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PAULA DÍAZ-SYLVESTER,[1,2] MYRIAM MAC LAUGHLIN,[1] AND CARLOS AMORENA[1,2]
[1]*Instituto de Investigaciones Cardiológicas-Consejo Nacional de Investigaciones Científicas y Técnicas, 1122 Buenos Aires; and* [2]*Escuela de Ciencia y Tecnología, Universidad Nacional de General San Martín, 1650 San Martín, Argentina*

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Díaz-Sylvester, Paula, Myriam Mac Laughlin, and Carlos Amorena. Peritubular fluid viscosity modulates H^+ flux in proximal tubules through NO release. *Am J Physiol Renal Physiol* 280: F239–F243, 2001.—We evaluated the effects of increasing the viscosity (η) in peritubular capillary perfusates (PCP; 20 mM $HNaPO_4^-$-Ringer, pH 7.4) on proximal convoluted tubule (PCT) acidification. Micropuncture experiments were performed with simultaneous luminal and peritubular perfusion. Changes in pH of a 20 mM $HNaPO_4^-$-Ringer (pH 7.4 at $t = 0$) droplet placed in PCT lumen were measured with H^+-sensitive microelectrodes. By adding neutral dextran (molecular wt 300,000–400,000) to the PCP, η was increased. The effect of 10^{-5} M ATP added to normal-η PCP was evaluated. High η increased H^+ flux (85 and 97% when η was increased 20 and 30%, respectively, above the control value). This increase was abolished by adding the nitric oxide antagonist N^{ω}-nitro-L-arginine (L-NNA; 10^{-4} M) or the purinoreceptor antagonists suramin (10^{-4} M) and reactive blue 2 (3×10^{-5} M). Addition of 5×10^{-3} M L-arginine to the peritubular perfusate overcame the inhibitory effect of L-NNA on high-η-induced increase in H^+ flux. ATP increased H^+ flux (80%), and this effect was blocked by L-NNA. These results suggest that changes in η can modulate proximal H^+ flux, at least in part, through ATP-dependent nitric oxide release from the endothelial cells of the peritubular capillaries.

nitric oxide; shear stress; adenosine 5′-triphosphate antagonists; N^{ω}-nitro-L-arginine; micropuncture; hydrogen ion secretion

OF THE RENAL BLOOD FLOW, ~80% circulates through the renal cortex. The peritubular capillaries stemming from the efferent arteriole form a large net in close vicinity to the renal tubules. The formation of a protein-free ultrafiltrate induces changes in the biophysical properties of blood leaving the glomeruli (19). One such property is viscosity (η), which depends on the plasma protein concentration and the hematocrit. Of the filtered load of volume and sodium, 70–80% is reabsorbed from the proximal tubular lumen toward the peritubular capillaries. The constancy of proximal fractional sodium and water reabsorption is called the glomerular-tubular balance (GTB). This is a highly regulated process and, under physiological conditions, is kept constant (14). The interplay of hydrostatic and oncotic pressures between peritubular capillaries and the interstitial space plays a critical role in maintaining GTB (21).

Recently, the endothelium-dependent relaxing factor, which is thought to be nitric oxide (NO) (22), was shown to be involved in the regulation of HCO_3^- reabsorption (or H^+ secretion) at the proximal convoluted tubule (PCT) of the rat kidney. Indeed, Wang (28), using micropuncture techniques, showed an increase in HCO_3^- reabsorption after NO formation. Simultaneously, we described a cGMP-dependent mechanism that promotes H^+ secretion in PCT by activating Na^+/H^+ exchange. This mechanism is stimulated by NO agonists added to peritubular capillary perfusates (1). Furthermore, Green et al. (13) showed that cGMP stimulates Na^+/H^+ exchange at the renal brush border. On the other hand, most of the Na^+ reabsorption in PCT depends on Na^+/H^+ exchange (4).

In large arteries, carbamylcholine, bradykinin, and ATP stimulate NO release and cGMP accumulation in endothelial cells (11). Another important agonist of NO release is shear stress (7, 23, 26). Moreover, shear stress also induces ATP release from endothelial cells (6, 7, 20). Between shear stress and the rate of change in shear strain, η is the constant of proportionality. The purpose of the present work was to address the question of whether changes in fluid η (i.e., shear stress) in peritubular capillaries could affect, by modifying NO release, proximal tubule H^+ transport, through a paracrine mechanism.

