Zealand white rabbits (2–3 kg) were obtained from Mohican Valley Rabbitry (Loudenville, OH) and housed for at least 1 wk before the experiments. All animals were fed normal rabbit Chow (Ralston Purina Co., St. Louis, MO) and allowed free access to water. Unilateral ureteral obstruction was carried out by a previously described procedure (8). Briefly, the animals were anesthetized with ketamine/xylazine (35/3 mg/kg, i.m.), and a small abdominal incision was made. A silk suture was tied around one of the ureter near the bladder. 150,000 IU of penicillin antibiotic was administered (s.c.) postoperatively, and the rabbit allowed to return to dorsal recumbancy before returning to its cage. 3 d after obstruction (8), rabbits were anesthetized again (ketamine/xylazine 35/3 mg/kg, i.m.) and heparinized (250 U/kg, i.v.), and the abdominal cavity was opened and the renal arteries were cannulated. Kidneys were removed, flushed with 30 ml ice-cold Krebs-Henseleit buffer, and perfused at 10 ml/min with oxygenated (95% O_2/5% CO_2, pH 7.4) Krebs-Henseleit buffer maintained at 37°C.

Ex vivo kidney perfusion. Changes in perfusion pressures (measured with a Cobe disposable pressure transducer on a Grass model 7D polygraph) reflect changes in renal resistance. Perfusion of the kidneys at a rate of 10 ml/min gave pressures of 78±1 mmHg in the CLK and 75±2 mmHg in the HNK (n = 36). Under these experimental procedures, papaverine (0.1 mM) did not affect basal perfusion pressure, which demonstrates that the perfused rabbit kidney lacks intrinsic tone. Tone could be increased by adding noradrenaline (0.3 μM) in the perfusion buffer and this allowed vasodilator responses to BK or AA to be monitored. We did not increase tone in our experiments so as not to complicate the model and thus the interpretation of the data. There was no need for the addition of albumin or other osmotic agents to the buffer because the perfusion pressure recordings were stable for the duration of the experiment (6 h). The drugs used in our study did not affect perfusion pressure. No change in kidney weights were observed after perfusion for up to 6 h (from 15±0.1 to 15±1 g in the CLK and from 26±1 to 27±1 g in the HNK, n = 36), suggesting the absence of significant edema formation over this time period.

Drug administration. BK was used to stimulate NO_2^- and PGE_2. Release of PGE_2 by BK requires activation of phospholipase A_2 (PLA_2), which in turn releases AA from endogenous phospholipid for conversion to prostaglandin endoperoxide by COX. Therefore, to determine whether or not the actions of NO on PGE_2 release were a consequence of an effect on PLA_2 activity we also tested the effects of NO on AA-mediated release of PGE_2 in the CLK. The isolated rabbit kidneys were allowed to equilibrate for 30 min before experimental manipulations. All drugs used in this study, except for sodium nitroprusside (SNP) or 3,3-cholamidopropyl dimethylammonio 1-propanesulfonate (CHAPS), were infused for 30 min after the equilibration period and before the first BK (1 μg) or AA (30 μM) injection (this is referred to as time 0). Drugs were infused for the entire duration of the experiment (e.g., 6 h). Bradykinin or AA were injected as a bolus intraarterially every hour from 0 to 6 h of perfusion to stimulate NO_2^- and PGE_2 release from the kidney. These agonists were injected with a 10-min lag period from each other. The profile of PGE_2 release by BK or AA revealed that maximal PGE_2 release peaked at the third minute after agonist injection and declined between the fourth and fifth minutes (n = 8). Therefore, in all our experiments we collected the venous effluents from the CLK/HNK for 3 min both before and after each BK or AA injection. The effects of the NO donors, SNP or glyceryl trinitrate (GTN), or of the detergent, CHAPS (4.7 mg/ml) on BK (1 μg) or AA (30 μM)-induced PGE_2 release from the CLK or HNK were tested at the sixth hour of perfusion. At this time point, after the bolus injection of BK or AA, the kidneys were perfused with SNP for 30 min or with CHAPS for 30 s and then rechallenged with the same dose of BK or AA. The concentration of CHAPS used was sufficient to remove renal vascular endothelium and thus endothelium-dependent responses without altering non–endothelium-mediated effects (29). In some experiments, indomethacin (1 μM) was perfused through the kidneys together with SNP. The venous effluent was assayed for PGE_2 by radioimmunoassay (30) or for NO_2^- release using the diaminonaphthalene assay (31). The production of NO_2^-, a breakdown product of NO, or PGE_2 was used respectively as a marker for NOS and COX activity.

Materials. The composition of Krebs-Henseleit buffer was (mM): NaCl 120, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, dextrose 10. All drugs were obtained from Sigma Chemical Co. (St Louis, MO).

Statistical analysis. Results are expressed as mean±SEM for (n) experiments. The results were analyzed by Student's unpaired t test to determine the significant difference between means, or by a two-way ANOVA followed by a least significance procedure to determine the nature of this response. This will be specified when appropriate. A P value of < 0.05 was taken as significant.

