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NO· IN THE PARACRINE CONTROL OF THE PROXIMAL CONVOLUTED TUBULE OF THE RAT.

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Proximal convoluted tubule reabsorption[1].

PCT is the main responsible for bulk reabsorption of solutes and volume in the nephron. Almost 80% of the glomerular filtrate is reabsorbed from the lumen of the PCT through the peritubular capillaries. Considering an individual of 70 Kg this amounts up to 140L of water and almost 0.5 Kg of Na^+ reabsorbed per day; almost three times the total body water content and ten times the total body Na content. The whole process of Na^+ reabsorption can be divided in three sequential steps: the first one driven mostly by the electrochemical gradient for sodium at the apical membrane, the second, which is pivotal and responsible for O_2 consumption of the kidney, involves the activity of the Na^+-K^+ ATPase located at the basolateral membrane which extrudes Na^+ out of the cytosol , and the third one, due to solvent drag, occurring through the endothelial cells of capillaries, consequence of the Starling's equilibrium forces between the peritubular interstitium and the peritubular capillary lumen. Indeed, as a result of glomerular filtration the balance between oncotic pressure and hydrostatic pressure favors the net flow of volume from the interstitium through the capillary including the solutes present in the reabsorbed fluid.

Glomerulotubular balance.

A very delicate equilibrium occurs between the amounts of ultrafiltrate formed in the Bowman's capsule and the total volume reabsorbed in the PCT. In fact, the fractional amount of volume as well as Na^+ reabsorbed in the PCT remains a constant fraction of the filtered values, around 70%. This relationship is known as GTB and, as indicated above, physical forces account for an important part of the maintenance of this equilibrium although factors, like Na^+ load and tubular fluid velocity among others have been implied in this process (1, 2, 3). The fall in the hydrostatic pressure in the efferent arteriole accompanies the increase of the oncotic pressure in the blood emerging from the glomerulus as a result of the ultrafiltration process. The water reabsorbed by the epithelium is incorporated to the circulation as a consequence of the difference between these pressures, and the solutes are included by solvent drag in the volume flux between the interstitium and the capillary lumen. Equation 1 shows the relationship between forces favoring and opposing volume flux through the endothelium of the peritubular capillaries,

$$J_v = \kappa(Ph_i - Ph_c + \pi_c - \pi_i) \quad \textbf{Eq. 1}$$

where κ is the hydraulic conductivity of the endothelium including the area, and *i* and *c* stands for interstitial an capillary hydraulic (*Ph*) and oncotic (π) pressures respectively. The simultaneous determination of these driving forces allows for an analysis of the net

[1] ***Glossary.*** PCT: Proximal convoluted tubule, GTB: Glomerulotubular balance, NO·: Nitric oxide, CCh: Carbamylcholine, BK: Bradykinin, NLA: Nitro-L-Arginine, L-NAME: Nitro-L-Arginine Methyl Ester, GB: Glibenclamide, NBCI: Na-HCO3-Cl cotransporter SNP: Sodiumnitroprusside. η: viscosity.

pressure favoring fluid absorption or filtration. Changes in the rate of glomerular filtration imply changes in the relative pressures operating in the postglomerular capillary bed. Indeed, these physical factors, as stated above, account for the maintenance of the glomerulotubular balance. Changes in glomerular filtration rate will also induce tubular modifications affecting the absolute proximal reabsorption (1, 2, 4).

In this review I will consider the putative role of a paracrine mechanism operating in the structure composed by the endothelium of the peritubular capillaries and the epithelium of the PCT, particularly to the effect of $NO^{\cdot}$ on PCT function.

First, we must consider the close relationship between the endothelial cells and the basolateral membrane of the PCT cells.

As can be seen in the **figure 1** only a thin layer of basement membrane from both types of cell separates the endothelium from the epithelium and this anatomical arrangement facilitates the interaction between them.

