Inhibition of Ecto-5'-nucleotidase by Nitric Oxide Donors

IMPLICATIONS IN RENAL EPITHELIAL CELLS

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We evaluated, in renal epithelial cells with a proximal tubule phenotype, the effect of nitric oxide (NO) on ecto-5'-nucleotidase (5'-NU), the underlying mechanism and its functional consequence. Sodium nitroprusside (SNP, 1-1000 μM), a NO donor, inhibited 5'-NU activity in a time- and concentration-dependent manner. Consequently, NO blunted the inhibition by extracellular cyclic AMP (cAMP, 10-1000 μM) of sodium-phosphate cotransport, a pathway which involves degradation of adenosine monophosphate (AMP) by 5'-NU. SNP-induced inhibition of 5'-NU was not mediated by cyclic GMP, since it was not mimicked by atrial natriuretic peptide, and was reproduced by isosorbide dinitrate and sodium nitrate, two NO donors. SNP and genuine NO decreased the activity of 5'-NU in renal homogenates, and the effect of SNP was potentiated by dithiothreitol and glutathione, but not by nicotinamide adenine dinucleotide.

1 The abbreviations used are: 5'-NU, ecto-5'-nucleotidase; AMP, adenosine monophosphate; cAMP, cyclic AMP; AC, adenylate cyclase; PKC, protein kinase C; FPI, fibroblast peroxidase; GSH, glutathione; HBS, Hank's balanced salt solution; LNAME, Nω-nitro-arginine methyl ester; NAD, nicotinamide adenine dinucleotide; SNAP, S-nitroso-N-acetylpenicillamine; SNP, sodium nitroprusside; OK cells, opossum kidney cells.

Ecto-5'-nucleotidase (5'-ribonucleoside phosphohydrolase, 5'-NU; EC 3.1.3.5) is a membrane-bound glycoprotein which hydrolyzes extracellular nucleotides into membrane-permeable nucleosides. In the kidney, 5'-NU is expressed mainly in plasma membranes of proximal tubular cells and, to a lesser extent, in glomerular mesangial cells, interstitial fibroblasts and intercalated cells of the collecting tubule (see Ref. 1 for review). Apical brush-border membranes of proximal cells are equipped with ectoenzymes (adenosine triphosphatase, phosphodiesterases, and 5'-NU) which convert adenine nucleotides, i.e. adenosine triphosphate, adenosine diphosphate, adenosine monophosphate (AMP), and cyclic AMP (cAMP), into adenosine (2, 3). Released adenosine can be taken up by proximal tubular cells through dipyridamole-sensitive carriers (4–7) and phosphorylated into adenine nucleotides. This cascade of events was shown to account for the protective effect of extracellular adenosine nucleotides on tubular function during and after anoxia (8). Our previous studies have evidenced that degradation of extracellular cAMP in the tubular lumen followed by adenosine uptake were mandatory steps in the well known inhibitory effect of extracellular cAMP on renal proximal phosphate (Pi) reabsorption (9). Through this pathway, luminal cAMP (nephrogenous cAMP), added to the tubular fluid under the influence of parathyroid hormone, is not only a marker of the activity of parathyroid hormone but also participates in the overall phosphaturic effect of the hormone (9–11). We have recently reported that parathyroid hormone-stimulated 5'-NU activity via a mechanism which involved protein kinase C activation and de novo protein synthesis (12).

Nitric oxide (NO) is a local mediator which is synthesized from l-arginine by numerous cell types including endothelial cells, activated macrophages, and renal tubular cells under physiological or pathological conditions (13–23). Cellular targets of NO and signaling pathways involved in its pleiotropic effects have been extensively studied in the past decade. Stimulation of soluble guanylate cyclase (24–26), ADP-ribosylation-like reaction with proteins (27–29) and, more recently, S-nitrosylation of proteins (30–37) have been reported as biochemical events which accounted for the actions of NO. S-Nitrosylation results from direct or indirect (via intermediate S-nitrosothiols) transfer of NO" to thiol groups of proteins (34, 35, 37). Such reactions were recently shown to affect the activity of nuclear, cytosolic, and membrane-bound proteins (34) including heterotrimeric G proteins (33), p21ras (36), and glyceraldehyde-3-phosphate dehydrogenase, a key enzyme of glycolysis (32). However, a direct interaction between NO and an ecto-enzyme has not been reported.