Results

Renal NO and PGE_2 production. The ex vivo perfusion of the HNK caused a time-dependent release in nitrite in the venous effluent (Fig. 1 *A*), which was not enhanced by injection of BK. In contrast, bolus injection of BK (1 μg) at 1, 3, and 6 h of perfusion caused a marked increase in the amounts of PGE_2 release that was dependent upon the time of perfusion (Fig. 1 *B*). In the CLK (e.g., control, unobstructed kidney), BK released NO_2^- (Fig. 2 *A*) and PGE_2 (Fig. 2 *B*) above basal levels but this release was not altered when the time of perfusion was increased from 0 to 6 h. Similar results were obtained with injections of the PG precursor AA (30 μM, n = 8, not shown).

Effect of indomethacin. Intrarenal injection of BK or AA into the CLK after 6 h of ex vivo perfusion released 242±22 and 202±11 pg PGE_2/ml per min and 495±55 and 550±33 pmol NO_2^-/ml per min (n = 8, respectively). Perfusion of the kidney with 1 μM indomethacin suppressed the release of PGE_2 (99±8 and 77±11 pg PGE_2/ml per min, n = 8) to near-basal values (n = 8), whereas it had no effect on the release of NO_2^- (473±44 and 495±22 pmol/ml per min, n = 8). Similarily, indomethacin blocked the exaggerated release of PGE_2 in response to BK stimulation in the HNK (from 3069±416 to 87±22 pg PGE_2/ml per min when BK was injected at 6 h of perfusion, n = 8) but had no effect on the release of NO_2^- (from 1,870±55 to 1,815±110 pmol/ml per min at 6 h of perfusion, n = 8).

Effect of detergent perfusion. Detergent perfusion results in loss of endothelial cells from the renal vasculature (29). In the CLK, the release of NO_2^- (Fig. 3 *A*) or PGE_2 (Fig. 3 *B*) elicited by BK (1 μg, n = 3) or AA (30 μM, n = 3) was abolished by a 30-s perfusion with CHAPS (4.7 mg/ml). CHAPS treatment did not alter the release of NO_2^- in the HNK (from 1,958±22 to 1,848±77 pmol/ml per min at 6 h, n = 3, Fig. 3 *C*) or the release of PGE_2 by BK (from 3,100±58 to 2,666±88 pg PGE_2/ml per min, n = 3, Fig. 3 *D*).

Influence of NO on PG production. Perfusion of the CLK kidney with 100 μM L-NMMA, but not with aminoguanidine blocked BK (1 μg, n = 6)-induced release of NO_2^- and attenuated PGE_2 release by at least 40% (Fig. 2, *A* and *B*). Similar results were obtained with AA (30 μM, n = 6, not shown). In the HNK, L-NMMA or AG (100 μM, n = 6) blocked NO_2^- release to a similar extent (Fig. 1 *B*) and attenuated BK-in-

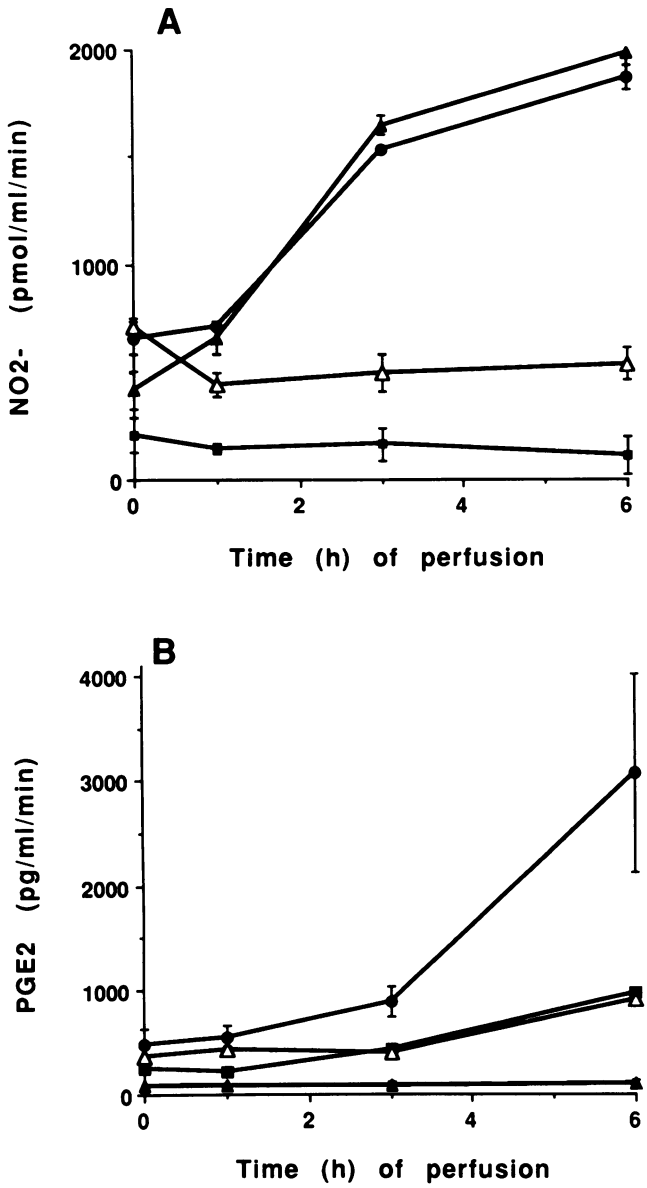

Figure 1. The effects of inhibitors of NOS (L-NMMA or AG) on nitrite and PGE_2 release in the perfused HNK. (*A*) The basal levels of NO_2- increased with time of ex vivo perfusion (▲); this release was not potentiated by BK (1 μg, ●). (*B*) In contrast, bolus injection of BK (1 μg, ●) led to a marked increase in PGE_2 release that increased with time. L-NMMA (100 μM, ■) or AG (100 μM, △) blocked nitrite (*A*) and PGE_2 (*B*) release. Each point is the mean±SEM for $n = 6$ experiments.

