Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12056–12060, December 1994
Pharmacology

Nitric oxide, cGMP, and hormone regulation of active sodium transport

(Na,K-ATPase/sodium pump/kidney/guanylyl cyclase)

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Communicated by Edwin J. Furshpan, July 29, 1994

ABSTRACT **The inter- and intracellular regulator nitric oxide (NO) has been suggested to play a role in the modulation of cellular excitability, but the mechanism(s) by which this occurs remain unclear. Using the kidney as a model system, we report here evidence that NO, produced in response to various hormones and cytokines, can effect long-term alterations in the activity of the membrane sodium pump. This regulation of Na,K-ATPase, which occurs in a system of NO-containing renal tubules, involves cGMP and cGMP-dependent protein kinase. Na,K-ATPase can also be regulated by alterations of cGMP initiated through NO-independent factors, such as atriopeptin, and in nonrenal tissues, such as cerebellum. Regulation of the membrane sodium pump by NO and cGMP, therefore, represents a mechanism for hormonal modulation of ion gradients, not only in kidney but also in other organs, including brain, where NO and cGMP play a prominent role in cellular function.**

Nitric oxide (NO) is a key paracrine and autocrine regulator in a number of tissues, including blood vessels, immune cells, and the nervous system (1). In this latter tissue, NO has been implicated in mechanisms of cell injury and in long-term physiological changes in cellular excitability. While considerable progress has been made in elucidating the regulation of NO synthesis and in identifying NO's immediate second messenger effectors [e.g., soluble guanylyl cyclase (GC)], much less is known about the downstream biochemical targets of NO.

In the kidney, acetylcholine (ACh), bradykinin (BK), and certain other endothelium-dependent factors promote salt and water loss through a mechanism involving formation of NO and production of cGMP (2, 3). Prior reports have suggested that these hormonal effects occur in renal blood vessels and result from an alteration of renal or glomerular hemodynamics. In the kidney-like choroid plexus and ciliary process, however, cGMP [formed from activation of atriopeptin (ANF) receptors] alters fluid secretion and stimulates protein phosphorylation through a direct effect on secretory epithelium (4–6). Because NO is a potent stimulus to cGMP production, these latter observations raise the possibility that NO (and hormones stimulating its production) might be capable of altering membrane ion movement through direct (i.e., nonvascular) effects on transporting epithelium. An intriguing target for such regulation in secretory (as well as excitable) cells would be the membrane sodium pump Na,K-ATPase, which has been shown (7, 8) to be regulated by dopamine and cAMP. The present study investigates this possible role for NO and cGMP.

METHODS

NO Synthase (NOS). NOS activity was measured (9) in high-speed supernatants from rat cerebellum, kidney medulla and cortex, porcine LLC-PK_1 epithelial cells, and purified cultured tubules incubated with 0.2 mM L-arginine/10 mM Hepes/0.425 mM EDTA/0.45 mM $CaCl_2$/80 units of calmodulin/1 μM tetrahydrobiopterin/4 μM FAD/4 μM FMN/0.5 mM dithiothreitol/0.16 M sucrose/±1 mM NADPH. For Ca^{2+}/calmodulin-free activity, EDTA was replaced by EGTA and $CaCl_2$ and calmodulin was omitted.

Diaphorase (NADPH-d) Histochemistry. Thick slices of rat or human kidney were fixed (3 hr at 4°C) in buffered 2% (vol/vol) paraformaldehyde, rinsed, cryoprotected with sucrose, frozen, and sectioned at 10 μM. The NADPH-d reaction (20–40 min at 37°C), as modified (10), contained 0.3% Triton X-100, 0.5 mM nitro blue tetrazolium, 10 mM sodium phosphate (pH 7.4), and ±1.25 mM NADPH. Reactivity was totally NADPH-dependent and inhibited by preincubation (30 min at 37°C) with diphenyliodonium (Fluka), a potent inhibitor of NOS (11).

Immunolocalization. Immunostaining was as described (4, 6, 12), except that some sections were first reacted for NADPH-d and then washed prior to antibody application. Controls utilizing nonimmune serum yielded low-to-moderate backgrounds distinct from staining patterns noted for specific antibodies.

NOS Isoforms. NOS isoforms were localized in human postmortem kidney and LLC-PK_1 cultures by using monoclonal antibodies directed against unique regions of NOS isotypes for bNOS (brain type), ecNOS (endothelial cell), and macNOS (macrophage) (Transduction Laboratories, Lexington, KY).