The aim of the present study was: (i) to evaluate whether NO-related activity affected 5'-NU activity of proximal tubular cells and the extent to which such a modulation might influence inhibition of sodium-Pi cotransport by extracellular cAMP; (ii) to elucidate the mechanism involved in the effect of NO; (iii) to identify possible conditions in which NO inhibits 5'-NU in vivo. We show that NO inhibits 5'-NU activity in a cyclic GMP (cGMP)- and protein synthesis-independent manner, most likely through S-nitrosylation of the enzyme, and that renal ischemia/reperfusion results in NOx-dependent inhibition of 5'-NU.

EXPERIMENTAL PROCEDURES

Materials—Insulin, transferrin, hydrocortisone, sodium nitroprusside (SNP), isosorbide dinitrate, sodium selenite, bovine serum albumin, adenosine nucleotides and nucleosides, α,β-methyleneadenosine

4659
5'-diphosphate (AMP-PCP), glutathione (GSH), 3-isobutyl-1-methylxanthine, dipiridamole, human aortic natriuretic peptide (ANP), N nitro-l-arginine methyl ester (l-NAME) were purchased from Sigma. 5-Nitro-N-acetyl-penicillamine (SNAP) was from Biomol Research Laboratories (Plymouth Meeting, PA). Tracers were from the following sources: K^37,PO_4 from DuPont NEN. [3^14C]-AMP and [3^3H]-adenosine from Amersham (Amersham, UK). Control reagents and enzymes were from Life Technologies, Inc. (Cergy-Pontoise, France). Plasticware was from Costar (Cambridge, MA). All other reagents were of analytical grade.

Preparation of NO Solutions—Hank's balanced salt solution (HBS) was first deoxygenated by bubbling through argon gas for 30 min, and then the medium was bubbled with the air space above the solution was briefly flushed with argon. This saturated solution, with a NO concentration of approximately 1.2 μM, was further diluted in deoxygenated HBS to reach specified NO concentrations (33, 38).

Cell Culture—Opossum kidney (OK) cells (passages 80-100) were grown to confluence in 24-well trays in a medium consisting of a 1:1 (v/v) mixture of Ham's F-12 and Dulbecco's modified Eagle's medium containing 15 mM Hepes, 21.5 mM HCO_3, 1 mM sodium pyruvate, 4 mM l-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 50 mM sodium selenite, 5 μg/ml insulin, 5 μg/ml transferrin, 50 mM hydrocortisone, and 2.5% fetal calf serum. Medium was changed on alternate days. Monolayers of OK cells reached confluence after 4 days, and they were used for experiments 2 or 3 days after confluence was achieved. Cells were subcultured weekly by trypsinization. The splitting ratio was 1 to 5.

On the day prior to experiments, culture medium was changed to hormone-free and serum-free medium, and, on the day of experiment, preincubations were usually performed in the same medium to which drugs were added as concentrated aliquots.

Determination of Cyclic GMP Content—After removal of culture medium, cells were washed with 1 ml/well of HBS supplemented with 15 mM Hepes and 2 mM L-glutamine (HBS-Hepes), and were then preincubated for 15 min at 37 °C in HBS-Hepes (500 μl/well) containing 0.5 mM 3-isobutyl-1-methylxanthine, a nonspecific phosphodiesterase inhibitor. The coverslips were then removed and cells were incubated usually for 5 min in a similar solution to which hormones or drugs were added. At the end of incubation, intracellular cGMP content was measured by radiommunoassay as described previously (39).