duced release by at least 50% (Fig. 1 *B*). The release of PGE_2 or NO_2- in response to BK or AA stimulation was not affected when the contralateral kidneys were perfused with L-Arg (1 mM, $n = 4$ not shown). However, perfusion of the HNK with L-Arg (0.1–1 mM) but not D-Arg (1 mM) enhanced the release of NO_2- observed with increased time of perfusion (Table I) and potentiated the release of PGE_2 in response to BK stimulation (Table I). The effects of L-Arg (0.1 mM) were abolished by co-infusion with L-NMMA or AG (0.1 mM, $n = 6$, Fig. 4, *A* and *B*). Fig. 5 shows that in the HNK, the marked potentiating effects of L-Arg on the exaggerated BK-induced release of PGE_2 was completely abrogated by co-infusion with indomethacin. Perfusion of the HNK with L-citrulline (1 mM), an amino acid produced together with NO, or with L-lysine (1 mM) an amino acid not involved in the generation of NO, had no effect on BK-promoted PGE_2 release ($n = 6$, not shown).

Perfusion (30 min) of the CLK or HNK with 0.125–1 mM SNP or GTN (exogenous sources of NO) augmented even further the production of PGE_2 observed after BK stimulation (Table II). The potentiating effect of the highest dose of SNP tested (1 mM) was abrogated by the simultaneous perfusion with indomethacin (1 μM, Fig. 6, *A* and *B*). The effects of SNP

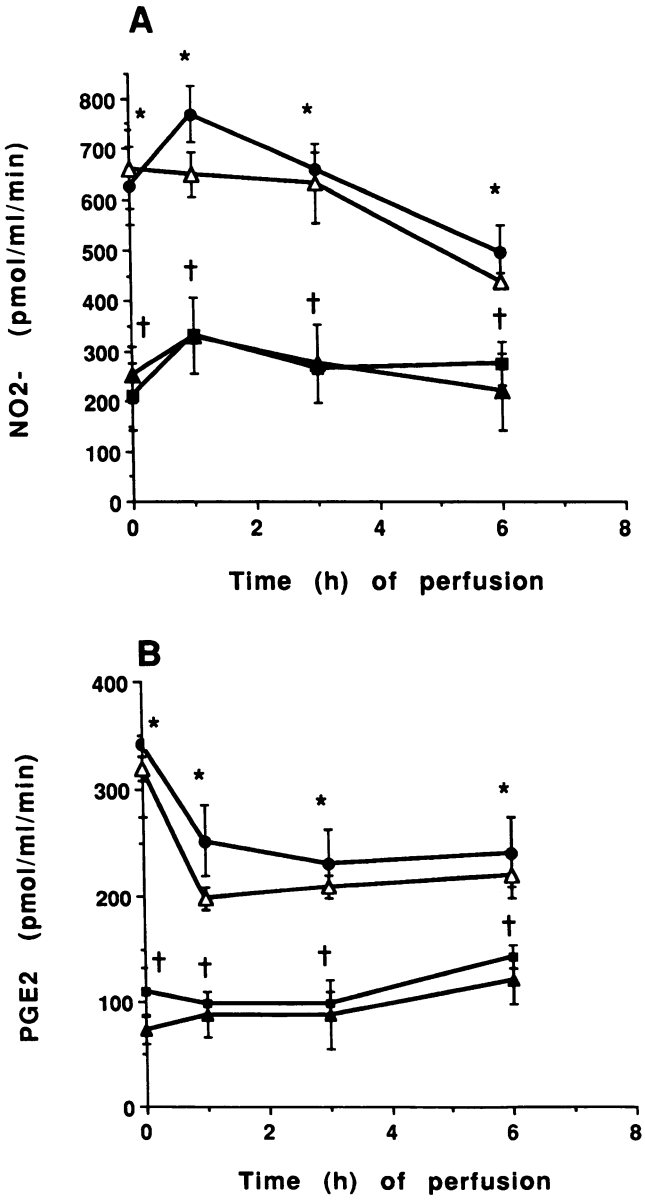

Figure 2. The effects of NOS inhibitors on nitrite and PGE_2 release in the perfused control CLK. Bolus injection of BK (1 μg, ●) increased NO_2- (*A*) and PGE_2 (*B*) release above basal (▲) but this did not increase with time of perfusion (*A* and *B*). Perfusion of the kidney with L-NMMA (■) but not AG (△) (both at 100 μM) attenuated the release of NO_2- (*A*) and PGE_2 (*B*) in response to BK (1 μg). Each value is the mean±SEM for $n = 6$ experiments. $^*P < 0.01$ when compared to basal values and $^+P < 0.01$ when compared to values obtained with BK (ANOVA).

