Autocrine Inhibition of Na^+/K^+-ATPase by Nitric Oxide in Mouse Proximal Tubule Epithelial Cells

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Abstract

An inducible nitric oxide synthase has recently been described in proximal tubule epithelium. To investigate the effects of proximal tubule NO on Na^+/K^+-ATPase, we induced NO production in mouse proximal tubule epithelial cells by treatment with lipopolysaccharide (LPS) and interferon-γ (IFNγ) followed by determinations of ouabain-sensitive ATPase activity. Na^+/K^+-ATPase activity decreased after 4 h of LPS/IFNγ treatment, reaching maximal inhibition after 24 h (34% reduction in activity). The inhibition of Na^+/K^+-ATPase activity by LPS/IFNγ was prevented by simultaneous incubation with N^ω-nitro L-arginine and markedly blunted by removal of L-arginine from the medium. The NO donors sodium nitroprusside and SIN-1 also inhibited Na^+/K^+-ATPase activity to a similar extent than LPS/IFNγ. However, treatment with 8-pCPT-cGMP only modestly reduced Na^+/K^+-ATPase activity. Interestingly, superoxide dismutase prevented the inhibitory effects of NO on Na^+/K^+-ATPase activity, suggesting a role for peroxynitrite in this inhibition. We conclude that NO generated by mouse proximal tubule epithelial cell iNOS inhibits Na/K ATPase activity in an autocrine fashion and that this inhibition is accompanied by a reduction in Na-dependent solute transport. (*J. Clin. Invest.* 1995. 95:2083–2088.) Key words: epithelial transport • sodium nitroprusside • cyclic guanosine monophosphate • L-arginine • peroxynitrite

Introduction

Nitric oxide (NO)[1] is an important modulator of vascular tone, neurotransmission and immune function (1). NO is synthesized from L-arginine by the enzyme NO synthase (NOS) in a complex reaction requiring NADPH, tetrahydrobiopterin and flavin nucleotides as cofactors (2). The cellular actions of NO are diverse and include activation of guanylate cyclase, inhibition of mitochondrial enzymes, modification of receptor and enzyme activity via protein nitrosylation, and cytotoxicity due to formation of reactive radicals (1, 3).

In the kidney, proximal tubular epithelium has recently been found to possess an inducible isoform of NOS (iNOS) and a soluble guanylate cyclase (4–6). Whereas NO generated in the renal vasculature and macula densa plays an important role in the regulation of renal hemodynamics and tubuloglomerular feedback (7, 8), the implications of NO generation by proximal tubular epithelium are currently unknown. However, the co-localization of NOS and guanylate cyclase suggests that NO may have autocrine effects in this nephron segment.

Nitric oxide has been shown to inhibit Na^+ transport by cortical collecting duct cells in vitro and to enhance natriuresis in vivo (9–11). The renal proximal tubule reabsorbs ~ 65% of the filtered luminal sodium load through a process driven by the activity of basolateral Na^+/K^+-ATPase (12). This pump actively transports Na^+ from the intracellular compartment into the circulation and creates a Na^+ gradient which facilitates Na^+-dependent luminal solute uptake as well as Na^+/H^+ exchange (12). The purpose of this study was to determine whether NO produced during induction of iNOS in proximal tubule epithelium affects Na^+/K^+ ATPase pump activity. We demonstrate that NO, either endogenously formed by induction of iNOS with immunostimulants or released from exogenous NO donors causes inhibition of proximal tubule Na^+/K^+-ATPase. The mechanisms of this inhibition appear, in part, to be mediated by the formation of reactive peroxynitrite radicals as well as by activation of guanylate cyclase and cyclic guanosine monophosphate (cGMP) formation.

Methods

Chemicals. Dulbecco's modified Eagle's medium (DME) and all cell culture materials were purchased from Fisher Scientific (Orlando, FL). Interferon-γ (IFNγ, rat recombinant) was purchased from GIBCO BRL (Gaithersburg, MD). Lipopolysaccharide (LPS, *E coli* serotype 026:B6), superoxide dismutase, ouabain, sodium nitroprusside, sulfanilamide, sodium nitrite, *N*-(1-naphthyl)ethylenediamine hydrochloride and N^ω-nitro-L-arginine (L-NNA) were purchased from Sigma Chemical Co. (St. Louis, MO). 8-(4-Chlorophenylthio)-guanosine-3′ 5′-cyclic monophosphate (8-pCPT-cGMP) was obtained from Biolog (La Jolla, CA). SIN-1 and SIN-1C were a kind gift of Dr. Rainer Henning, Casella AG (Frankfurt, Germany). [^{3}H]L-Arginine and [^{3}H]*myo*-inositol were purchased from Amersham (Arlington Heights, IL) and AG50WX-8 Dowex (100–200 mesh) was obtained from Bio-Rad (Melville, NY).