Determination of 5'-NU Activity—The activity of ecto-5'-NU was determined on intact OK cells using a method adapted from Gentry and Olsson (40) as described previously (12). Briefly, after removal of the culture medium, cells were rinsed twice with HBS-Hepes, and then incubated in the same solution (500 μl/well) in the presence of [1^33P]-AMP (0.02 μM/ml), unlabeled 5'-AMP (usually 10 μM except for determination of kinetic parameters), and 20 μM dipiridamole which abolishes cellular uptake of generated adenosine (6, 7, 9). At the end of incubation, 400 μl/well of incubation medium were mixed with 100 μl of 0.1 M NaOH. After vigorous shaking or vigorous stirring for 15 min, the mixture was centrifuged (10,000 × g; 5 min) in order to separate adenosine from 5'-AMP and 400 μl of supernatant, which contains adenosine, were counted by liquid scintillation. Blank values were measured in the absence of cells and represented less than 0.5% of added radioactivity and, in any case, less than 10% of the signal attributed to genuine 5'-NU. In each experiment, recovery of adenosine was evaluated with [3^3H]-adenosine and was found to average 75-80%.

Activity of 5'-NU from renal homogenates was determined by incubating aliquots of the homogenate (2-4 μg of protein/ml) in HBS-Hepes solution in the presence of labeled and unlabeled 5'-AMP as described above during 20 min at 37 °C. The reaction was terminated as for OK cells.

Uptake Studies—Uptake of P_i was performed as described previously with minor modifications (9). Briefly, uptake studies were performed at 37 °C in a buffered solution with the following composition (millimoles/liter): 137 NaCl, 5.4 KCl, 1 CaCl_2, 1.2 MgSO_4, 15 Hepes (pH 7.4). The sodium-free solution was made isosmotic by replacing sodium chloride with Na2-ethyl-g-glutamate. After removal of culture medium, cells were washed with 1 ml/well of the uptake solution, and were incubated for various periods of time in the presence of K^37,PO_4 (0.5 μM/ml) and 100 μM KH_2PO_4. All these steps were performed at 37 °C. At the end of incubation, the uptake was stopped by washing the cells three times with 1 ml/well of ice-cold solution (137 mM NaCl, 15 mM Hepes, pH 7.4). Cells were then solubilized in 0.5% Triton X-100 (250 μl/well) and aliquots were counted by liquid scintillation.

In Vivo Studies—Sprague-Dawley rats, weighing 180-200 g, were anesthetized and infused as described previously (9-11). Two groups of four rats (n = 4 in each group) were studied. In the first group, the pedicle of the left kidney was clamped during 15 min. At the end of that period, reperfusion was allowed during 60 min. In the second group, rats were treated similarly except that l-NAME infusion (50 μg/min/100 g body weight), starting after a priming dose of 5 mg/100 g body weight, was initiated 15 min prior to renal ischemia.

Presentation of Data—5'-NU activity and P_i uptake were expressed as nanomoles/mg of protein (41). 5'-NU activity was calculated as 10 μM AMP-PCP-sensitive 5'-AMP hydrolysis. Sodium-dependent uptakes were calculated by subtracting uptake values measured in the presence of N-methyl-D-glucamine from those measured in the presence of sodium. Intracellular cGMP content was expressed as picomoles/mg of protein. Results were presented as mean ± S.E. of three to five different experiments (n) in which duplicates were obtained. One-way or two-way analyses of variance were performed and, when allowed by the F value, results were compared by the modified t test (42).

RESULTS

Effect of NO Donors on 5'-NU Activity in OK Cells—As previously reported, OK cells exhibited ecto-5'-NU activity (Fig. 1). This activity increased linearly with the time of incubation up to 120 min, both under control conditions and after preincubation with 1 mM SNP (Fig. 1, panel A). A 60-min incubation time was therefore chosen in subsequent experiments. SNP decreased 5'-NU in a time- and concentration-dependent manner (Fig. 1, panels B and C). The effect of SNP was already apparent after 30 min of incubation and reached significance after 60 min (Fig. 1, panel B). After 3 h of incubation, SNP was inhibitory at the lowest concentration of 10 μM. The magnitude of this effect increased with SNP concentration up to 1 mM (Fig. 1, panel C). We next examined the effect of SNP on the kinetic parameters of 5'-NU. As shown in Fig. 1, panel D, SNP, at 1 mM, affected the V_max value of 5'-NU which decreased significantly from 46 ± 4.3 to 27 ± 4.5 nmol/mg of protein/60 min (n = 3, p < 0.05). In contrast, apparent K_m values were not different whether cells were pretreated with SNP or vehicle (71 ± 12 versus 57 ± 8 μM, n = 3, NS).