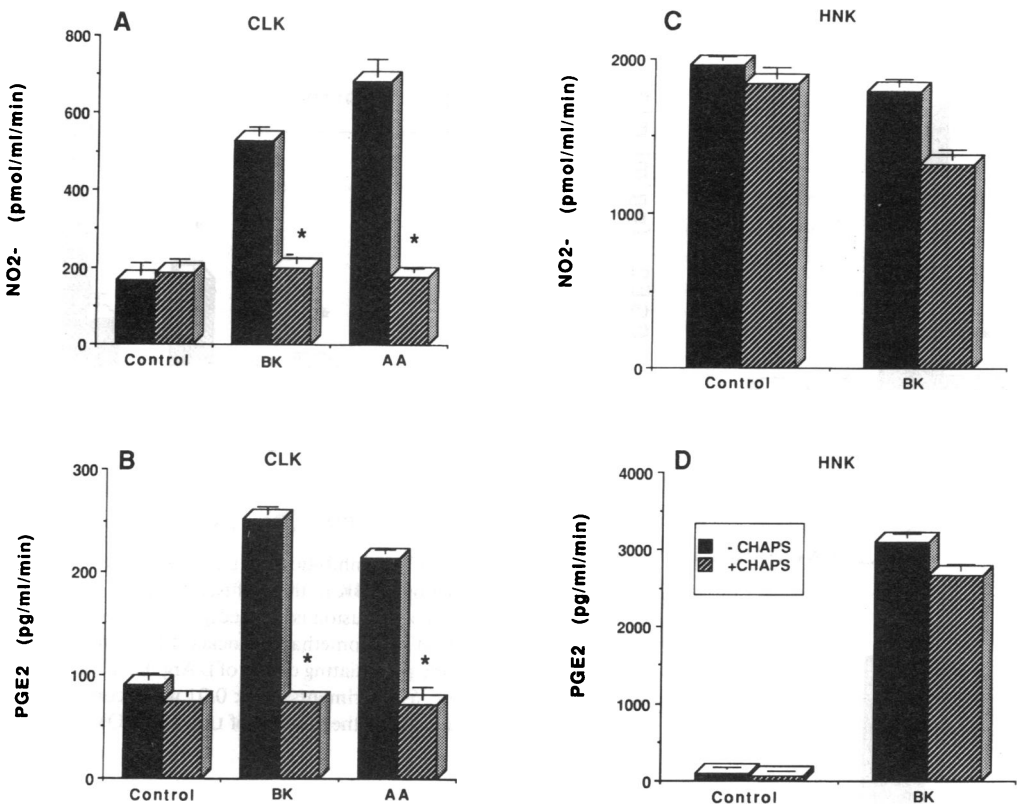

Figure 3. The effects of endothelium removal by the detergent CHAPS on nitrite and PGE_2 release in the perfused CLK and HNK. Brief exposure (30 s) of the CLK with CHAPS (4.7 mg/ml) blocked the release of nitrite (*A*) and PGE_2 (*B*) elicited by BK (1 μg) or AA (30 μM). However, CHAPS did not block perfusion-mediated release of nitrite in the HNK (*C*) nor did it block the BK-stimulated PGE_2 production (*D*). Vertical bars represent the mean±SEM for n = 3 experiments. Filled bars represent experiments done in the absence of CHAPS and hatched bars in the presence of CHAPS. $^*P < 0.01$ when compared to the value obtained in the absence of CHAPS.

on PGE_2 do not likely involve PLA_2, since SNP also increased PGE_2 production by AA (Fig. 6 *B*), which is known to bypass PLA_2 activation.

Effects of dexamethasone or cycloheximide. Fig. 7 *A* shows that dexamethasone or cycloheximide abolished the time-dependent release of NO_2^- in the HNK; this inhibition was very similar to the time taken for these drugs to block the BK-induced release of PGE_2 (Fig. 7 *B*). The release of NO_2^- from the CLK by BK or AA was not affected (n = 8, not shown).

Discussion

By using recombinant enzymes and intact cell systems, we have recently demonstrated that NO activates both COX-1 and COX-2 (17). Our in vitro observation has now been extended to intact tissue (e.g., perfused kidney). In this study we observed that in a model of renal inflammation, there is a clear NO-driven activation of COX resulting in an enhancement of PG release.

Table I. Effects of Exogenous L-Arg at 6 h of Perfusion on Nitrite and PGE_2 Release in the Perfused HNK

Agents used	NO_2^-	PGE_2
	pmol/ml per min	
None	1980±100	99±33
BK	1870±55	3069±416
BK + L-Arg (0.1 mM)	4100±77*	5390±110*
BK + L-Arg (1 mM)	6600±65‡	8646±162‡
BK + D-Arg (1 mM)	1650±50	2607±264

Perfusion of the HNK with L-Arg (0.1–1 mM) at 6 h of perfusion increased both NO_2^- and PGE_2 release. D-Arg had no effect. Each point is the mean±SEM for n = 6 experiments. $^*P < 0.05$ and $^‡P < 0.01$ when compared to the value obtained in the absence of L-Arg.