Regulation of NO and cGMP in Intact Tubules. Hormone-stimulated production of NO and cGMP was measured in intact tubules prepared and purified as described (unpublished data), preincubated (30 min) in medium 199 (GIBCO) with 1 mM isobutylmethylxanthine (for cGMP) or medium 199 with [^{3}H]arginine at 1 μCi/ml (for citrulline production) (1 Ci = 37 GBq). Drugs were added for 10 min, and incubation was terminated, respectively, by boiling or by ice-cold 0.2 mM arginine/0.425 mM EDTA and sonication. cGMP was measured by RIA, and citrulline was measured by column separation (9).

Na,K-ATPase. Slices (0.4 × 0.4 × 1 mm) of adult rat medulla were prepared on a tissue chopper, washed extensively to remove small particles, cooled to 4°C, and suspended (25–30 mg/ml) in 137 mM NaCl/5 mM KCl/0.8 mM $MgSO_4$/0.25 mM $CaCl_2$/1 mM $MgCl_2$/10 mM Hepes/≈2 mM NaOH to adjust pH to 7.4 at 34°C. Drug was added to tubes (five replicate tubes per drug) containing 1-ml aliquots of slice

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Abbreviations: ACh, acetylcholine; BK, bradykinin; NO, nitric oxide; NOS, NO synthase; OT, oxytocin; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; ANF, atriopeptin; GC, guanylyl cyclase; NADPH-d, diaphorase; bNOS, brain type NOS; ecNOS, endothelial cell NOS; Hb, hemoglobin; TAL, thick ascending limb; SNP, sodium nitroprusside; PPI, protein phosphatase inhibitor; I-1, inhibitor 1; SOD, superoxide dismutase.
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suspension, incubated (15 min at 34°C), and rapidly frozen on dry ice. Tubes were thawed and centrifuged, and the supernatant (containing drug) was removed. Supernatant was heated (5 min at 90°C), NaOAc was added to 75 mM, and the mixture was dried and stored for cAMP/cGMP assay by RIA. Tissue pellet was rapidly refrozen in 1 ml of ATPase reaction buffer (modified from ref. 8; 85 mM NaCl/20 mM KCl/4 mM $MgCl_2$/0.2 mM EGTA/30 mM histidine/≈20 mM NaOH, to adjust to pH 7.2 at 31°C). Tubes were thawed on water ice. Alamethacin was added at a concentration (10–20 μg/ml) known not to alter Na,K-ATPase (13), and slices were incubated (10 min at 34°C) and then centrifuged. Supernatant was removed, and fresh reaction buffer, containing a V_{max} concentration (10 mM) of ATP and 0.3–0.5 μCi of [γ-^{32}P]ATP (DuPont/NEN), was added. Slices were then incubated (1 hr at 31°C), during which time aliquots of buffer were periodically withdrawn for assay of hydrolyzed $^{32}P_i$, measured by scintillation counting after centrifuge separation of labeled ATP by addition of 5 vol of 5% (wt/vol) trichloroacetic acid containing 10% (wt/vol) activated charcoal and 1 mM NaH_2PO_4. For ouabain-sensitive Na,K-ATPase, $^{32}P_i$ release in complete buffer was compared, for each drug condition, with activity of identically treated slices incubated in buffer containing 3 mM ouabain, choline chloride substituted for NaCl and KCl, and Tris base for pH adjustment. This latter ouabain-insensitive ATPase (Mg-ATPase) activity was subtracted from that above, and activity was normalized for protein content. The permeabilized slice procedure yielded ouabain-sensitive Na,K-ATPase activity that was linear (60 min) and similar in degree (typically 75–114 nmol of P_i per mg of protein per min) to that (120 nmol per mg of protein per min) determined in untreated fresh tissue homogenates. Although drugs were removed prior to ATPase assay, their effects persisted for at least 1 hr. cGMP released from permeabilized slices after the initial freezing/thawing was equivalent to that measured directly by homogenization. In studies with oxytocin (OT), BK, and ACh, tubes contained superoxide dismutase (SOD). SOD and hemoglobin (Hb) were added 3 min prior to drug.