Cell cultures. Murine proximal convoluted tubule (MCT) cells were grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum, 25 mM Hepes buffer, 10 μM non-essential amino acids and 100 μM sodium pyruvate. L-Arginine concentration in this

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1. *Abbreviations used in this paper:* ATPase, adenosine triphosphatase; NO, nitric oxide; NOS, nitric oxide synthase; L-NNA, N^ω-nitro-L-arginine; IFNγ: interferon-gamma; cGMP: guanosine-3′ 5′-cyclic monophosphate; 8-pCPT-cGMP: 8-(4-Chlorophenylthio)-guanosine-3′ 5′-cyclic monophosphate; 8-Br-cGMP: 8-Bromo-guanosine-3′ 5′-cyclic monophosphate; MCT cells: murine proximal convoluted tubule cells; SIN-1: 3-morpholinosyndnomine; SNP: sodium nitroprusside.

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medium is 84 mg/liter. The cultures were maintained on plastic cell culture plates at 37°C in a 5% CO_2/95% O_2 environment. The MCT cell lines were kindly provided by Dr. Eric Neilson (University of Pennsylvania). These cells were established from microdissected mouse proximal tubules after stabilization by transfection with SV40 virus and maintain many of the properties of differentiated proximal tubule epithelial cells (13).

Nitrite assay. Nitrites were measured by the addition of 200 μl of freshly prepared Greiss reagent (0.75% sulfanilamide in 0.5N HCl and 0.075% *N*-(1-naphthyl)ethylenediamine dihydrochloride in double distilled water) to 200 μl of the culture medium from treated and control cells, followed by spectrophotometric absorbance readings at 543 nm (Beckman DU 650) (14). Nitrite concentrations were determined by comparisons with a standard sodium nitrite curve with double distilled water as blank and expressed as nanomoles per 1,000 cells.

Determination of Na/K ATPase activity. Na/K ATPase activity was determined by measuring the difference between total cellular ATPase activity and Mg-ATPase (ouabain inhibitable) activity as previously described (15, 16). This method is based on a fluorometric assay that follows the oxidation of NADH coupled to ATP hydrolysis. Control and experimentally treated MCT cells were washed thoroughly with PBS and permeabilized by freeze-thawing followed by measurements of enzyme activity in the supernatant. The reaction was carried out in the presence or absence of ouabain (10 mM), using an incubation mixture having the following composition: NaCl 100 mM; NH_4Cl 66.7 mM; imidazole 50.3 mM; $MgCl_2$ 3.7 mM; EDTA 0.08 mM; Na_2ATP 1.1 mM; phosphoenolpyruvate 0.2 mM; NADH 0.017 mM; pyruvate kinase 2.3 U/ml; and lactate dehydrogenase 3.3 U/ml. After incubating the cells in the reaction mixture for 30 min, fluorescence was measured and Na/K ATPase activity determined using the corresponding formula as described by Garg (16). Results are expressed in picomoles of ATP hydrolyzed per min per 1,000 cells. Cell viability assessed by trypan blue exclusion was invariably > 95% under all experimental conditions studied.

Determination of NOS activity. NOS activity in intact MCT cells was determined by measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline after separation of these amino acids by anion exchange chromatography (AG50WX-8 DOWEX, Na^+ form) as previously described (17). [^{3}H]L-citrulline was quantified by liquid scintillation spectroscopy and NOS activity expressed as [^{3}H]L-citrulline DPMs/mg of protein. Proteins were determined by the method of Lowry (18).

Determination of myo-inositol uptake. Initial rates of *myo*-inositol (Ins) uptake were measured over 10 min at 37°C using [^{3}H]Ins (10–20 Ci/mmol) as previously described (19). Na^+-dependent specific Ins uptake was calculated as the difference between uptake in the presence and absence of Na^+ (replaced with 140 mM choline chloride) and constituted between 92 and 98% of total uptake.

Statistical analysis. Results are presented as mean±standard error of the indicated number of experiments performed in triplicate samples. All experiments in a series were performed in paired fashion with the N in the figure legend representing the number of paired experiments each of which contained all control and experimental protocols. Statistical analysis was performed using Student's *t* test for paired and unpaired data, or analysis of variance (ANOVA) and subsequent Scheffe's F-test (StatView® II, Abacus Concepts, Inc. Berkeley, CA) for multiple group comparisons as appropriate.