Inhibition of endogenous NO production attenuates PG release. Ex vivo perfusion of the HNK, but not the CLK led to a perfusion time-dependent release of NO_2^- (Fig. 1, *A* and *B*), a breakdown product of NO. L-NMMA which blocks the activity of both cNOS and iNOS (19) or AG, a recently described selective iNOS inhibitor (26–28), abolished the release of NO_2^-. The renal effects of L-NMMA or AG were reversed in a stereospecific manner by co-infusion with L-arginine, the precursor for NO synthesis, but not by D-arginine (Fig. 4). In addition, dexamethasone or cycloheximide abolished the release of NO_2^- in the HNK (Fig. 7 *A*), further supporting the evidence for the presence of an inducible NOS isoform in the HNK.

Ex vivo perfusion of the HNK, but not CLK induces COX since perfusion with dexamethasone or cycloheximide blocked the exaggerated production of PGE_2. When endogenous NO production in the HNK was inhibited with either L-NMMA or AG, the increased production of PGE_2 observed by the hourly injection of BK was markedly reduced (Fig. 1 *B*). In the CLK, injection of BK or AA causes PGE_2 release via activation of COX-1 (e.g., non-cycloheximide, non-dexamethasone sensitive). The NO production of the CLK appears to be via cNOS, since only L-NMMA (not AG) abolished NO_2^- production (Fig. 2 *A*). L-NMMA by blocking NO_2^- also attenuated BK or AA-elicited PGE_2 production (Fig. 2 *B*). Electron spin resonance studies demonstrated that NO forms a complex with the heme center of COX (32) and we have recently reported that NO can directly activate COX (17). These studies suggests that a most likely mechanism by which NO augments PGs release

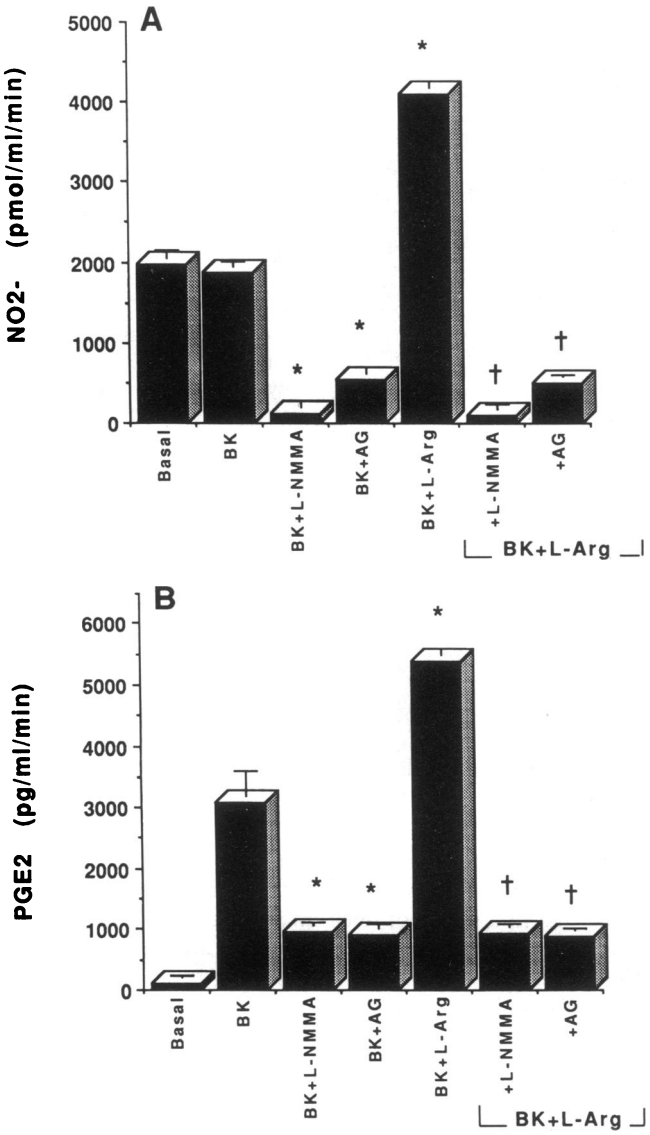

Figure 4. The effects of inhibitors of NO synthase on L-Arg-promoted potentiation of nitrite and PGE_2 production in the perfused hydronephrotic kidney. The release of nitrite (*A*) and PGE_2 (*B*) at 6 h of perfusion was potentiated by L-Arg (100 μM). This potentiating effect of L-Arg was abrogated by co-infusion with L-NMMA or AG (100 μM) (*A* and *B*). Each value is the mean±SEM of $n = 6$ experiments. $^*P < 0.05$ when compared to release obtained by BK alone and $^+P < 0.05$ when compared to the value obtained in the presence of L-Arg.