RESULTS AND DISCUSSION

NOS. Kidney was used as a model to investigate NO/cGMP regulation of Na,K-ATPase because of this organ's almost exclusive enrichment in a single isoform (α1) of Na,K-ATPase (12). Renal supernatants (Fig. 1*A*) demonstrated a surprising amount of NADPH- and Ca^{2+}-dependent NOS activity (10–20% that of the highly NOS-enriched cerebellum), with a 3-fold greater specific activity in medulla than cortex. This medullary enrichment (inhibitable by N^{ω}-nitro-L-arginine) was of considerable interest, given that prior *in situ* and immunological studies (14, 15), utilizing bNOS probes, had reported none of this isozyme in medulla and only small amounts in cortex, localized to macula densa. These biochemical data suggested that additional NOS isozymes might exist in medulla (16, 17).

Localization. To localize the sites of NO synthesis, we used the NOS isozyme-independent marker NADPH-d (9, 18) and observed a prominent and, to our knowledge, previously unreported array of diaphorase-positive tubules (Fig. 2). In cortex, reactivity was particularly intense in a small subgroup of subcapsular and periglomerular tubule segments (Fig. 2 *a* and *c*), as well as in glomerular mesangial-like cells and adjacent macula densa (14, 15). Outer medulla (Fig. 2 *b* and *d*) contained a much greater concentration of NADPH-d-positive tubules, with particularly intense staining of a large subgroup prominent in both inner and outer stripe, with a sharp demarcation at the point of origin of the thick ascending limb (TAL) (Fig. 2*e*).