Results

Treatment of MCT cells with a combination of bacterial lipopolysaccharide (LPS, 0.1 μg/ml) and IFNγ (100 U/ml) for 24 h resulted in marked iNOS induction as indicated by the accumulation of nitrites in the medium (1.39±0.12 vs. 26.9±2.8 nmoles of nitrite/1,000 cells/24 h for control versus treated cells, respectively, $P < 0.005$, N = 9). Therefore, this combination of LPS and IFNγ was used throughout the study to induce iNOS activity. To determine whether endogenous generation of NO had any effects on MCT Na^+/K^+-ATPase, we measured

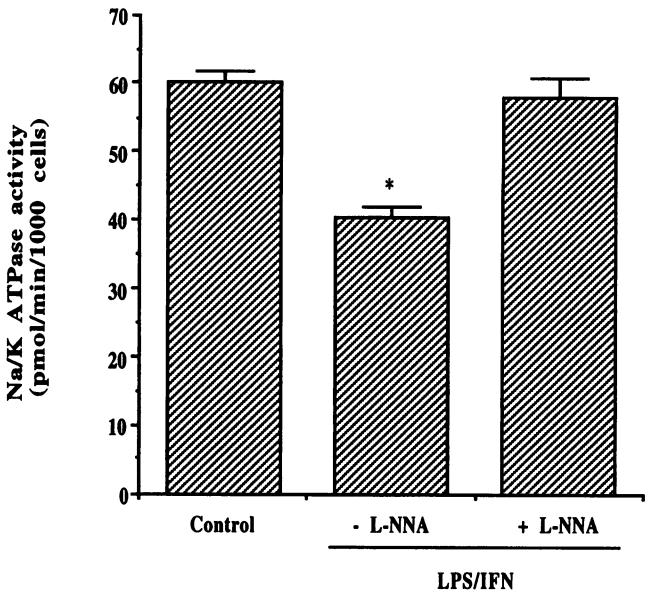

Figure 1. Na^+/K^+-ATPase activity in MCT cells after 24 h of treatment with LPS (0.1 μg/ml) and IFNγ (100 U/ml) in the absence (− L-NNA) or presence (+ L-NNA) of the NOS inhibitor N^{ω}-L-nitro arginine (300 μM). L-NNA prevents the inhibitory effect of LPS/IFNγ treatment (*$P < 0.01$ vs. control, N = 9).

the activity of this enzyme after 24 h of treatment with LPS and IFNγ. As illustrated in Fig. 1, induction of iNOS was associated with a 34% reduction in Na^+/K^+-ATPase activity. This effect was completely prevented by the competitive inhibitor of iNOS, N^{ω}-L-nitro arginine (L-NNA, 300 μM) (Fig. 1) which, as expected, also prevented the synthesis of nitric oxide during iNOS induction (nitrite levels were 1.21±0.10 nmoles/1,000 cells/24 h in the presence of L-NNA, not statistically different from control, N = 9). Incubation with L-NNA alone did not affect Na^+/K^+-ATPase activity (data not shown). When MCT cells were treated with LPS and IFNγ for 24 h in medium devoid of L-arginine, the inhibition of Na^+/K^+-ATPase activity was markedly blunted (Fig. 2). Nitric oxide synthesis after LPS and IFNγ treatment was also minimal in the absence of L-arginine (nitrite concentrations were 1.89±0.18 nmoles/1,000 cells/24 h, $P < 0.05$ as compared with control, N = 9). However, NO synthesis was not completely suppressed in medium devoid of L-arginine possibly because of the reported ability of proximal tubule epithelium to synthesize this amino acid (20). Thus, these results indicate that endogenous generation of NO by proximal tubule epithelium inhibits Na^+/K^+-ATPase activity in an autocrine fashion.

To investigate the time course of this inhibition, we treated MCT cells with LPS and IFNγ for various periods of time followed by determination of Na^+/K^+-ATPase activity. As shown in Fig. 3, inhibition of Na^+/K^+-ATPase activity could be observed as early as 4 h after treatment with LPS and IFNγ and was maximal at 24 h. Periods of treatment with LPS and IFNγ longer than 24 h did not cause further inhibition of Na^+/K^+-ATPase (data not shown). The time course of inhibition of Na^+/K^+-ATPase was closely correlated with that of iNOS induction as measured by [^{3}H]L-citrulline conversion (Fig. 3). This assay is very sensitive for measuring NOS activity (17, 21, 22). Thus, these studies demonstrate a close correlation between the time course of iNOS induction and the inhibition of Na^+/K^+-ATPase activity.