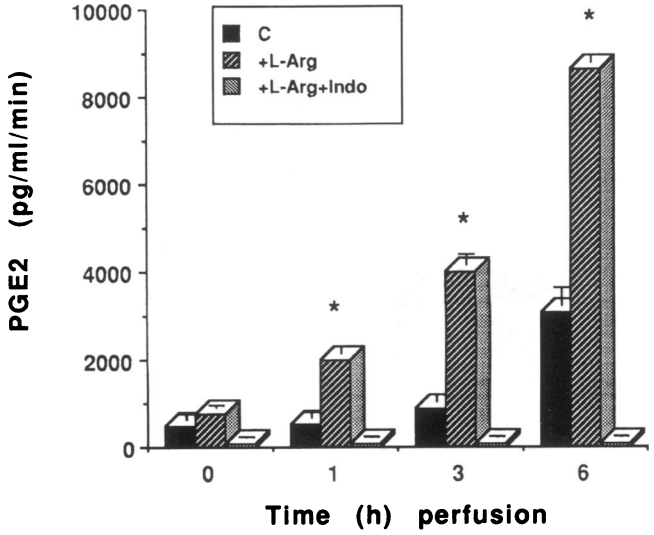

Figure 5. The effects of COX inhibition on L-Arg-promoted potentiation of PGE_2 production by BK in the perfused HNK. PGE_2 release by BK at 0, 1, 3, and 6 h of perfusion is markedly potentiated by an infusion of L-Arg (1 mM). Indomethacin blocked BK-induced PGE_2 release and abolished the potentiating effects of L-Arg. Each value is the mean±SEM for $n = 6$ experiments. $^*P < 0.01$ when compared to the value obtained with BK in the absence of L-Arg (ANOVA).

from the kidneys is through activation of the COX enzyme(s). Nevertheless other possibilities exist. For instance, NO interacts with oxygen-derived free radicals and as such NO can be considered a free radical scavenger (33). Free radicals are known to modulate the COX pathways (34). Whether this or other properties of NO account for part of the mechanism by which NO increases prostaglandin production in whole organs or in vivo is a possibility which remains to be explored in future studies. The effects of L-NMMA or AG on PGE_2 release are not due to a direct effect on COX activity (17).

Renal localization of NOS and COX. In the perfused normal rat kidney, release of NO by BK or AA likely requires a functional endothelium, since its removal by the detergent CHAPS abolishes its release (29). Likewise, on the one hand, we have found that CHAPS prevented the release of NO_2^- and PGE_2 by BK and AA (Fig. 2, *A* and *B*), suggesting an endothelium-dependent mechanism for the formation of these mediators. On the other hand, in the HNK, CHAPS failed to block the time-dependent release of NO_2^- and the exaggerated BK-induced PGE_2 release (Fig. 2, *C* and *D*), thereby suggesting a nonendothelial localization for induced NOS and COX. Cell culture, ex vivo and in vivo experiments have provided substantial evidence which suggested that fibroblasts expressed

Table II. Effects of SNP or GTN on BK-induced PGE_2 Release in the Perfused CLK and HNK

Additions	CLK	HNK
	pg/ml per min	
None	121±2	99±33
BK	242±33	3069±946
BK + SNP (0.125 mM)	223±20	4329±40
BK + SNP (0.25 mM)	380±25*	6000±120‡
BK + SNP (0.5 mM)	455±40‡	8200±50‡
BK + SNP (1 mM)	485±35‡	8138±120‡
BK + GTN (0.125 mM)	300±15	5000±75*
BK + GTN (0.25 mM)	320±20*	6300±100‡
BK + GTN (0.5 mM)	400±15‡	8000±100‡
BK + GTN (1 mM)	600±20‡	8005±75‡

Perfusion (30 min) of the CLK or HNK with SNP or GTN (0.125–1 mM) increased BK-induced production of PGE_2. Each value represents the mean±SEM for $n = 6$ experiments. $^*P < 0.05$ and $^‡P < 0.01$ when compared to the value obtained in the absence of SNP or GTN.