We have previously reported that 5'-NU played a key role in the inhibitory effect of extracellular cAMP on sodium-P_i co-transport (9, 12). In order to evaluate the influence of NO on this inhibitory pathway, we measured sodium-dependent P_i uptake after that OK cells had been preincubated with increasing concentrations of cAMP in the presence or absence of SNP or AMP-PCP. As expected, extracellular cAMP (10-1000 μM) inhibited P_i uptake in a concentration-dependent manner (Table I). AMP-PCP, a potent inhibitor of 5'-NU, blunted significantly the inhibition by CAMP. The effect of AMP-PCP was mimicked by SNP, although to a lesser extent. It is noteworthy that neither AMP-PCP nor SNP affected P_i uptake by themselves.

In order to confirm that NO_3 was indeed responsible for the inhibitory effect of SNP on 5'-NU, we evaluated: (i) the effect of another NO donor, isosorbide dinitrate; (ii) the effect of the ferricyanide and ferrocyanide moieties. As shown in Fig. 2, panel A, l-isosorbide dinitrate and SNP, each of them at 1 mM, inhibited 5'-NU to a similar extent. In contrast, neither K_Fe(CN)_6 nor K_Fe(CN)_6, at the same concentration, affected significantly 5'-NU activity.

Because NO was reported in many cellular systems to act through generation of cGMP, we evaluated whether this signaling pathway was involved in inhibition of 5'-NU. For that purpose, we compared the effects of SNP and ANP on cGMP accumulation and 5'-NU activity. SNP-induced increase in intracellular cGMP content was modest and did not reach signif-
Inhibition of Ecto-5'-nucleotidase by NO Donors

In previous studies from several groups including ours (12), modulation of 5'-NU activity was reported to depend on de novo protein synthesis. Regarding the effect of NOx, this possibility was evaluated by a pretreatment of OK cells with cycloheximide or actinomycin D at concentrations previously reported to abolish the modulation of the enzyme by protein kinase C activators (12). Cycloheximide or actinomycin D did not prevent NO-induced inhibition of 5'-NU activity (Table II).

S-Nitrosylation of proteins with NOx was recently described (30–37) and was reported in some instances to account for inhibition of enzymatic activities (32, 34). The possibility that a similar mechanism was involved in NOx-induced inactivation of 5'-NU was investigated. The effect of NO donor SNP was potentiated by addition of the reducing agent glutathione (GSH) to the incubation medium (Fig. 3). Incubation of OK cells during 3 h with GSH alone, 0.01 to 1 mM, had no effect on 5'-NU activity. However, the presence of GSH together with SNP during the preincubation period increased markedly the effect of the NO donor: SNP, at 10 μM, decreased 5'-NU activity by 7, 13, 39, and 40% in the presence of GSH at a concentration of 0, 0.01, 0.1, and 1 mM, respectively.

Effect of NO Solutions, NO Donors, and Renal Ischemia on Rat Renal 5'-NU Activity—Finally, we asked the question of whether inhibition of renal 5'-NU by NOx might occur under pathophysiological conditions. For that purpose, we induced an ischemia/reperfusion injury in rat kidneys because hypoxia/reoxygenation injury was recently shown to increase NO production, likely through activation of inducible NO-synthase in proximal tubules (20). As shown in Fig. 4, panel A, 15-min ischemia followed by 60-min reperfusion resulted in a marked decrease of 5'-NU activity. This inhibition was entirely prevented by infusion of L-NAME at a rate which did not affect renal 5'-NU activity (Fig. 4, panel C).