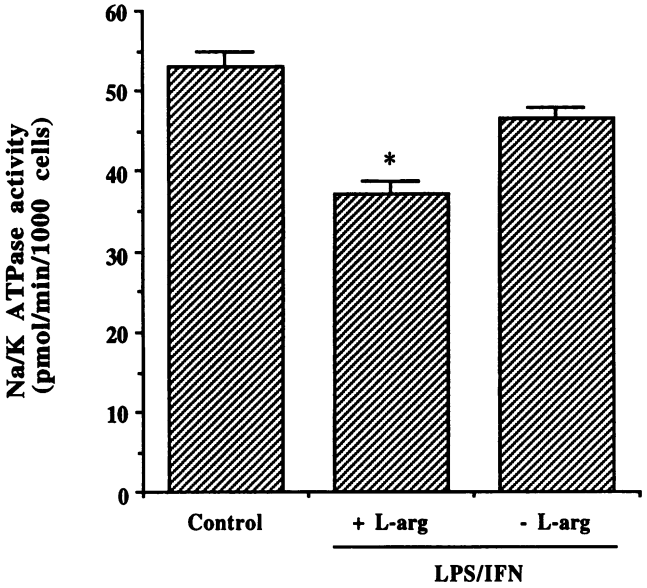

Figure 2. Effects of L-arginine on the inhibition of Na^+/K^+-ATPase activity caused by iNOS induction. MCT cells were treated with LPS and IFNγ for 24 h in the presence (+ L-arg) or absence (− L-arg) of L-arginine followed by determinations of Na^+/K^+-ATPase activity. The inhibition of Na^+/K^+-ATPase by LPS and IFNγ treatment is blunted in the absence of L-arginine (* $P < 0.05$ versus control and versus LPS/IFNγ in the presence of L-arginine). Na^+/K^+-ATPase activity in the absence of L-arginine was not different from control ($P = 0.09$, N = 6).

We next studied the effects of exogenous NO donors on MCT Na^+/K^+-ATPase. Treatment with SIN-1 (30 μM) or sodium nitroprusside (SNP, 400 μM) for 2 h inhibited Na^+/K^+-ATPase activity to a similar degree than endogenous NO (Fig. 4). The inhibition by SIN-1 and SNP was observed as early as 30 min and reached maximum at 2 h (data not shown). Conversely, the inactive metabolite of SIN-1, SIN-1C, failed to

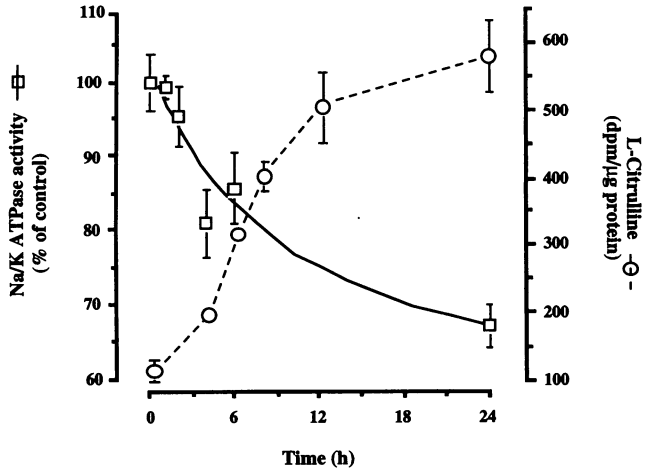

Figure 3. Correlation between the time course of induction of iNOS activity (○) and the inhibition of Na^+/K^+-ATPase (□) during treatment with LPS and IFNγ. iNOS activity was determined by measuring the conversion of L-arginine to L-citrulline in intact MCT cells as described in Methods. Both Na^+/K^+-ATPase and iNOS activity became statistically different from controls ($P < 0.05$) after 4 h of LPS and IFNγ treatment. Where standard error bars are not indicated these fall within symbols (N = 9).