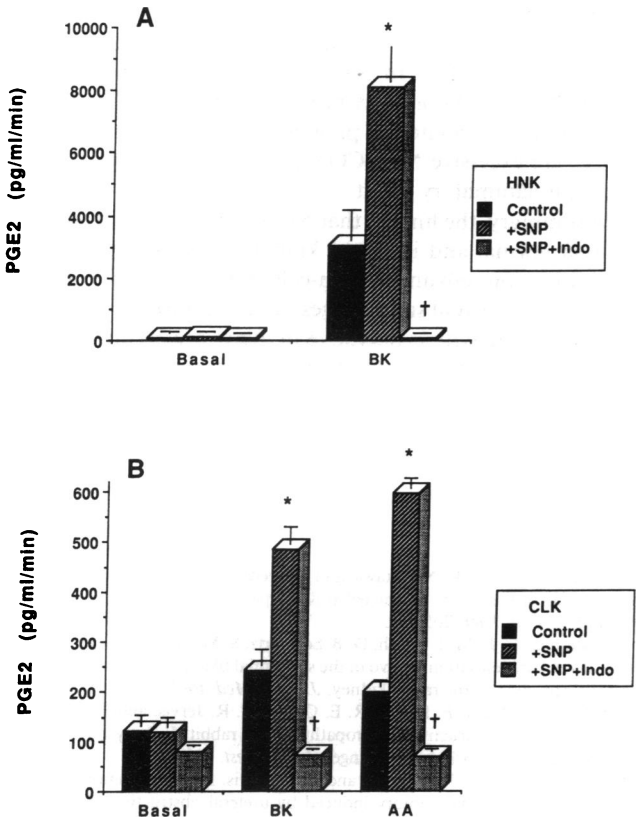

Figure 6. The effects of sodium nitroprusside on BK-induced PGE_2 release in the perfused CLK and HNK. (*A*) Perfusion of the CLK with SNP (1 mM) for 30 min increased BK or AA-induced PGE_2 release. (*B*) In the HNK the exaggerated BK-induced production of PGE_2 was increased further by SNP. Indomethacin (1 μM) prevented the effects of SNP. Vertical bars represent the mean±SEM for $n = 6$ experiments. $^*P < 0.01$ when compared to the value obtained in the absence of SNP and $^+P < 0.001$ when compared to the value obtained in the presence of SNP.

COX-2 upon stimulation by factors released from activated macrophages and that the former were in turn responsible for increased PGE_2 elicited by BK stimulation in the HNK (35, 36). Macrophages are also good candidates for the observed release of NO that takes place in the HNK. Indeed, iNOS is known to be expressed in macrophages upon appropriate immunostimulation or perfusion (19, 37) and in inflamed tissues characterized by macrophage infiltration (e.g., in glomeruli taken from glomerulonephritic rats; 38–40).

Endogenous effects of NO are mimicked by exogenous NO. L-Arg further potentiated the time-dependent release of NO_2- in the HNK and accentuated the exaggerated production of PGE_2 by BK (Table I). Activation of COX by NO was also substantiated by demonstrating that SNP or GTN, nitrovasodilators which release NO (41–45), increased PGE_2 release in the CLK and in the HNK in response to both BK or AA (Table II; Fig. 6). The finding that L-Arg increased NO_2- and PGE_2 release in the HNK, but not CLK suggests that in the inflamed HNK there has been an alteration in the synthesis or an increased utilization of L-Arg resulting in a decreased level of this substrate necessary for NO synthesis.

Model of interaction of NOS and COX in inflammation. In light of the observations in this paper, the model for the changes occurring in the HNK that were proposed earlier (5, 11) can be updated as shown in Fig. 8. Unilateral ureter obstruction causes a mechanical disruption and/or an immunologic stimulus in the cortex that triggers a regional inflammatory response resulting in accumulation of macrophages, fibroblast proliferation, and markedly increased AA metabolism. We propose that macrophages are activated by ex vivo kidney perfusion to release factor(s) that stimulate fibroblast proliferation and that induces COX-2 presumably in the fibroblast. At the same time, induction of iNOS takes place in the macrophage, NO is released and COX-2 activated. Release of endogenous renal AA after stimulation with BK leads to an exaggerated release of PGE_2. Removal of endogenous NO (e.g., by the use of NOS inhibitors) prevents the NO-driven activation of