METHODS

Materials

H^+ ionophore, *cocktail A*, was purchased from Fluka (Ronkonkoma, NY). Other chemicals were from Sigma (St. Louis, MO), ICN (Costa Mesa, CA), and Pharmacia (Uppsala, Sweden).

General Procedure

Male Wistar rats, weighing ~300 g, were anesthetized with pentobarbital sodium (50 mg/kg ip) and prepared for mi-

Address for reprint requests and other correspondence: C. Amorena, M. T. de Alvear 2270, 1122 Buenos Aires, Argentina (E-mail: Carlos.Amorena@unsam.edu.ar).

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cropuncture experiments as previously described (2). Briefly, the right jugular vein was cannulated, a cannula was placed in the trachea, and the left kidney was exposed through a flank incision. Peritubular capillaries were perfused with micropipettes made of 1.5-mm-outer-diameter borosilicate glass tubing (Hilgenberg, Malsfeld, Germany) that had a tip diameter of 5–10 μm. PCTs in the perfused area were impaled with double-barreled micropipettes, one barrel containing Sudan black castor oil and the other, the luminal perfusion solution. The oil column injected in the tubule lumen was split by a droplet of perfusion solution, and a single-barreled pH-sensitive microelectrode, positioned two to three loops downstream, was employed to continuously measure the pH of the droplet.

pH Measurements

Liquid-membrane pH-sensitive microelectrodes were made as previously described (3) and had slopes of 54–58 mV/pH unit. Microelectrodes were calibrated in phosphate-Ringer buffer. The voltage difference between the pH microelectrode and a reference calomel electrode placed in contact with the skinned tip of the tail is proportional to the pH of the luminal fluid. Voltages were measured with a high-impedance electrometer (FD 223; World Precision Instruments, Sarasota, FL).

Solutions

The control peritubular perfusate contained (in mM) 105 NaCl, 5 KCl, 1 $CaCl_2$, 20 $HNaPO_4^-$, 1.25 $MgSO_4$, and 10 glucose. The control luminal perfusate contained (in mM) 75 NaCl, 5 KCl, 1 $CaCl_2$, 20 $HNaPO_4^-$, 1.25 $MgSO_4$, 10 glucose, and 90 raffinose. In Na^+-free experiments, Na^+ was replaced by the addition of choline chloride to a 5 mM HEPES solution. Na^+-free peritubular perfusate contained (in mM) 4 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 10 glucose, 5 HEPES, and 138 choline chloride. Na^+-free luminal perfusate contained (in mM) 4 KCl, 1 $CaCl_2$, 1 $MgSO_4$, 10 glucose, 5 HEPES, 98 choline chloride, and 90 raffinose. Final pH of luminal and peritubular solutions was adjusted to 7.4, and the osmolality, measured with a vapor pressure osmometer (model 5100C, Wescor, Logan, UT) was 290 mosmol/kg.

Theoretical Assumptions

During phosphate perfusion, H^+ secretion results in acidification of the luminal solution and titration of alkaline phosphate. Therefore, acid phosphate concentration rises and reaches steady state. Detailed treatments of this model have been previously published (2, 8, 12). Using pH values recorded from microelectrode measurements, H_2NaPO_4 concentration at time t was calculated according to

$$[H_2NaPO_4]_t = T/(10^{(pH_t - pK)} + 1)$$

where T is the total phosphate concentration ($[H_2NaPO_4] + [HNa_2PO_4]$) present in the luminal solution (20 mM) and the $pK = 6.8$ (12), where brackets indicate concentration. To calculate acidification rates, the log of ($[H_2NaPO_{4\,\infty}] - [H_2NaPO_{4\,t}]$), where $[H_2NaPO_{4\,\infty}]$ and $[H_2NaPO_{4\,t}]$ are the concentration of the injected phosphate at steady state and at time t, respectively, is plotted against time, in seconds. This plot can be fitted to a straight line, meaning that $[H_2NaPO_4]$ approaches exponentially to its steady-state value. The slope of this line is the acidification rate constant (κ). Net proton flux (J_{H^+}) was calculated according to

$$J_{H^+} = ([H_2NaPO_{4\infty}] - [H_2NaPO_{4t=0}]) \times \kappa \times r/2$$

where r is the tubule radius in centimeters (0.0015 cm).

Experimental Groups

The luminal perfusion solution was the same for all the experimental groups, except for the Na^+-free groups. The composition of peritubular capillary perfusion solutions varied as follows.