In a last set of experiments, the in vitro effect of NO solutions and of two NO donors, SNP and SNAP, was evaluated on 5'-NU activity in homogenates prepared from control rat kidneys. 5'-NU activity (nmol/mg of protein/min, means ± S.E. n = 3 in each group) decreased from 5.4 ± 0.30 under basal conditions to 4.2 ± 0.21, 3.5 ± 0.25, 3.9 ± 0.30, and 2.8 ± 0.17 after incubation with 0.1 mM SNP, 1 mM SNAP, 10 μM NO, and 100

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**TABLE I**

| [AMP] (μM) | Sodium-dependent P<sub>i</sub> uptake (nmol/mg protein/5 min) |
|------------|-------------------------------------------------------------|
|            | Control | SNP (100 μM) | AMP-PCP (10 μM) |
| 0          | 4.0 ± 0.23 | 4.2 ± 0.29 | 4.4 ± 0.23 |
| 10         | 3.3 ± 0.20<sup>a</sup> | 3.9 ± 0.22<sup>b</sup> | 4.2 ± 0.18<sup>a</sup> |
| 100        | 2.4 ± 0.17<sup>a</sup> | 3.3 ± 0.12<sup>a,b</sup> | 3.6 ± 0.13<sup>a</sup> |
| 1000       | 1.7 ± 0.09<sup>a</sup> | 2.4 ± 0.21<sup>a,b</sup> | 3.1 ± 0.17<sup>a,b</sup> |

<sup>a</sup>Significantly different from the homologous basal value without cAMP, p < 0.05.

<sup>b</sup>Significantly different from the homologous control value without SNP or AMP-PCP, p < 0.05.
Inhibitory effect of NO donors in renal epithelial cells

The main results of the present study are that: (i) in renal epithelial cells, NO donors inhibited 5'-NU activity in a cGMP- and protein synthesis-independent manner; (ii) this effect resulted in impairment of cAMP-induced inhibition of sodium-Pi cotransport; (iii) S-nitrosylation of the enzyme, either direct or indirect, is likely to underlay enzymatic inactivation; and (iv) in vivo, NO overproduction during ischemia/reperfusion injury led to inhibition of 5'-NU activity. To our best knowledge, this is the first demonstration of a direct interaction between a nitrogen oxide and an ecto-enzyme.

The mechanism of 5'-NU inhibition by NOx differs from that involved in previously reported hormonal modulation of renal 5'-NU (12, 43–45). In cultured glomerular mesangial cells, cAMP-protein kinase A activating substances, such as dopamine, and tumor necrosis factor-α or interleukin-1β were shown to stimulate 5'-NU in a cycloheximide-dependent manner (43–45). In OK cells, we established that parathyroid hormone stimulation of 5'-NU through protein kinase C was dependent on cycloheximide and actinomycin D (12). In contrast, de novo protein synthesis was not involved in the effect of NO as evidenced by the short-term action of this compound (Fig. 1) and its persistence in the presence of cycloheximide and actinomycin D (Table II).

The inhibitory effect of NOx on 5'-NU did not result from
Inhibition of Ecto-5'-nucleotidase by NO Donors

Fig. 3. Effect of glutathione on SNP-induced inhibition of 5'-NU in OK cells. OK cells were incubated during 3 h in the absence or presence of SNP and GSH at the indicated concentrations prior to determination of 5'-NU activity. Results are expressed as means ± S.E. of four different experiments (n = 4) in which duplicates were obtained. *, significantly different from the homologous control value, without SNP, p < 0.05; §, significantly different from the homologous value without GSH, p < 0.05.

Fig. 4. Effect of renal ischemia/reperfusion on 5'-NU activity in rats. Left kidneys were subjected to 15-min ischemia followed by 60-min reperfusion while right kidneys served as controls. All along the experiments, rats were infused either with saline (panel A) or with L-NAME (50 μg/min/100 g body weight after a priming dose of 5 mg/100 g body weight) (panel B). 5'-NU activity was determined on renal homogenates. *, significantly different from the value of the right (control) kidneys, n = 4, p < 0.05.

Table III

Effect of NAD, glutathione, and dithiothreitol on SNP-induced inhibition of rat renal 5'-NU

Renal homogenates were incubated in the absence or presence of 0.1 mM SNP with or without NAD, glutathione (GSH), or dithiothreitol (DTT) during 30 min prior to determination of enzymatic activity. Results are expressed as means ± S.E. of three different experiments in which duplicates were obtained.

| 5'-NU activity (nmol/mg protein/min) | No SNP | 0.1 mM SNP |
|-------------------------------------|--------|------------|
| Control                             | 5.4 ± 0.3 | 3.8 ± 0.2* |
| NAD (0.1 mM)                        | 5.4 ± 0.3 | 3.7 ± 0.2* |
| GSH (0.1 mM)                        | 5.5 ± 0.2 | 3.0 ± 0.2* |
| GSH (1 mM)                          | 5.2 ± 0.2 | 2.3 ± 0.1* |
| DTT (0.1 mM)                        | 5.3 ± 0.2 | 2.5 ± 0.2* |

*Significantly different from the homologous value without SNP, p < 0.05.