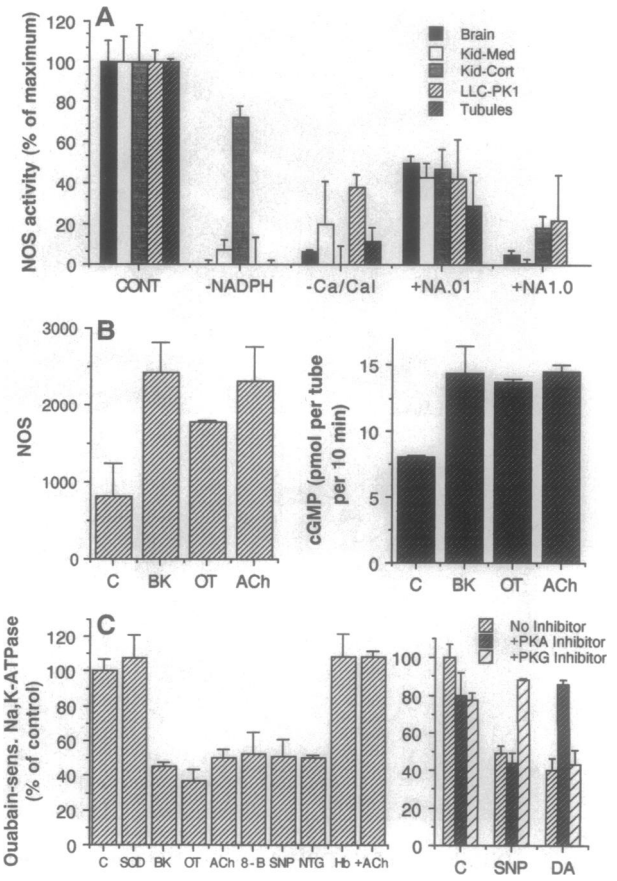

FIG. 1. Biochemical studies demonstrating NOS activity in kidney and renal epithelial cell tubules, regulation by hormones, and ability of this system, through cGMP and PKG, to regulate ouabain-sensitive Na,K-ATPase in medulla. (*A*) NOS activity in supernatants from cerebellum, renal medulla and cortex, LLC-PK_1 cells, and purified cultured tubules and the effects of omitting NADPH or exogenous Ca^{2+}/calmodulin (Ca/Cal) or of adding 0.01 or 1 mM N^{ω}-nitro-L-arginine methyl ester (NA.01 or NA1.0, respectively). Values shown (percentage of maximum activity) are the mean ± SEM of triplicate samples from typical experiments replicated at least twice. For all tissues, activities in the absence of NADPH or Ca^{2+}/calmodulin and in the presence of N^{ω}-nitro-L-arginine methyl ester were significantly less ($P < 0.05$) than control. Range of basal NOS activities (nmol per mg of protein per hr) in kidney were as follows: medulla, 6.8–10.1; cortex, 2.3–3.3; LLC-PK_1, 5–22; tubules, 6–23. Note that cortex had significant amounts of NADPH-independent activity. (*B*) Levels of [^{3}H]citrulline (cpm per tube) (*Left*) and cGMP (*Right*) after incubation of intact purified tubules with 1 μM BK, 10 μM OT, or 100 μM ACh. Values are the mean ± SEM of triplicate determinations (*Left*, $P < 0.05$; *Right*, $P < 0.01$). (*C*) (*Left*) Ouabain-sensitive Na,K-ATPase activity in slices of rat renal medulla after incubation (15 min at 34°C) with the following agents: SOD, 100 units/ml; BK, 10 μM; OT, 10 μM; ACh, 100 μM; 8-Br-cGMP (8-B), 4 mM; SNP, 100 μM; nitroglycerine (NTG), 500 μM; Hb (38), 20 μM; ACh, 100 μM, in the presence of Hb. (*Right*) Differential effects of 0.5 μM KT5720 (Kamiya Biomedical, Thousand Oaks, CA), a selective inhibitor of PKA, and 2 μM KT5823, a selective inhibitor of PKG, in blocking effects of either 100 μM SNP or 100 μM dopamine (DA) on ouabain-sensitive Na,K-ATPase. Values shown are the mean ± SEM of quintuplicate samples from representative experiments replicated on average two or three times. Of 12 experiments with SNP or NO agonists, there was a significant decrease (ANOVA, $P < 0.01$) of Na,K-ATPase in 10. Cont or C, control.

McK1, a monoclonal antibody specific for α1 Na,K-ATPase (Fig. 3 *a* and *b*) (12), is known to be highly enriched in the TAL and convoluted portion of distal renal tubules (12, 19, 20). Colocalization of NADPH-d-reactive tubules with this marker (Fig. 3) confirmed that most of the diaphorase