Control. Peritubular capillaries were perfused with the peritubular perfusate solution described above as the control.

Increased η-perfusion. Four sets of experiments were performed, in which η in peritubular capillary perfusates was increased 10, 20, and 30% over the normal Ringer values of η by addition of neutral dextran (molecular wt 300,000–400,000). In another experiment, Percoll (colloidal silica coated with polyvinyl pyrrolidone) was added to increase η 30% over the control value. The alteration in osmolality of peritubular perfusion solution by the addition of dextran or Percoll was negligible. The final η relative to Ringer-phosphate solution was measured with an Oswald viscosimeter at room temperature.

ATP. ATP (10^{-5} M) was added to the peritubular perfusion solution.

Antagonists of purinergic receptors. Suramin (10^{-4} M) or 3×10^{-5} M reactive blue 2 (RB2) was added to the high-η (η increased 30% by dextran) peritubular perfusate. Suramin is a nonselective P_2-purinoreceptor antagonist (9), and RB2 is a more selective P_2 type-Y-purinoreceptor antagonist (10).

Antagonist of NO. N^{ω}-nitro-L-arginine (L-NNA; 10^{-4} M) was added to the high-η (η increased 30% by dextran) or the ATP-containing peritubular perfusate. L-NNA inhibits the synthesis of NO, acting as a competitive antagonist of L-arginine, the substrate of NO synthase (15, 17, 27).

L-Arginine+L-NNA+high η. L-Arginine (5×10^{-3} M) was added to the high-η (η increased 30% by dextran) perfusate containing L-NNA.

Na^+ free. Composition of Na^+-free peritubular and luminal solutions is detailed above. Effect of high η in Na^+-free experiments was evaluated by increasing η 30% in peritubular Na^+-free perfusate by adding dextran.

ATP and L-NNA solutions were prepared freshly for every experiment.

Statistics

Results are shown as means ± SE. Data were evaluated with one-way analysis of variance, and multiple comparisons including all experimental groups were performed with a Student-Newman-Keuls test.

RESULTS

Kinetics of Proximal Luminal Acidification in Different Experimental Groups

The steady-state pH was the same in all groups studied. The acidification rate constant increased by increasing η in the peritubular perfusate with dextran as well as with Percoll. The addition of L-NNA blocked the effect of high η. The effect of L-NNA was overcome by addition of L-arginine to the high-η, L-NNA-containing perfusate. The addition of ATP to the control perfusate also increased the rate constant, and this effect was abolished by L-NNA. Suramin or RB2, antagonists of purinergic receptors, abolished the effect of high η on the acidification rate constant (Table 1).

Table 1. *Effects of peritubular pefusates with high viscosity, ATP, NO synthase blockade, and purinoreceptor blockade on the kinetics of proximal luminal acidification in micropuncture experiments with simultaneous luminal and peritubular perfusion*

	κ, s^{-1}	pH_∞	*n*
Control	0.22 ± 0.013	6.77 ± 0.012	82
Percoll (η increased 30%)	0.41 ± 0.048*	6.83 ± 0.022	23
Dextran			
η increased 10%	0.23 ± 0.012	6.73 ± 0.015	39
η increased 20%	0.40 ± 0.032*	6.80 ± 0.006	63
η increased 30%	0.45 ± 0.034*	6.83 ± 0.096	60
+L-NNA	0.14 ± 0.007*	6.84 ± 0.018	58
+L-NNA+L-arginine	0.37 ± 0.030*	6.76 ± 0.007	29
+RB2	0.20 ± 0.012	6.65 ± 0.019	28
+Suramin	0.23 ± 0.012	6.75 ± 0.021	58
ATP	0.41 ± 0.028*	6.79 ± 0.059	105
ATP+L-NNA	0.20 ± 0.012	6.76 ± 0.018	52

Values are means ± SE. *n*, No. of observations; NO, nitric oxide; L-NNA, N^w-nitro-L-arginine; RB2, reactive blue 2; κ, acidification rate constant; pH_∞, steady-state pH. *$P < 0.05$, significantly different from control.