§Significantly different from the value without GSH or DTT, p < 0.05.

activation of the soluble guanylate cyclase-cGMP-dependent protein kinase pathway, a common mode of action of NOx in several systems (24–26) which accounts for relaxation of smooth muscle cells and for the classical vasodilatory effect of NOx (15, 16, 24). Our data argue against the involvement of this pathway in 5'-NU inhibition since: (i) NOx had a modest effect on cGMP generation in OK cells; (ii) ANP, a well known agonist of particulate guanylate cyclase, which increased dramatically cGMP generation in OK cells (Fig. 2 and Ref. 46), did not affect 5'-NU activity; (iii) the effect of NOx on the enzyme was also observed in homogenates of renal tissue.

Nitrosylation was recently reported to affect the activity of a large number of membrane-bound, cytosolic and nuclear proteins (30–37). This expression of a wide variety of effects is achieved through interaction of nitrogen oxides with targets via a complex redox signaling and additive chemistry (34, 35, 37). As regards inhibition of 5'-NU, it may result from interaction with NO or with congeners NO + and NO –. Indeed, SNP, which inhibited 5'-NU in the two preparations used in the present study, is better regarded as an NO + donor rather than an NO donor (34, 37). The observation that the effect of SNP was potentiated in the presence of thiols such as dithiothreitol or glutathione (Fig. 3 and Table III) raises the possibility that these compounds first interact with NO – and that RSNO compounds then inhibit 5'-NU, probably by S-nitrosylation. It is noteworthy that neither glutathione nor dithiothreitol alone affected 5'-NU activity, a feature which contrasts with the reported inhibition of bull seminal plasma 5'-NU by dithiothreitol (47). This apparent discrepancy can be attributed to the fact that dithiothreitol concentrations used in our study were 2 to 3 orders of magnitude lower than those reported to inhibit 5'-NU (47). Alternatively, our data showing that genuine NO solutions decreased the activity of 5'-NU from kidney homogenates is also consistent with the possibility of a direct interaction between NO and the enzyme. It can be pointed out that NOx was active within a concentration range similar to that reported to affect the activity of heterotrimeric G proteins and P21ras (33, 36). Along the same line, SNAP, which can be regarded as an NO donor, inhibited renal 5'-NU as well. Finally, the possibility that decreased activity of the enzyme resulted from an interaction between a nitrogen oxide and the zinc moiety of 5'-NU, which is a zinc metalloprotein (48, 49), is unlikely since nitric oxide does not react with zinc, an element which was shown to be crucial for 5'-NU activity in the mammalian membrane-bound form of the enzyme (48, 49).

The interaction between NOx and 5'-NU differs from that described between NO and glyceraldehyde-3-phosphate dehydrogenase: in the latter case, S-nitrosylation of the protein precludes to covalent linkage of NAD, a cofactor of the enzyme (31). This reaction was first interpreted as ADP-ribosylation since NO was also reported to stimulate an ADP-ribosyltransferase (27–29). In our model, the observation that NAD, alone or in combination with SNP, had no effect on 5'-NU activity rules out such a possibility. It should be stressed that the eczonymatic situation of 5'-NU made unlikely an interaction with NAD.