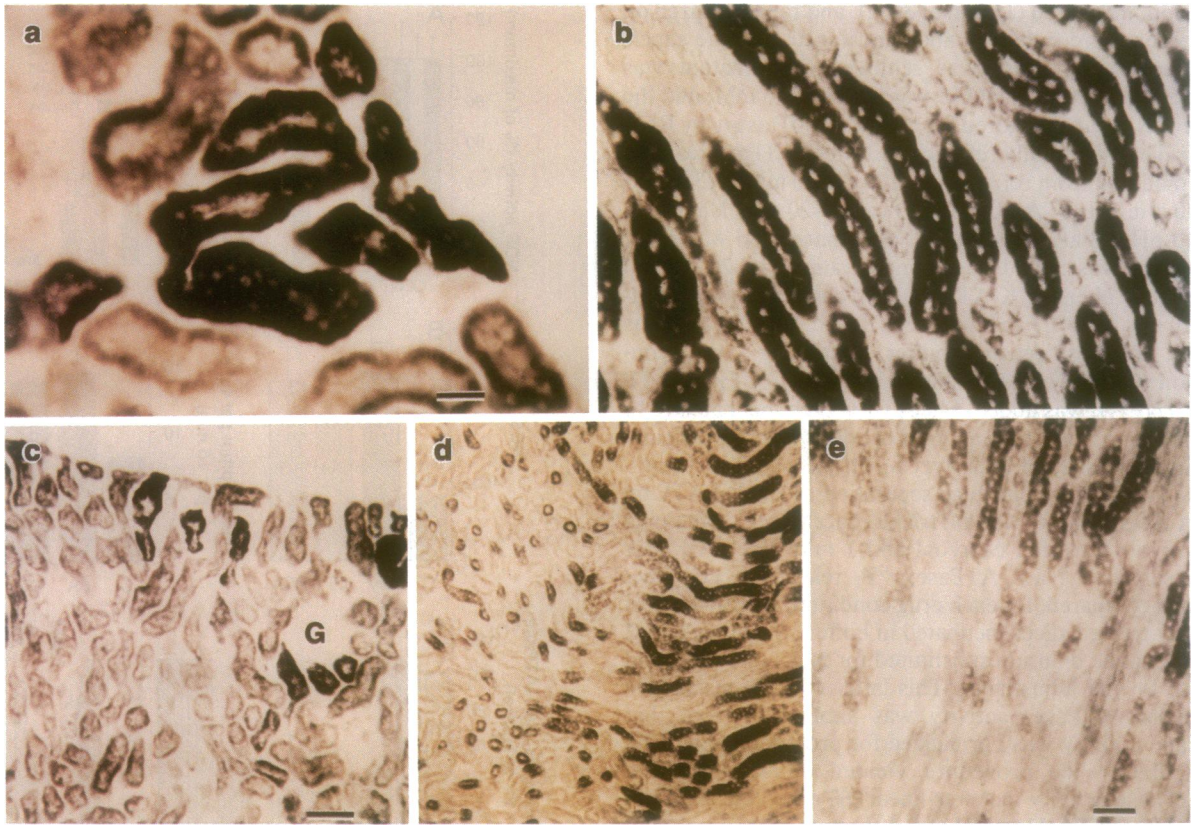

FIG. 2. Distribution of NADPH-d-positive NO-producing tubules in renal cortex and medulla. In cortex (*a* and *c*), NADPH-d-positive tubules appear singly and in small clusters, often localized subcapsularly (*a*) or near glomeruli "G" (*c*). Colocalization studies indicate that most are distal convoluted tubules. Outer medulla (*d*) contains an extensive array of intensely positive segments appearing in both the outer (to the left) and, especially, inner (to the right) stripes. Higher magnification of the inner stripe (*b*) shows segments to be the TAL of Henle (see also Fig. 3). Neighboring collecting ducts are negative except for intercalated cells. (*e*) Abrupt loss of reactivity is noted at junction of the inner stripe (upper part) with the inner medulla (lower part) (*e*), consistent with the TAL origin. (Bars: *a* and *b*, 25 μm; *c* and *d*, 100 μm; *e*, 50 μm.)

reactivity seen in Fig. 2 occurs in the TAL and distal segment. About 20% of NADPH-d-positive tubules failed to stain for McK1, indicating that the renal diaphorase system is heterogeneous and that observed reactivity was not simply the result of nonspecific labeling of Na,K-ATPase-enriched cells.

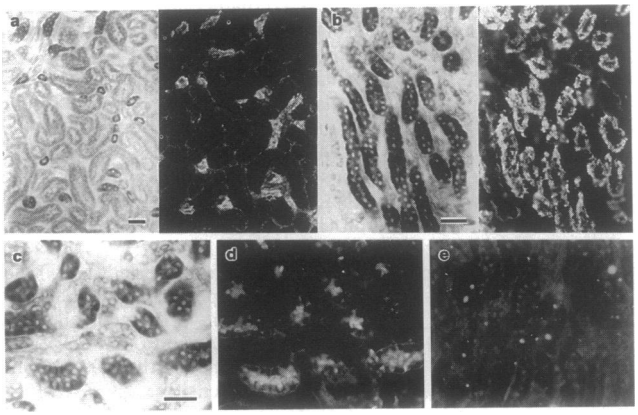

FIG. 3. Colocalization of NADPH-d with Na,K-ATPase (*a* and *b*) and DARPP-32 (*c* and *d*). In *a* (outer stripe) and *b* (inner stripe), the same section is seen by transmitted light (*Left*), showing diaphorase staining, and by immunofluorescence (*Right*), showing colocalization of McK1, a monoclonal antibody specific for the αI isozyme of Na,K-ATPase (12), known to be enriched in the TAL and convoluted distal tubule. (*c* and *d*) Sections are labeled for diaphorase (*c*) and immunostained for DARPP-32 (*d*) (4). (*e*) Control section incubated with nonimmune serum shows background fluorescence and a few rhodamine particles. (Bars = 40 μM.)

Isolated Tubules. Experiments using porcine LLC-PK_1 renal epithelial cells (21–24) provided additional evidence of the specificity of NOS for differentiated renal tubules. When cultured, this tubule cell line initially grew as a diaphorase-negative monolayer. However, at 3–5 days, scattered areas of cells assembled into distinct tubule-like structures (Fig. 4 *A* and *B*) that reacted intensely for NADPH-d (data not shown). Such tubules (an arrangement of LLC-PK_1 cells not previously described) could be harvested and purified using selective trypsinization, panning, and differential centrifugation (J.A.N. and M.M., unpublished data). High-speed supernatants prepared from such tubule homogenates demonstrated NADPH- and Ca^{2+}-dependent NOS activity inhibited by N^{ω}-nitro-L-arginine (IC_{50} = 10 μM) (Fig. 1*A*), and intact recultured tubules, pulse-labeled with [^{3}H]arginine, produced NO and could be stimulated by modulators known to increase renal sodium and/or water loss (2, 25) (Fig. 1*B*). In the presence of isobutylmethylxanthine, hormone stimulation was accompanied by an increase in tubule cGMP (Fig. 1*B*), consistent with activation, by hormone-stimulated NO, of soluble GC (1).