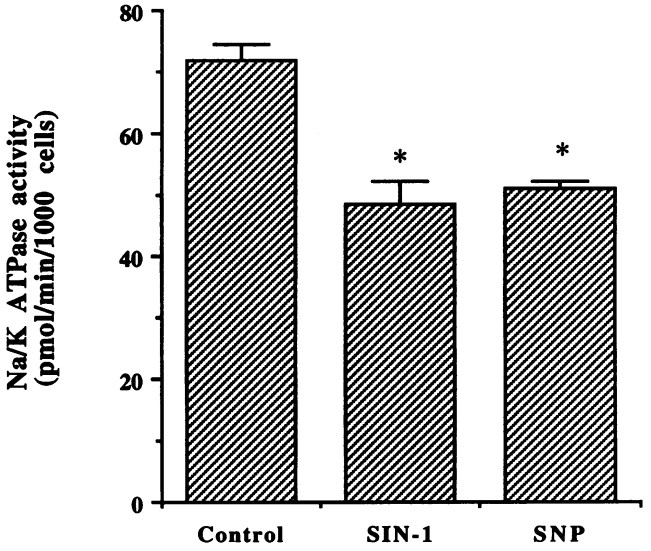

Figure 4. Na^+/K^+-ATPase activity in MCT cells after 2 h of treatment with the NO donors SIN-1 (30 μM) and sodium nitroprusside (SNP, 400 μM) (* $P < 0.01$ vs. control, N = 9).

inhibit Na^+/K^+-ATPase activity (data not shown). Thus, these results indicate that NO generated from exogenous sources also inhibits Na^+/K^+-ATPase and therefore confirm our previous observations suggesting an autocrine inhibitory effect of NO generated endogenously by MCT iNOS.

To investigate the possible mechanisms of the inhibition of Na^+/K^+-ATPase by NO, we used the potent and stable membrane permeant analogue of cGMP, 8-pCPT-cGMP (0.5 mM) (23). As illustrated in Fig. 5, treatment with 8-pCPT-cGMP resulted in a modest but significant decrease in Na^+/K^+-ATPase activity. The magnitude of Na^+/K^+-ATPase inhibition achieved with 8-pCPT-cGMP was < 50% of that observed after induction of iNOS or treatment with NO donors. This suggests that although activation of guanylate cyclase and cGMP forma-

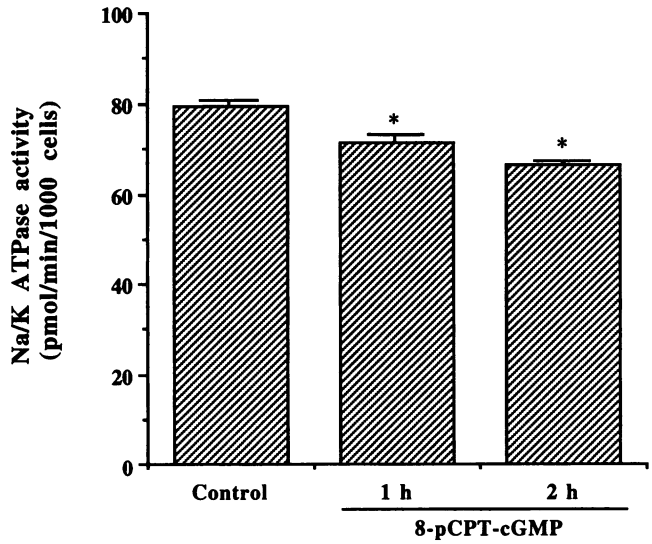

Figure 5. Effects of the stable cGMP analogue 8-pCPT-cGMP (500 μM) on Na^+/K^+-ATPase activity in MCT cells (* $P < 0.05$ vs. control, N = 9). Cells were treated with this analogue for 1 and 2 h before measurements of Na^+/K^+-ATPase activity were made.

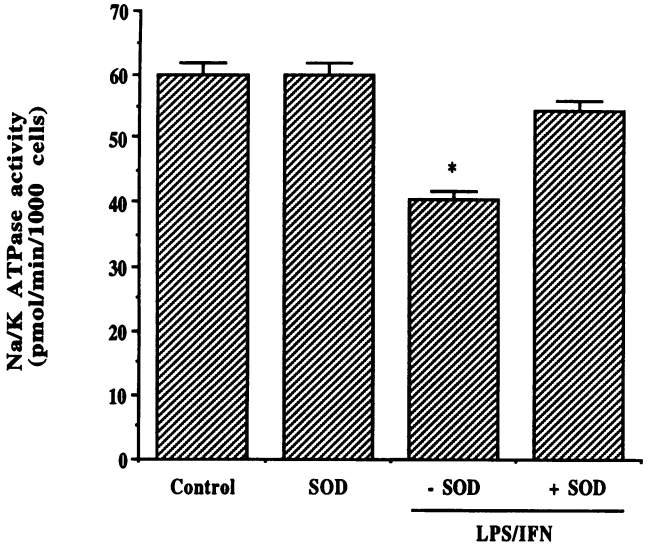

Figure 6. Prevention of the inhibitory effect of NO on Na^+/K^+-ATPase activity by superoxide dismutase (SOD, 100 U/ml). MCT iNOS was induced by treatment with LPS and IFNγ for 24 h in the absence (− SOD) or presence (+ SOD) of SOD (* $P < 0.01$ vs. control, N = 9).