Effects of Changes in η of the Peritubular Perfusate on PCT H^+ Flux

The control value for H^+ flux was 0.970 ± 0.050 nmol·cm^{-2}·s^{-1}. Perfusing peritubular capillaries with high-η perfusates induced an increase in H^+ flux (Fig. 1). Increasing η 20 or 30% above normal values with dextran-stimulated H^+ flux, which reached values of 85 and 97% higher than control (1.791 ± 0.140 nmol·cm^{-2}·s^{-1} by increasing η 20%; 1.907 ± 0.156 nmol·cm^{-2}·s^{-1} by increasing η 30%). This effect was not seen with an increase in η of 10%. Increasing η 30% by addition of Percoll also stimulated H^+ flux (1.655 ± 0.208 nmol·cm^{-2}·s^{-1}, 71% higher than control). The effect of Percoll was similar to that observed when η was increased with dextran. The stimulatory effect of high η on H^+ flux was abolished in the presence of the NO antagonist L-NNA, which decreased H^+ flux to 0.554 ± 0.037 nmol·cm^{-2}·s^{-1} ($P < 0.01$) (Fig. 2). This value was also significantly different from control ($P < 0.05$). To further test the specificity of NO synthase mediating the effects elicited by high η, perfusion was made with high-η solutions containing L-NNA together with L-arginine. In these experiments, H^+ flux was stimulated by high η (1.789 ± 0.140 nmol·cm^{-2}·s^{-1}) (Fig. 2). Thus the inhibitory effect of L-NNA was abolished by L-arginine.

Effects of ATP on PCT H^+ Flux

Addition of 10^{-5} M ATP to the peritubular perfusate induced a significant increase in H^+ flux. The value of H^+ flux was 1.746 ± 0.132 nmol·cm^{-2}·s^{-1}, and L-NNA inhibited the stimulatory effect of ATP (0.894 ± 0.044 nmol·cm^{-2}·s^{-1}) (Fig. 3).

Effects of Purinergic-Receptor Antagonists on Increased PCT H^+ Flux Induced by Dextran

The presence of antagonists of P_2 purinoreceptors in peritubular perfusates prevented the high-η-induced increase in H^+ flux (Fig. 4). The values of H^+ flux were 1.026 ± 0.046 and 0.855 ± 0.069 nmol·cm^{-2}·s^{-1} for dextran plus suramin and dextran plus RB2, respectively.

Effect of Na^+-Free Solutions on H^+ Flux in PCT

Net H^+ flux was significantly reduced with Na^+-free peritubular and luminal perfusion. In such conditions, H^+ flux was 0.125 ± 0.010 nmol·cm^{-2}·s^{-1} ($n = 27$). Increasing η 30% with dextran in peritubular Na^+-free perfusate did not induce any change in H^+ flux

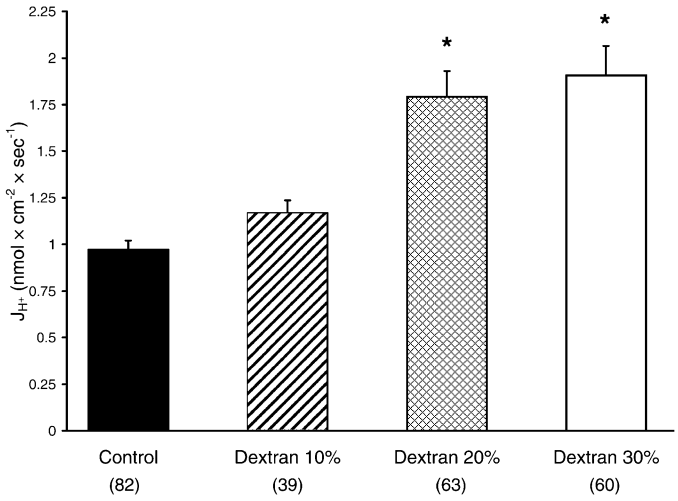

Fig. 1. Effect of increasing viscosity of peritubular perfusion solutions on H^+ flux (J_{H^+}) in proximal convoluted tubule (PCT). Addition of different amounts of dextran increased viscosity 10, 20, and 30% over the viscosity of the control solution. Nos. in parentheses, no. of observations. Values are means ± SE. *$P < 0.05$, significantly different from control.