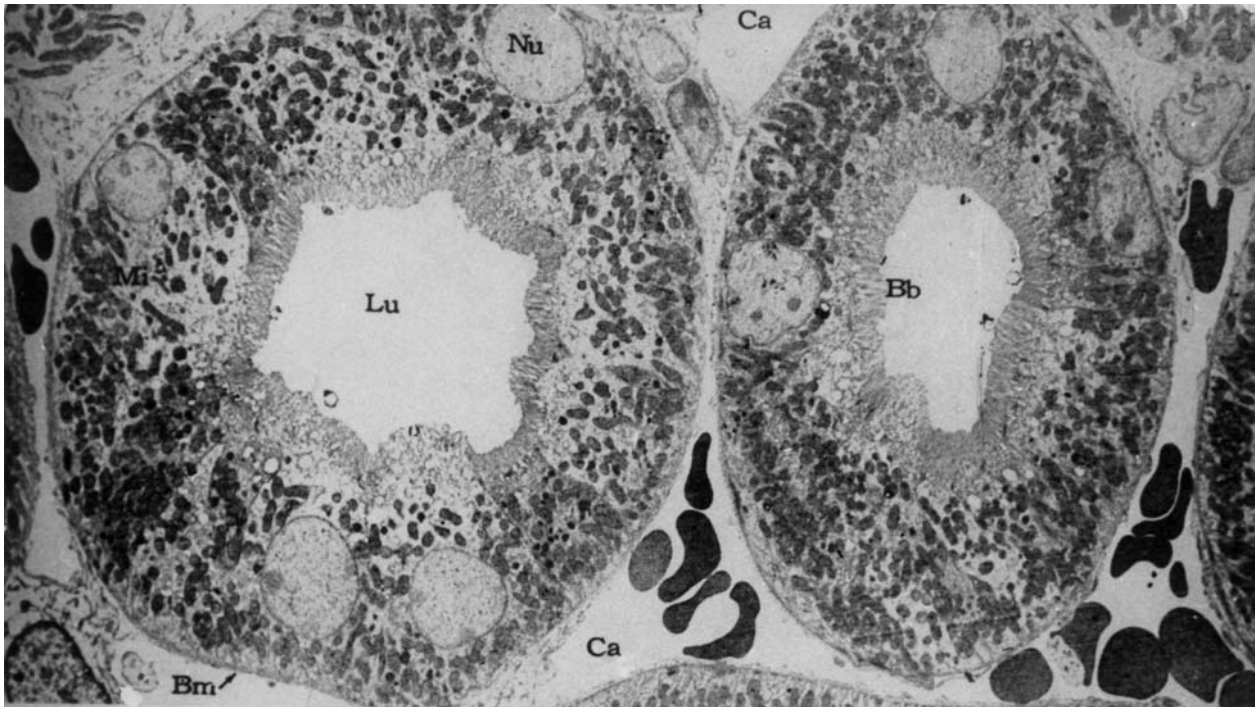

***Figure 1. Lu**: Proximal tubular lumen. **Nu**: Nucleus. **Bm**: Basolateral membrana. **Bb**: Brush border. Ca: Capillary.*

Hemodynamic changes due to $NO^{\cdot}$.

The observation, by Furchgott and Zawadzki, in 1980 that a factor of short average life, released by endothelial cells accounted for vasodilatation lead not only to a Nobel Prize but also to extensive research in several areas of the biological sciences looking for the role of $NO^{\cdot}$ in the control of different process, from immune response to neural differentiation. Nitric Oxide, produced in endothelial cells in response to different agonists like CCh, BK, ATP, and shear stress, acts in a paracrine way on the underlying smooth muscle cells of the vascular tree, stimulating the soluble guanylate cyclase and the formation of cGMP (4, 5, 6)

Experiments with nitro-l-arginine (NLA) and N-nitro-l-arginine methyl ester (l-NAME) have demonstrated that NO participates in the control of normal vascular tone in the kidney (7). Intra-arterial infusion of NLA in anaesthetized dogs increase renal vascular resistance 50% and reduces renal blood flow, 25% (8). Renal cortical NO activity increases steeply and linearly within the autoregulatory range, with increments in perfusion pressure. It has been suggested that it results from increased endothelial cell shear stress (9).

$NO^{\cdot}$ and tubular transport.

It is well known that the distal nephron is responsible for the fine tuning of final Na excretion. There are data showing that pressure natriuresis depends on decreased tubular sodium reabsorption through an amiloride sensitive transport process, implicating the distal tubule as an important site of $NO^{\cdot}$ action. This is in agreement with extensive data showing the inhibitory effect of $NO^{\cdot}$ on distal Na reabsorption (10).