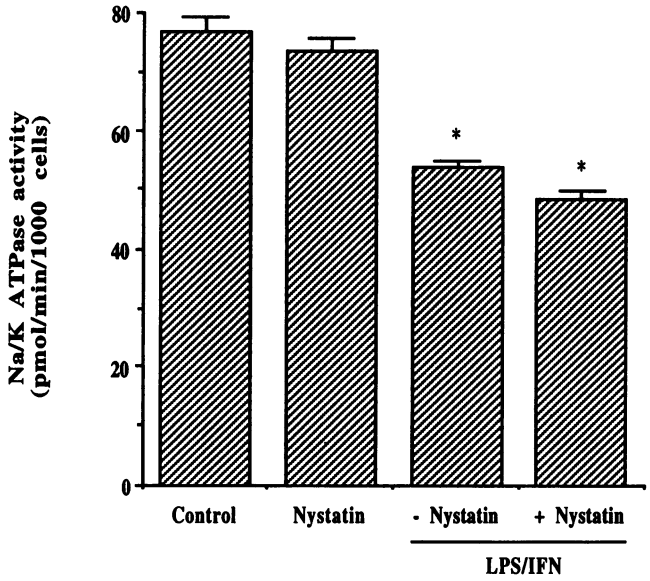

Figure 7. Lack of effect of nystatin on the inhibition of Na^+/K^+-ATPase activity caused by MCT iNOS induction. MCT cells were treated with a combination of LPS and IFNγ for 24 h and nystatin was added during the last 30 min of this induction (* $P < 0.01$ vs. control, N = 9).

tion may in part mediate the inhibitory effects of NO, other important mechanisms are also involved.

Since NO can combine with endogenous superoxide to form reactive peroxynitrite which may alter the function of membrane proteins (24, 25), we investigated the effects of the superoxide radical scavenger superoxide dismutase (SOD) on the inhibition of Na^+/K^+-ATPase by endogenous NO. Addition of SOD (100 U/ml) to the tissue culture medium markedly blunted the inhibition of Na^+/K^+-ATPase caused by treatment with LPS and IFNγ (Fig. 6). Thus, these results suggest that the formation of reactive peroxynitrite radicals or its decomposition products plays a major role in mediating the inhibitory effects of NO on Na^+/K^+-ATPase.

To determine whether NO could be inhibiting Na^+/K^+-ATPase activity indirectly through a reduction in intracellular Na^+ levels due to Na^+/H^+ antiporter or Na^+ channel blockade as opposed to through direct inhibition of the pump, we investigated the effects of nystatin (26) on Na^+/K^+-ATPase activity following iNOS induction. MCT cells were treated with LPS and IFNγ for 24 h in the presence or absence of nystatin (100 U/ml) added during the last 30 min of induction followed by measurements of Na^+/K^+-ATPase activity. As illustrated in Fig. 7, the inhibition of Na^+/K^+-ATPase activity caused by iNOS induction was unaffected by nystatin. Similarly, nystatin failed to prevent the inhibition of Na^+/K^+-ATPase activity caused by SNP (data not shown). Thus, these findings support the conclusion that NO inhibits Na^+/K^+-ATPase through a direct effect on this pump and not indirectly through a reduction in intracellular Na^+.

Last, to determine whether the observed inhibition of Na^+/K^+-ATPase activity caused by NO was associated with a reduction in Na^+-dependent solute transport, we measured *myo*-inositol uptake into MCT cells after iNOS induction or treatment with SNP. Similarly to what has been previously described in other renal and non-renal cells (19, 27–29), *myo*-inositol uptake by MCT cells was found to be largely Na^+-dependent (92 to 98% of total uptake) and linear over at least 30 min (data not shown). Therefore we chose to determine initial rates of uptake over a 10 min period. As shown in Fig. 8, treatment with LPS and IFNγ for 24 h or SNP for 2 h both resulted in a significant reduction in Na^+-dependent *myo*-inositol uptake by MCT cells (34.4 and 38.8% reduction, respectively). Interestingly, the decrease in *myo*-inositol uptake was comparable in magnitude to the inhibition of Na^+/K^+-ATPase activity observed after similar treatments. Conversely, induction of MCT iNOS with LPS and IFNγ did not affect Na^+-independent [^{3}H]L-arginine uptake (1.24±0.05 vs. 1.19±0.06 pmol/mg protein/min for untreated versus LPS/IFNγ-treated cells, respec-