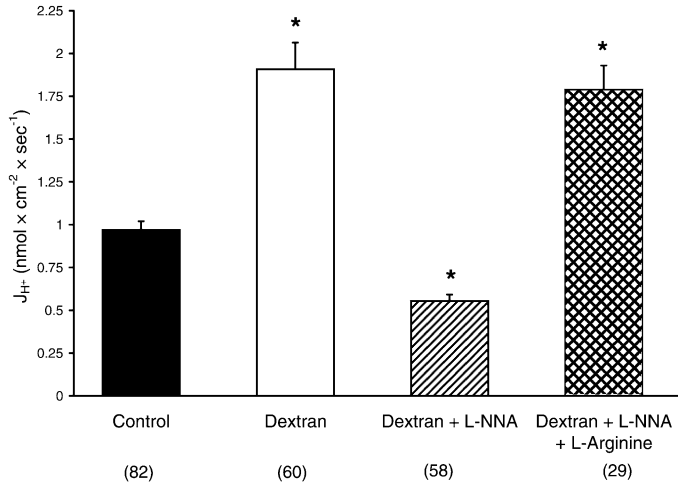

Fig. 2. Requirement of nitric oxide synthesis (NOS) for high-viscosity-induced increase in J_{H^+} in PCT. Dextran added to the peritubular perfusates increased viscosity 30% over the control solution. Concentration of N^ω-nitro-L-arginine (L-NNA) was 10^{-4} M, and concentration of L-arginine was 5×10^{-3} M. Nos. in parentheses, no. of observations. Values are means ± SE. *$P < 0.05$, significantly different from control.

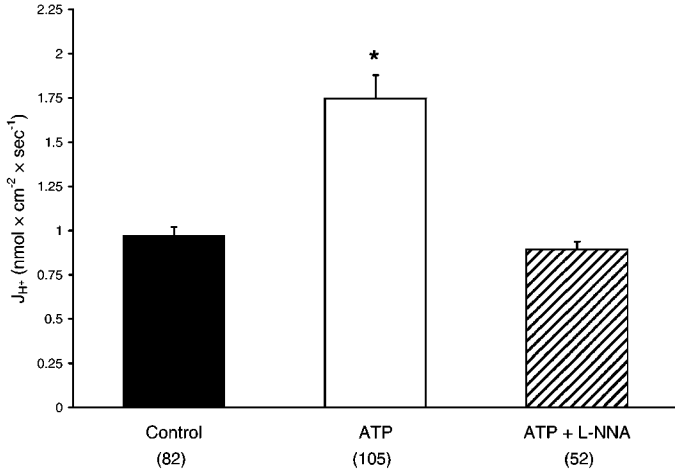

Fig. 3. Effect of ATP peritubular perfusion on J_{H^+} in PCT. ATP and L-NNA were added to peritubular perfusates at concentrations of 10^{-5} and 10^{-4} M, respectively. Nos. in parentheses, no. of observations. Values are means ± SE. *P < 0.05, significantly different from control.

compared with the Na^+-free solution with normal η (0.109 ± 0.010 nmol·cm^{-2}·s^{-1}; n = 39).

DISCUSSION

NO synthesis is involved in the control of kidney hemodynamic and tubular functions (16). Endothelial cells affect the function of epithelial cells by a paracrine-like mechanism involving the release of NO (1, 18, 27, 28). Moreover, NO induces an increase in proximal tubule acidification, which depends on cGMP production in proximal tubule epithelial cells (1, 28).

A consequence of glomerular filtration is an increase in the concentration of plasmatic proteins and hematocrit in the efferent arteriole. With a filtration fraction of 30%, the hematocrit will increase from 40 to 50% and the apparent η of blood would increase ~30% (29). We increased the η of the peritubular perfusate solution in this range. In the present work, we found that an increase in the η of the peritubular capillary perfusate induces an increase in proximal tubule acidification. This effect appears to be independent of the agent used to increase flow η, because peritubular perfusates containing either dextran or Percoll evoked the same response. The effect of high η was observed when the increase in η was >20%. An increase in η of 10% was without effect. The η-induced increase in PCT H^+ flux was blocked by L-NNA, suggesting that it depends on NO release. L-NNA not only abolished the effect of dextran but also inhibited H^+ flux below the control value. This result is coincident with previous observations, where we found that 10^{-4} M L-NAME decreased H^+ flux 37% below the control value (1). These results suggest that there is a basal NO-dependent H^+ flux. Shear stress is directly related to η and is a strong agonist of NO release in large arteries (7, 11, 23, 26). Moreover, the inhibitory effect of L-NNA on H^+ flux stimulated by high η was abolished by L-arginine. This result gives support to the hypothesis that NO release was involved in the modulation of H^+ flux by η. Furthermore, the effect of high η was absent in Na^+-free conditions, suggesting that the Na^+/H^+ exchanger is involved in the high-η-induced increase in H^+ flux. On the other hand, the effect of increased η was abolished by P_2-purinoreceptor blockade with suramin and RB2. Several subtypes of P_2 purinoreceptors have been characterized. Generally, vascular P_{2X} purinoreceptors are located on smooth muscle to mediate contraction, whereas P_{2Y} purinoreceptors, present on endothelial cells and on smooth muscle, are involved in vasodilatation (24). Although not highly specific, RB2 seems to be more selective for P_{2Y} (10); thus the effect of high η could be mediated, at least in part, by these receptors. ATP is a strong agonist of NO release from endothelial cells of large arteries (11). It has been shown that ATP is released from the endothelium by shear stress (6, 20). Therefore, changes in η could modify NO synthesis through ATP release. However, we cannot exclude that shear stress per se could stimulate NO release directly through activation of another kind of mechanism (5). In the present work, the peritubular perfusate containing 10^{-5} M ATP induced an increase in H^+ flux similar to that observed with dextran or Percoll. The effect of ATP resembled the effect previously found with bradykinin and carbamylcholine (1) and was also blocked by L-NNA, suggesting an involvement of NO release.