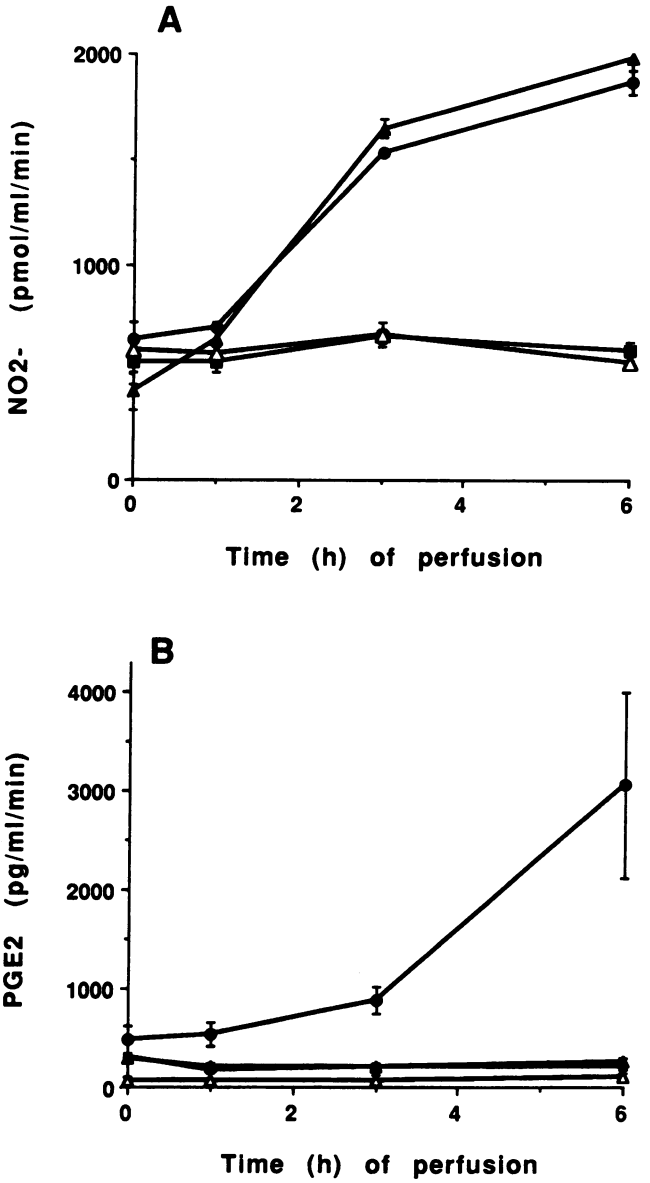

Figure 7. The effects of dexamethasone or cycloheximide on nitrite and PGE_2 production in the perfused HNK. (▲) Basal; (●) BK. Perfusion of the HNK with dexamethasone (10 μM, ■) or cycloheximide (5 μg/ml, △) blocked both (*A*) NO_2- and (*B*) PGE_2 release. Each point is the mean±SEM for $n = 8$ experiments.

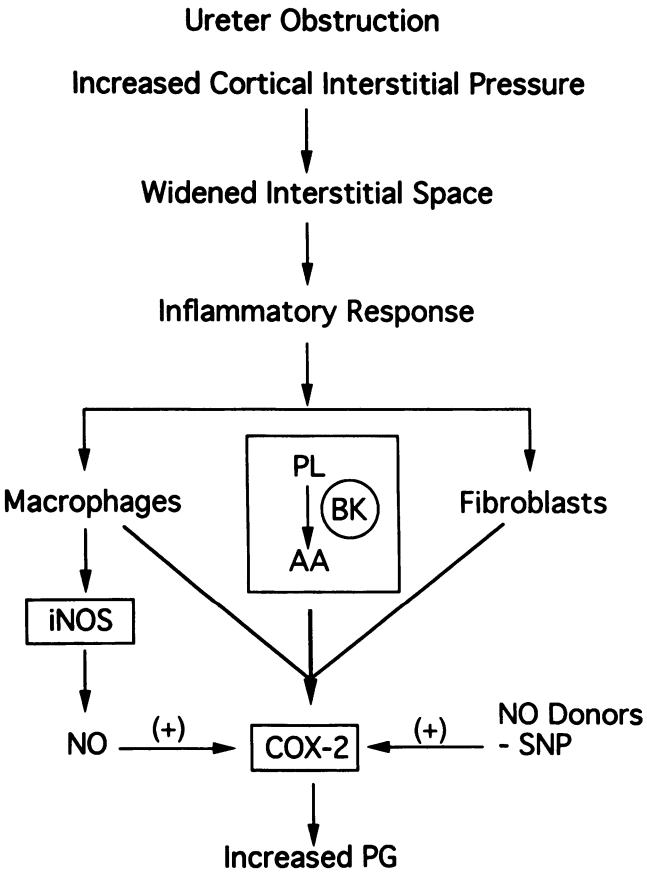

Figure 8. Proposed model of interactions between the NOS and COX pathways in hydronephrosis.

COX-2 and hence blunts PG production. Conversely, exogenously administered NO (e.g., SNP or GTN) directly stimulate COX-2 resulting in an amplification of the endogenous NO-driven COX-2 activation and thus PG production. Induction of iNOS and COX-2 may initially function as a homeostatic mechanism to return glomerular function toward normal by counteracting severe renal vasoconstriction and decreased glomerular rate elicited by uncontrolled increased circulating/locally vasoconstrictor agents produced in hydronephrosis. PGE_2 is known to be natriuretic and diuretic. In the HNK, renal PG production is markedly enhanced by the direct activation of COX by NO. This enhanced renal PGE_2 may be in part responsible for the increased excretion and reduced capacity to concentrate urine, a phenomena associated with postobstructive diuresis/obstructive uropathy. When released in large amounts, NO has proliferative and cytotoxic effects (19, 37) and could contribute to fibrosis and medullary damage observed in hydronephrosis. Dexamethasone, by blocking the induction of both iNOS and COX-2, will abrogate excessive NO and PGs release while leaving unaltered that from cNOS and COX-1. Similar effects can also be obtained with selective iNOS inhibitors such as AG, which by inhibiting NO release abrogate PGE_2 production. Such therapeutic strategies could possibly in turn restore renal function by restoring optimal NO-driven prostaglandin production. Thus, blockade of the constitutively expressed NOS and COX (e.g., pharmacological inhibitors, pathological circumstances such as renal ischemia) results in decreased renal perfusion pressure, renal blood flow, glomerular filtration rate, sodium excretion, edema, increased responsiveness to vasoconstricting agents, and ultimately acute renal failure (46, 47). In this respect, the combined use of iNOS-COX-2 inhibitors may prove to be a powerful tool that can be used to regulate the progression of those types of diseases where excessive NOS-COX products are known to support the inflammatory insult.

In summary, the finding that NO activates COX in isolated perfused normal and inflamed kidneys extends our previous observations on cells and broken-cell enzymatic reactions. Furthermore, the current study suggests that the nitric oxide regulation of COX activity is operative in intact organs and is a key player in the production of prostaglandins under normal and tissue inflammation conditions. The broader implication of the finding that NO regulates the production of prostaglandins is that the COX isozymes are potential NO receptors.

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