In proximal tubular cells, 5'-NU was shown to be involved in modulation of P, reabsorption and in restoration of intracellular stock of ATP following ischemia (8, 9, 12). (i) The inhibitory effect of extracellular cAMP on sodium-P, cotransport was previously shown to require extracellular degradation of the nucleotide by phosphodiesterases and 5'-NU and subsequent uptake of adenosine (9); (ii) the protective effect of extracellular nucleotides on intracellular ATP content during ischemia also requires degradation of extracellular nucleotides followed by adenosine uptake (8). Our present finding that impairment of 5'-NU activity by NOx blunts the phosphaturic effect of extracellular cAMP demonstrates that 5'-NU inhibition has functional implications in terms of P, homeostasis: the relief of tonic
inhibition exerted by cAMP on sodium-P cotransport may help to maintain normal P reabsorption in case of impaired ATP content. In those situations, the fall in ATP content results in a decrease of Na,K-ATPase activity. In turn, the impairment of this pump may potentially increase intracellular sodium concentration and thus affect the magnitude of membrane sodium gradient. Sodium gradient across plasma membranes is mandatory for the efficiency of secondary active transport systems such as sodium-P cotransport.

Increased NO production, either by activated macrophages, or by proximal tubular cells under the influence of cytokines or of hypoxia (15–20), was shown to occur in pathologic conditions such as ischemia or in kidney diseases with macrophage infiltration, whatever their cause (20, 21–23). The present data, however, cannot definitely discriminate between activation of inducible NO-synthase and increased activity of constitutive NO-synthase as the source of NOx overproduction. Our observation that transient ischemia induced inhibition of 5'-nucleotidase, and that this effect is prevented by inhibition of NO-synthase by L-NAME, clearly demonstrates that 5'-nucleotidase inhibition can indeed occur in vivo in relation with NOx overproduction whatever its origin.

In summary, we have demonstrated that NOx interacts with ecto-5'-nucleotidase and that S-nitrosylation of the enzyme is likely to result in inhibition of its activity with functional implications in renal epithelial cells. Under in vivo conditions such as renal ischemia, overproduction of NO may lead to impairment of 5'-nucleotidase.