NOS Isoforms. LLC-PK_1 monolayers revealed very low levels of specific immunoreactivity for bNOS, ecNOS, and macNOS. However, coincident with the formation of diaphorase-positive tubules, there was a selective increase in immunoreactive ecNOS, localized almost exclusively to tubules and pretubule thickenings (Fig. 4 *A* and *B*), a distribution identical to that of diaphorase.

In postmortem human kidney, outer medulla demonstrated a subgroup of tubules with bright specific staining for ecNOS (Fig. 4*C*). Reactivity for bNOS was present in nerve fibers, but tubular bNOS was much less intense, and there was no

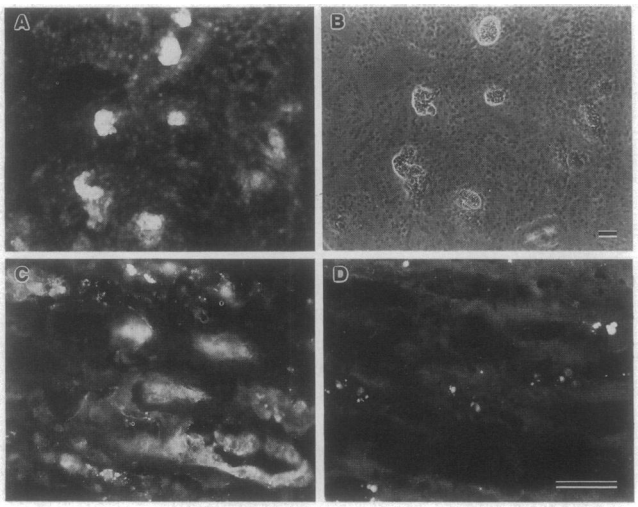

FIG. 4. Immunocytochemical localization of NOS in LLC-PK$_1$ tubules (*a* and *b*) and human renal outer medulla (*c* and *d*) with a monoclonal antibody specific for ecNOS, the major isoform found in renal tubules. Note selective labeling of differentiating tubules but not cells in monolayer in *a*, shown in phase-contrast in *b* (fluorescence seen in cell nuclei is nonspecific). Labeling is similar to that seen with NADPH-d (data not shown). In intact kidney, labeling is in epithelium of a subset of collecting tubules (*c*) and in blood vessels (data not shown). (*d*) Control labeling using mouse IgG shows a small amount of nonspecific labeling present throughout medulla. (Bar = 200 μm.)

tubular immunostaining for macNOS. Colabeling with NADPH-d diminished ecNOS immunoreactivity in both LLC-PK$_1$ cultures and renal medulla, a quenching effect (due to the dense nitro blue tetrazolium reaction product) that supported the colocalization of NADPH-d with ecNOS-like immunoreactivity.

However, while tubule NOS appears immunologically similar to ecNOS, it may not be identical or, indeed, consist of only a single isoform. For example, LLC-PK$_1$ monolayers exposed to tumor necrosis factor α or interleukin 6 for 48–96 hr showed a substantial increase in diaphorase staining (J.A.N. and M.M., unpublished data), suggesting that tubule NOS activity(ies) not only are hormone-responsive but also share characteristics with activities of inducible NOS isozymes (1). If so, NO production, *in vivo*, would be expected to increase during periods when circulating levels of cytokines are elevated (such as in septic shock). Because the TAL and distal tubule are known to be particularly sensitive to damage during hypoxia and endotoxemia (26), such NO elevations may have pathophysiological implications: either harmful, if NO is cytotoxic (1), or beneficial (26), if NO down-regulates Na,K-ATPase.