The effect of $NO^{\cdot}$ on salt and water transport in the proximal tubule, which is responsible for reabsorbing 70% of filtered sodium and water, are controversial. Whilst some studies suggest that $NO^{\cdot}$ inhibits proximal tubule transport, others conclude that $NO^{\cdot}$ stimulates proximal tubule transport. NOS activity measured as the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline and/or accumulation of $NO^{\cdot}$ end-metabolites NO_2 or NO_2/NO_3 was detected in isolated rat proximal tubules, primary cultures of rat or human proximal tubule cells, and proximal tubule cell lines (11, 12, 13). The mRNA for soluble guanylate cyclase, which mediates the effect of $NO^{\cdot}$, seems to be more abundant in the proximal tubule than in most other tubular segments (14). Indeed, $NO^{\cdot}$ appears to modulate several types of transporters, from ionic channels trough modulation of Na^+-K^+-ATPase, Na^+/H^+ exchangers, and also paracellular permeability of opossum kidney cells (11, 15, 16, 17). Wang reported a biphasic effect of $NO^{\cdot}$ on proximal tubular transport of sodium and bicarbonate (15). An intravenous bolus injection of L-NAME followed by addition of L-NAME to the luminal perfusate modestly decreased proximal tubular fluid reabsorption and bicarbonate reabsorption. When 1μM sodium nitroprusside (SNP) or *S*-nitroso-*N*-acetylpenicillamine, both of which are $NO^{\cdot}$ donors, was added to the luminal perfusion solution, in proximal convoluted tubules, both were increased by 30~50%. However, proximal tubular volume and bicarbonate decreased by 50~70% with 1mM SNP. On the other hand, perfusion of the tubule lumen with dibutyryl cGMP increased net proton flux. Agents that elicit $NO^{\cdot}$ production, like BK, Cch, and ATP increase proton flux when added to the peritubular capillary perfusate. These effects were blocked byL-NAME. Bradykinin increased cGMP content of isolated proximal convoluted tubules, but only if they were co-incubated with endothelial cells (17, 18).

Basal $NO^{\cdot}$ production stimulates Na^+/H^+ exchange in proximal tubules of rats (16, 17, 18), whereas higher $NO^{\cdot}$ concentrations from exogenous sources inhibit Na^+/H^+ exchange and Na^+ reabsorption. It is important to note that doses of the SNP inhibit Na^+-K^+ ATPase and Na^+ reabsorption in proximal cells in the range of 0.4-1 mM which are substantially higher than the dose shown to stimulate proximal tubular reabsorption in vivo (19, 20). Interestingly, cholinergic agents increase the activity of the renal Na^+-HCO^-_3 cotransporter, this effect is mimicked by SNP and blocked by L-NAME (21). The increase in the Na^+/H^+ activity due to NO is consistent with this effect.

Another important agonist of $NO^{\cdot}$ formation in the vascular system is shear stress (22, 23). A viscous fluid (blood) moving along a solid boundary will undergo a shear stress on that boundary. In this condition the fluid has zero velocity relative to the boundary, but at some distance from the boundary the flow speed must equal that of the fluid. The shear stress is imparted onto the boundary (in our case the apical membrane of the endothelial cells) as a result of this loss of velocity and can be expressed as (Eq 2)

$$ss = \eta \frac{dv}{dy} \qquad \textbf{Eq. 2}$$

where η is the viscosity of the fluid, v is the velocity of the fluid along the boundary, and y is the distance from the boundary. Thus we can consider shear stress as an agonist of NO production like like BK, Cch etc. This point is important since as result of glomerular filtration the viscosity of the emerging blood in the efferent arteriole must increase. The increased η results from the increase of hematocrit (Htc)—with a filtration fraction of 0.3 Htc should raise from 0.45 to 0.64—as well as of the protein concentration. From a physiological point of view, increased η at the efferent arteriole would justify the generation of $NO\cdot$ that is able to affect functionally epithelial structures in the surroundings of the arteriole.