REFERENCES

1. Le Hir, M., and Kaislings, B. (1993) Am. J. Physiol. 264, F377-F387
2. Boumenidi-Podevin, E. F., and Podevin, R. A. (1977) J. Biol. Chem. 252, 6675–6681
3. Culli, O., Sabolic, I., and Zanic-Grubisic, T. (1990) Biochim. Biophys. Acta 1030, 143–151
4. Trickle, M. E., and Coulson R. (1984) Am. J. Physiol. 246, F794-F803
5. Le Hir, M., and Dubach, U. C. (1988) Eur. J. Clin. Invest. 15, 121–127
6. Franco, R., Centelles, J. J., and Kinne, R. K. H. (1990) Biochim. Biophys. Acta 1024, 241–248
7. Plegmann, P. G. W., and Aran, J. M. (1990) Biochim. Biophys. Acta 1028, 289–298
8. Mandel, L. J., Takano, T., Sotoff, S. P., and Murdacha, S. (1988) J. Clin. Invest. 81, 1255–1264
9. Friedlander, G., Couette, S., Coureau, C., and Amiel, C. (1992) J. Clin. Invest. 90, 848–858
10. Kuntziger, H., Amiel, C., Roined, N., and Morel, F. (1974) Am. J. Physiol. 227, 905–911
11. Amiel, C., Kuntziger, H., Couette, S., Coureau, C., and Bergounioux, N. (1976) J. Clin. Invest. 57, 256–263
12. Siegfried, G., Vrtovsnik, F., Prié, D., Amiel, C., and Friedlander, G. (1995) Endocrinology 136, 1267–1275
13. Morris, S. M., and Biliar, T. M. (1994) Am. J. Physiol. 266, E829-E839
14. Nathan, C. (1992) FASEB J. 6, 3051–3064
15. Moncada, S., and Higgs, E. A. (1991) Eur. J. Clin. Invest. 21, 361–374
16. Moncada, S., and Higgs, E. A. (1993) New Engl. J. Med. 329, 2002–2012
17. Marletta, M. A. (1993) J. Biol. Chem. 268, 12231–12234
18. McClay, J. S., Chatterjee, P., Nicolson, A. G., Jardine, A. G., McKay, N. G., Ralston, S. H., Grabowski, P., Hailes, N. E., Macled, A. M., and Hawkesworth, G. M. (1994) Kidney Int. 46, 1043–1049
19. Markewitz, B. A., Michael, J. R., and Kohan, D. E. (1993) J. Clin. Invest. 91, 2138–2143
20. Yu, L., Gengaro, P. E., Niederberger, M., Burke, T. J., and Schrier, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1691–1695
21. Ketteler, M., Border, W. A., and Noble, N. A. (1994) Am. J. Physiol. 267, F197-F207
22. Nathan, C., and Xie, Q. W. (1994) J. Biol. Chem. 269, 13725–13728
23. Raij, L. (1993) Circulation 87, V26-V29
24. Lüscher, T. F., Bock, H. A., Yang, Z., and Diederich, D. (1992) Kidney Int. 40, 575–590
25. Drexel, J. G., and Gabers, D. L. (1994) Endoov. Res. 15, 135–162
26. Wong, S. K.-F., and Gabers, D. L. (1992) J. Clin. Invest. 90, 299–305
27. Brüne, B., and Lapetina, E. G. (1990) Arch. Biochem. Biophys. 279, 266–290
28. Zhang, J., and Snyder, S. H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9382–9385
29. Dinneler, S., Lottspeich, F., and Brüne, B. (1992) J. Biol. Chem. 267, 16711–16717
30. Stamler, J. S., Simon, D. I., Osborne, J. A., Mullins, M. E., Jakari, O., Michel, T., Singel, D. J., and Loscalzo, J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 444–448
31. McDonald, L. J., and Moss, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6238–6241
32. Molina y Vedia, L., McDonald, B., Reep, B., Brüne, B., Di Silvio, M., Billiar, T. R., and Lapetina, E. G. (1992) J. Biol. Chem. 267, 24929–24932
33. Lander, H. M., Sehadjipal, P. K., and Novogrodsky, A. (1993) J. Immunol. 151, 7182–7187
34. Stamler, J. S. (1994) Cell 78, 933–936
35. Lipton, S. A., Chol, Y. B., Pan, Z. H., Lee, S. Z., Chen, H. S. V., Suche, N. J., Loscalzo, J., Singel, D. J., and Stamler, J. S. (1993) Nature 364, 626–632
36. Lander, H. M., Ogiste, J. S., Pearse, S. F. A., Levi, R., and Novogrodsky, A. (1995) J. Biol. Chem. 270, 7017–7020
37. Arndt, D. R., and Stamler, J. S. (1995) Arch. Biochem. Biophys. 318, 279–285
38. Clancy, R. M., Miyazaki, Y., and Cannon, P. J. (1990) Anal. Biochem. 191, 138–143
39. Le Gaos, F., Amiel, C., and Friedlander, G. (1991) Am. J. Physiol. 261, F587-F592
40. Gentry, M. K., and Olsson, R. A. (1975) Anal. Biochem. 64, 624–627
41. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
42. Snedecor, G. W., and Cochran, W. G. (1972) Statistical Methods, 6th Ed., pp. 593, Iowa State University Press, Ames, IA
43. Savic, V., Stefanovic, V., Ardailou, N., and Ardaillou, R. (1990) Immunology 107, 321–326
44. Savic, V., Blanchard, A., Vlahovic, P., Stefanovic, V., Ardaillou, N., and Ardaillou, R. (1991) Arch. Biochem. Biophys. 290, 202–206
45. Vlahovic, P., and Stefanovic, V. (1994) Arch. Int. Physiol. Biochem. Biopt. 102, 171–173
46. Nakal, M., Fukase, M., and Fujita, T. (1988) Biochem. Biophys. Res. Commun. 152, 1416–1420
47. Fini, C., Minelli, A., Camidi, M., and Floridi, A. (1985) Biochem. Biophys. Acta 827, 403–409
48. Zimmermann, H. (1992) Biochem. J. 285, 345–365
49. Fini, C., Parmentier, C. A., Damiani, P., Stochaj, U., Mannherz, H. G., and Floridi, A. (1990) Biochim. Biophys. Acta 1038, 18–22
50. Van Waarde, A., Stromski, M. E., Thuin, G., Gaudio, K. M., Kashgarian, M., Shulman, R. G., and Siegel, N. J. (1989) Am. J. Physiol. 256, F298-F305
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