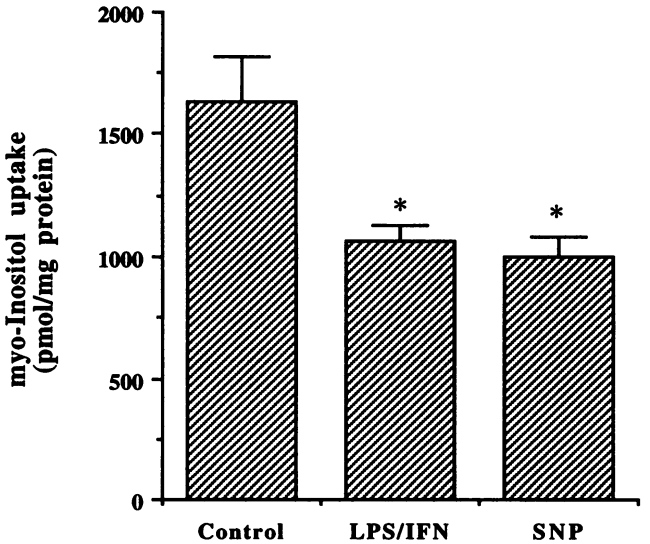

Figure 8. Inhibition of Na^+-dependent *myo*-inositol uptake in MCT cells treated with LPS and IFNγ for 24 h or sodium nitroprusside (SNP, 400 μM) for 2 h (* $P < 0.01$, N = 9). Na^+-dependent *myo*-inositol uptake was measured over 10 min (see Methods).

tively, N = 9, ns) or Na^+-independent insulin-insensitive 2-deoxy-D-[^{3}H]glucose uptake (140.8±17.7 versus 126.4±6.7 pmol/mg protein/min for untreated versus LPS/IFNγ-treated cells, respectively, N = 9, ns), suggesting that the inhibition of Na^+/K^+-ATPase by NO is not likely to be the result of nonspecific membrane effects of peroxynitrite.

Discussion

We report that NO produced after induction of proximal tubule epithelial cell iNOS by immunostimulants causes autocrine inhibition of Na^+/K^+-ATPase activity, an effect that is mimicked by exogenous NO donors and prevented by the competitive iNOS antagonist L-NNA or by removal of L-arginine from the medium. This inhibition is associated with a comparable reduction in Na^+-dependent *myo*-inositol uptake and appears to result from a direct effect of NO on the Na^+/K^+-ATPase. The mechanisms of this inhibition involve mainly the production of peroxynitrite but may also involve activation of guanylate cyclase and cGMP production.

Our present observations have several important implications. First, they constitute the first description of a direct effect of NO on renal epithelial Na^+/K^+-ATPase, which is the major physiological determinant of Na^+ handling by the kidney. In addition, the finding of an autocrine effect of NO in proximal tubule epithelium raises the interesting possibility that local L-arginine:NO systems may play important regulatory roles in discrete segments of the nephron.

Recently, inducible NOS and soluble guanylate cyclase were found in proximal tubule epithelium (4–6). The proximal tubule is a major site of L-arginine synthesis which may serve as substrate for local NO actions (20). However, the function of the proximal tubule L-arginine:NO pathway is unknown. In a microperfusion study, cGMP produced from the activation of particulate guanylate cyclase by atrial natriuretic peptide was shown to inhibit water transport in the proximal tubule (30). Thus, it is conceivable that production of cGMP resulting from activation of soluble guanylate cyclase by NO may also modulate transport processes. Indeed, NO reportedly inhibits Na^+ transport in cultured cortical collecting duct cells by mechanisms involving cGMP production (9, 31). These studies differ from ours, however, in that the authors were unable to find a direct inhibitory effect of NO on Na^+/K^+-ATPase and attributed its effects to modulation of apical Na^+ channel activity (9). It should be noted however that the source of NO used in these studies was pulmonary artery endothelium stimulated with bradykinin, and therefore the amount of NO generated is likely to be substantially lower (picomolar amounts synthesized by the endothelial cNOS) than that generated by iNOS (nanomolar amounts) and only transiently active (1). It is therefore possible that this may account for the lack of effects on Na^+/K^+-ATPase reported by these investigators. Although we did not measure NO levels directly, it is likely based on our measurements of accumulated nitrite that the amount of NO produced by proximal tubule iNOS is comparable with that reported for iNOS in other systems and therefore substantially higher than that produced by the endothelial constitutive isoform. In addition, the concentrations of the NO donors SIN-1 and SNP used in our study reportedly result in the release of nanomolar to μM amounts of NO (32). Thus, our finding of a direct autocrine inhibitory effect of NO on renal epithelial Na^+/K^+-ATPase constitutes an important novel observation and raises the possibility that similar local L-arginine:NO systems may exist in other discrete nephron segments to modulate ion transport.