Na,K-ATPase. In intact choroid plexus and brain, elevation of cGMP, induced by ANF or sodium nitroprusside (SNP), causes phosphorylation of the protein phosphatase inhibitors (PPIs) inhibitor 1 (I-1) and DARPP-32 through cGMP-dependent protein kinase (PKG) (4, 6, 27, 28). I-1 and DARPP-32 are present in kidney (7), and their phosphorylation by dopamine, through cAMP and cAMP-dependent PK (PKA), has been proposed to mediate the regulation of Na,K-ATPase that underlies dopamine's action in promoting diuresis (7, 8). In the present studies, identification and colocalization of NOS in tubule segments showing high expression of Na,K-ATPase (Fig. 3) and the known effects of cGMP in choroid plexus (above) suggested that activation of the NOS system, with subsequent cGMP synthesis and stimulation of PKG, might regulate Na,K-ATPase and mediate the actions of hormones (e.g., ACh, BK, or OT) known to affect fluid and Na^+ balance.

Fig. 1*C* shows that short-term incubation of renal medullary slices with ACh, BK, or OT caused a substantial and long-lasting (>60 min) decrease in ouabain-sensitive Na,K-ATPase activity. This effect was potentiated by SOD, which is known to augment NO action. Ouabain-insensitive [Mg-ATPase] was unaffected. The induced decrease in Na,K-ATPase (which was the same as or larger than that observed with dopamine) was mimicked by 8-Br-cGMP and by the NO agonists SNP and nitroglycerine and was antagonized by reduced Hb, which binds and blocks NO. N^{ω}-Nitro-L-arginine also inhibited ACh's effect, and, along with Hb, caused a small increase in basal activity (data not shown), consistent with NOS experiments (Fig. 1 *A* and *B*), indicating some ongoing basal synthesis of NO.

The effect of SNP on Na,K-ATPase (also blocked by Hb) was associated with an increase in cGMP, but not cAMP, indicating that SNP's action was not through an indirect effect on the cAMP/PKA system (data not shown). Furthermore, the action of SNP was inhibited preferentially by KT5823, a selective inhibitor of PKG, but not by KT5720, a selective inhibitor of PKA (Fig. 1*C*). Conversely, the inhibitory effects of dopamine on Na,K-ATPase were blocked by KT5720 and not KT5823 and were associated with an increase in cAMP but not cGMP. At the concentrations used, protein kinase inhibitors had >8-fold selectivity for inhibiting their respective protein kinase (29). Use of protein kinase inhibitors, increases in cGMP, and mimicry by 8-Br-cGMP indicated that the effects of SNP on decreasing Na,K-ATPase activity were not due to a direct action (toxic or physiological) of NO on Na,K-ATPase but rather were mediated through cGMP and PKG.

cGMP also mediated changes in Na,K-ATPase activity in response to hormones acting independently of NO (C.S., J.A.N., M.M., and C. Scanlon, unpublished data). That is, like ACh, the natriuretic factor ANF inhibited ouabain-sensitive Na,K-ATPase in renal slices and increased cGMP. Unlike the cholinergic response, actions of ANF were not blocked by Hb, a result consistent with the mechanism by which each hormone stimulates cGMP: ACh via NO-mediated activation of soluble GC vs. ANF via direct stimulation of receptor-associated membrane GC. cGMP also regulated Na,K-ATPase activity in nerve tissue (J.A.N., C.S., M.M., and C. Scanlon, unpublished data), and the directionality of changes appeared to depend upon the predominant Na,K-ATPase isotype. In cerebellum, e.g., both NO-dependent elevation of tissue cGMP (by glutamate) and NO-independent stimulation of cGMP [by hydroxyl free radical (·OH) or carbon monoxide (CO)] increased Na,K-ATPase activity in α3-enriched cells. Both NO-dependent and -independent actions were mimicked by 8-Br-cGMP and blocked by inhibition of PKG, but only NO-dependent effects were blocked by Hb. These observations suggest that cGMP may play a wide role in regulating Na,K-ATPase in many cell types.

Colocalization of NO, Na,K-ATPase, DARPP-32, and I-1. DARPP-32 localized only to those tubule segments that stained for diaphorase (Fig. 3 *c* and *d*). Conversely, most, but not all, NADPH-d-positive tubules also stained for DARPP-32. A similar colocalization was observed with I-1 in medulla and for DARPP-32 and NADPH-d in renal cortex. Also, triple labeling of sections for diaphorase, McK1, and the PPI I-1 showed that most diaphorase-positive tubules that contained I-1 were also enriched in Na,K-ATPase (data not shown). Within tubules, basolateral labeling by the PPIs was less intense than that found apically, suggesting that, in addition to regulation of Na,K-ATPase (Fig. 1*C*), DARPP-32, I-1, and NO may also be involved in other membrane actions.