Our results agree with those reported by Wang (28), who found an increase in HCO_3^- flux in PCT induced by 10^{-6} M sodium nitroprusside (SNP), a NO donor. However, Linas and Repine (18) reported experiments, performed on cocultures of endothelial and epithelial cells, in which cGMP production induced by endothelium-derived NO decreased apical Na^+/H^+ exchange. Also, in rabbit proximal tubule cells, NO stimulated soluble guanylate cyclase and caused inhibition of Na^+/H^+

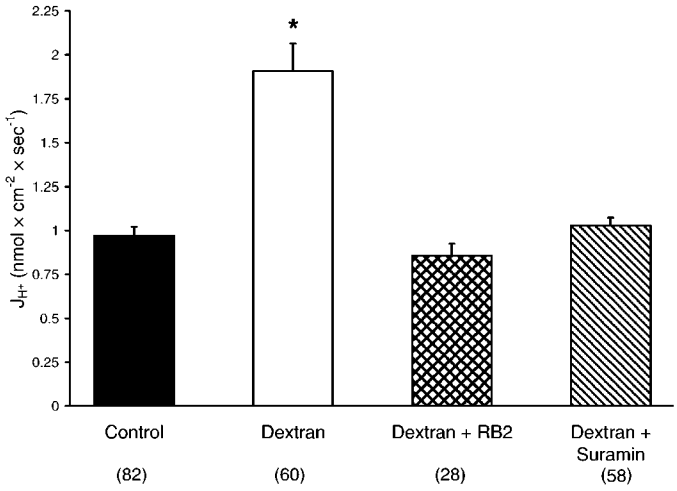

Fig. 4. Effect of antagonists of purinoreceptors on high-viscosity-induced increase in J_{H^+} in PCT. Reactive blue 2 (RB2) and suramin were added to peritubular perfusates at concentrations of 3 × 10^{-5} and 10^{-4} M, respectively. Dextran added to the peritubular perfusates increased viscosity 30% over the control solution. Nos. in parentheses, no. of observations. Values are means ± SE. *$P < 0.05$, significantly different from control.

exchange (25). Although we do not have a clear explanation for this discrepancy, there are at least two possible causes. First, we, like Wang (28), used an in vivo model, which implies a specific geometry between both structures, the epithelial cells of the proximal tubule and the underlying endothelium. Second, and perhaps more importantly, in our model the exposure to the agonists lasts a short time, no longer than 3 min, which, however, was long enough to perform H^+ flux measurements. Na^+ flux assessment, as measured by Linas and Repine (18), required longer exposure to the agonists. We did not subject our preparation to more prolonged NO exposure, which may have effects different from those reported here. On the other hand, Roczniak and Burns (25) used 1 mM SNP concentration. Wang (28) found that SNP has dual effects depending on its concentration, stimulating HCO_3^- reabsorption at low (1 μM) and inhibiting it at high concentration (1 mM).

In summary, NO produced by increased η stimulates the synthesis of cGMP in tubular epithelial cells, activating the Na^+/H^+ exchanger, and thus coupling filtered Na^+ load to proximal tubule Na^+ and water reabsorption. The present results suggest that a mechanism of this type could contribute, in addition to the Starling forces in the peritubular capillaries, to the control of GTB.

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