Changes in the viscosity of peritubular perfusate modify the reabsorption of bicarbonate in proximal convoluted tubules of rat's kidney. 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. These results suggest that there is a basal NO-dependent H^+ flux. Moreover, the inhibitory effect of L-NNA on H^+ flux stimulated by high η was abolished by L-arginine and by P_2-purinoreceptor blockade with Suramin and RB2 (20). It has been shown that ATP is released from the endothelium by shear stress (22, 23). Therefore, changes in η could modify $NO^{\cdot}$ synthesis through ATP release. These results strongly suggest that 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.

Given the structure of the peritubular capillaries net, it would not be strictly proper to consider blood flow to be Newtonian, thus the concept of viscosity turns to be rather misapplied. The apparent viscosity of blood in glass tubes declines with decreasing diameter (Fåhraeus-Lindqvist effect) and exhibits a distinctive minimum at 6–7 μm. However, flow resistance in vivo in small vessels is substantially higher than predicted by in vitro viscosity data. However, Lipowsky et al. (24) showed that the apparent blood viscosity is much higher in relatively straight, unbranched microvessels than in glass tubes with corresponding inner diameters.

Effect of $NO^{\cdot}$ on basolateral membrane voltage.

Finally, an additional effect of $NO^{\cdot}$ on proximal convoluted tubules cells has been documented. It has been reported that $NO^{\cdot}$ affects the activity of K^+ channels in the distal nephron segments, such as cortical collecting duct and thick ascending limb (25, 26).

Nakamura et al showed that $NO^{\cdot}$ modulates the activity of an inwardly rectifying K^+ channel in cultured human proximal tubule cells (27). These authors also show that the stimulatory effects of $NO^{\cdot}$ donors is abolished by a PKG-specific inhibitor and concluded that $NO^{\cdot}$ stimulated channel activity through the activation of soluble guanylate cyclase, consequent elevation of intracellular cGMP, and PKG-mediated phosphorylation. With micropuncture experiments we obtained evidence that agonists of $NO^{\cdot}$ formation at the peritubular capillaries affects basolateral membrane potential (V_{blm}) . Changes in the η of the peritubullar perfusate modified membrane potential at the basolateral membrane of PCT epithelial cells. As PCT epithelium is leaky, changes in V_{blm} affect also the apical membrane potential and thus the electrical gradient potential for Na^+ uptake. Also, BK, a chemical agonist, hyperpolarized the cells and both increased viscosity and BK effects were diminished by l-NAME. The effects of high η seem to involve the ATP sensitive K^+ conductance as GB abolishes the effect of increased viscosity (28). As it was discussed above, there is evidence that $NO^{\cdot}$ stimulates Na^+–H^+ exchanger at the apical membrane of PCT. This would lead to an incremented Na^+-K^+ ATPase activity. There is evidence that the activity of the Na^+–K^+ ATPase is linked to the K^+ conductance (29). An increase in Na^+–K^+ ATPase activity from an increase in Na^+ transport at the apical membrane domain, could lead to a decrease in ATP level and, consequently, to an increase in K^+ conductance. The NBC1 activity induces a depolarizing current at the basolateral membrane of the PCT (30). The effects of shear stress enhancing Na^+/H^+ exchange at the apical membrane and HCO^-_3 reabsorption through the NBC1 would generate a depolarizing current at the basolateral membrane. The hyperpolarizing effect at the basolateral membrane could counterbalance the depolarizing current, preserving the driving force for Na^+ from the lumen. The effects of HCO^-_3 transport at the basolateral membrane lead to a decrease in the electrical gradient for Na^+ across the apical membrane. The increase in gKATP must counterbalance the depolarizing effect of an increase in HCO^-_3 transport across the basolateral membrane.

All these results suggest the presence of a delicate equilibrium among these conductive transport processes in PCT that could be, at least in part, modulated naturally by glomerular filtration rate. The analysis of the mechanism as a whole seems to indicate that different control systems concur to damp fluctuation due to changes in GFR, i.e., tubuloglomerular feedback, thus maintaining the proximal Na^+ and water reabsorption constant in spite of changes in Na^+-filtered load. From a functional point of view, it looks like changes in shear stress through $NO^{\cdot}$ release modulate PCT epithelial cells function, synchronizing different transport mechanisms affecting the reabsorption process and coupling GFR with PCT reabsorption.

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