It is as yet unclear whether the modulation of Na,K-ATPase by NO occurs solely as a result of phosphorylation/dephosphorylation reactions or whether additional steps (e.g., an alteration of intracellular [Na^+]) are involved. A

direct effect of [Na^+] on altering substrate kinetics of Na,K-ATPase was unlikely, given the fact that permeabilization of medullary slices after drug incubation effectively clamped [Na^+] during the subsequent ATPase assay. However, because [Na^+] has been proposed to alter the phosphorylation/dephosphorylation state of Na,K-ATPase itself (30), a modulatory effect of this ion might occur if, during drug incubation, cGMP (generated by NO and released from the TAL) acted to decrease intracellular [Na^+] by inhibiting ANF- and amiloride-sensitive cation channels, known to be enriched in collecting duct epithelium (15). The previously described effects of cGMP on this channel are, in part, phosphorylation-independent (31), an observation that contrasts with our finding (Fig. 1*C*) that inhibition of PKG nearly completely blocks the effect of SNP. We also found (data not shown) that the effect of SNP was unaltered when slices were exposed to drug under conditions of low (20 mM) extracellular Na^+ and that effects of SNP could still be observed in the presence of amiloride or after prepermeabilization of medullary slices to fix [Na^+] during drug incubation. In some experiments, a partial inhibition of the SNP effect observed with amiloride or after prepermeabilization suggested that both Na^+-dependent and Na^+-independent mechanisms may be contributing to the observed regulation of Na,K-ATPase.

Given that DARPP-32 and I-1 colocalized with NOS and Na,K-ATPase and that cGMP can stimulate phosphorylation of these PPIs (6), it is possible that regulation of Na,K-ATPase by NO involves inhibition of PPI activity. Consistent with this possibility, addition of the PPI okadaic acid to slices exposed to SNP did not further increase the inhibitory effect on renal Na,K-ATPase (data not shown). Although we cannot be definitive about the site of phosphorylation changes, the observed paucity of DARPP-32 and I-1 in medullary blood vessels compared with the enrichment of these PPIs, along with Na,K-ATPase and diaphorase, in the TAL and distal tubule (22, 23), strongly suggests a tubular localization. Also supporting these findings is the recent identification of a brain PKG (CGKII) (32) that, in kidney, is much more abundant than the type 1 PKG known to be enriched in renal contractile cells (33). Our experiments do not rule out other effects of NO on renal hemodynamics (2, 18) nor preclude involvement of other second messengers, such as arachidonic acid (2, 34), inositol trisphosphate, or protein kinase C (34–37). NO-generated cGMP could also have additional direct or phosphorylation-dependent effects on amiloride-sensitive cation channels (15, 16, 31, 35). These and other actions of cGMP may occur at the sites of renal NO synthesis that we have identified (Fig. 2) or, if NO or NO-generated cGMP is released from the TAL (as is likely), may occur more distally and in neighboring cells (including collecting ducts).

Thus, the present studies indicate that NO, through generation of cGMP and stimulation of PKG, mediates the actions of several intercellular messengers to regulate renal tubular Na,K-ATPase. cGMP-mediated regulation of sodium transport, by both NO-dependent and NO-independent mechanisms, is not restricted to epithelium and appears to be part of a more wide-spread regulatory mechanism. In kidney, inhibition of the sodium pump (e.g., by ACh, BK, OT, and ANF) would be expected to decrease transcellular water and sodium reabsorption from the tubule lumen, resulting in diuresis and natriuresis. In brain, such regulation provides a mechanism by which certain transmitters (e.g., glutamate) and other messengers (e.g., CO, NO, and ·OH) can modulate ion gradients that affect a variety of cellular processes, including transcellular transport and reuptake, energy metabolism, and membrane excitability.

We thank C. Scanlon for measuring cyclic nucleotides, K. Sweadner for advice and McK1, and H. Hemmings and colleagues for antibodies to I-1 and DARPP-32. This work was supported by National Eye Institute Grant 05077. C.S. is a FAPESP Fellow, Department of Pharmacology, University of Sao Paulo, Sao Paulo, Brazil.

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