In the present study we found that stable analogues of cGMP cause a modest but significant inhibition of Na^+/K^+-ATPase activity suggesting that cGMP may at least in part mediate the inhibitory effects of NO. However, the magnitude of this inhibition was 50% or less of that observed with endogenous and exogenous NO. This suggests that additional mechanisms are involved in the actions of NO on proximal tubule Na^+/K^+-ATPase. In fact, the inhibition of Na^+/K^+-ATPase activity observed during iNOS induction was prevented by superoxide dismutase suggesting that endogenously generated superoxide is also involved. This observation taken together with the fact that both L-NNA and L-arginine depletion also prevented Na^+/K^+-ATPase inhibition during iNOS induction strongly suggests that an interaction between NO and superoxide anion is required for the full inhibitory effect to occur. In fact, the products of this interaction, peroxynitrite radical and its decomposition products, have all been reported to cause lipid oxidation and alter membrane proteins (24, 33) and could well be responsible for disrupting the function of the membrane-bound Na^+/K^+-ATPase. Nitric oxide can also inhibit protein function by binding thiol groups and forming *S*-nitrosothiols (33). Thiol groups have been reported to be important for the activity and, possibly, subunit assembly of the Na^+/K^+-ATPase (34, 35). It is therefore possible that NO may disrupt Na^+/K^+-ATPase function by nitrosylating critical thiol groups.

The functional significance of our present observations remains unknown. Our studies are limited by the fact that they were performed in cultured cells and may not necessarily reflect the in vivo situation. However, there is evidence from animal studies that NO inhibits Na^+ transport in the renal tubule, although the exact site or mechanism is not clear (10, 11). In vivo, inhibition of NO synthesis by intrarenal infusion of nitro-L-arginine in dogs reportedly causes a marked reduction in urinary Na^+ excretion independently of hemodynamic changes (36). Recent studies also in the dog have reported a reduced natriuresis and diuresis in response to volume expansion during NO synthesis inhibition which was attributed to altered proximal tubular reabsorption (37). However, other studies have suggested an acute natriuretic effect of NO blockade (38). These discrepancies in the reported effects of NO blockade on natriuresis may only reflect differences in the experimental settings and do not permit definitive conclusions about the possible effects of NO on proximal tubular function. However, our present results raise the possibility that inhibition of proximal tubule Na^+/K^+-ATPase may, in part, explain the reported natriuretic effects of NO.

For NO to exert a tonic modulatory effect on tubular Na^+/K^+-ATPase and Na^+ handling, continuous production of this molecule by tubular cells themselves or other neighboring cells would be required. Although until recently the best recognized renal source of NO that could play a role in regulating tubular Na^+ handling was cNOS localized in macula densa and vascular endothelium (7, 8), recent evidence suggests that tubular epithelial and vascular smooth muscle iNOS can also be "constitutively" expressed in the kidney (39, 40). This iNOS would provide another potential source for continuous NO release and long term tonic modulation of NO sensitive processes in the kidney. Whether proximal tubular iNOS can be expressed constitutively in vivo and generate NO for tonic modulation of Na^+/K^+-ATPase under physiologic conditions remains to be established. However, marked induction of iNOS occurs in in-

flammatory kidney disorders such as allograft rejection and glomerulonephritis (3, 41–43). In addition, NO synthesized in proximal tubular epithelium appears to play an important role in acute tubular ischemia-reperfusion injury (25). It is therefore possible that the large amounts of NO that are likely to be produced locally by proximal tubule epithelium and infiltrating inflammatory cells during pathologic conditions may result in sustained inhibition of Na^+/K^+-ATPase activity and alterations in tubular Na^+ handling.

In conclusion, NO generated after induction of proximal tubule epithelial cell iNOS inhibits Na^+/K^+-ATPase activity in an autocrine manner. This inhibition is accompanied by a reduction in Na^+-dependent solute transport and may play an important role in mediating the natriuretic effects of NO.

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