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Cross-talk between arterioles and tubules in the kidney

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Abstract

In hypertension the pressure natriuresis set point is shifted to a higher pressure due to an increase in both renal vascular resistance and sodium (Na) reabsorption. The afferent arterioles (Af-Arts) and efferent arterioles (Ef-Arts) account for most renal vascular resistance; they control glomerular filtration rate (GFR) and peritubular pressure, and, consequently, renal function. Af-Art and Ef-Art resistance is regulated by factors similar to those in other arterioles and also by tubuloglomerular feedback (TGF). TGF operates via the macula densa, which senses increases in sodium chloride (NaCl) and sends a signal that constricts the Af-Art and dilates the Ef-Art. In the outer renal cortex, the connecting tubule (CNT) returns to the glomerular hilus and contacts the Af-Art. This morphology is compatible with cross-talk between the CNT and Af-Art, so-called connecting tubule glomerular feedback (CTGF). Our studies show that increasing NaCl delivery to the CNT results in Af-Art dilatation that can be blocked by inhibitors of Na transport. We believe cross-talk between the CNT and Af-Art is a novel mechanism that may contribute to regulation of renal blood flow and GFR.

Keywords

Afferent arteriole; Macula densa; Connecting tubule; Tubuloglomerular feedback; Na transporter

The afferent arteriole (Af-Art) accounts for most renal vascular resistance. It controls glomerular filtration rate (GFR) and peritubular pressure, and consequently renal function. The hormonal and neural influences on vascular resistance that participate in the control of blood flow and GFR have been fairly well defined. Anatomical contact between the end of the thick ascending limb of the loop of Henle and its own glomerulus was described for the first time more than 100 years ago. In each nephron of the mammalian kidney, the tubule returns to the hilus of the parent glomerulus, forming the juxtaglomerular apparatus (JGA). The JGA is made up of (a) a plaque of specialized tubular epithelial cells called the macula densa; (b) the extra-glomerular mesangium; and (c) the glomerular Af-Art and efferent arteriole (Ef-Art). This anatomical connection is thought to be crucial for tubuloglomerular feedback (TGF) control of GFR [1–3]. Therefore, Af-Art resistance is regulated not only by factors similar to those in other arterioles but also by the macula densa. TGF describes a functional connection between the tubular epithelium at the site of the macula densa and the underlying smooth muscle cells of the Af-Art and Ef-Art. An increase or decrease in sodium chloride (NaCl) concentration in the luminal fluid at the macula densa cells activates arteriolar constriction or dilation, respectively, and hence alters single-nephron GFR (SNGFR).

Experimental studies of TGF began with the microinjection experiment designed and initiated by Klaus Thurau [4]. After the proximal segments belonging to the same nephron had been

outlined, loops of Henle were microperfused from the last superficial proximal segment. Flow in an upstream segment was interrupted by injection of an immobile block, and fluid was collected upstream to measure GFR. When the perfusion rate increased, there was an increase in NaCl concentration at the distal tubule and a parallel decrease in SNGFR [4,5]. Using a similar approach, Schnermann et al. [6] first measured stop-flow pressure (SFP), a close correlate of glomerular capillary pressure that shows the same sigmoidal relationship as perfusion rate. The concept of applying closed-loop measurements to study TGF was first proposed by Mason and Moore [7]. Later, Holstein-Rathlou [8] performed a closed-loop analysis of the TGF system by videometric flow velocitometry in hydropenic rats. In this study, the tubule was not blocked, and flow through the loop was perturbed by the addition or withdrawal of fluid at low rates. Using a similar technique, Thomson and Blantz [9] evaluated the ability of TGF to stabilize tubular flow (the so-called homeostatic efficiency of TGF). For this, they measured tubular flow upstream from the perturbation by a quasi-continuous videometric method in order to assess the compensatory power of the entire feedback loop. We studied TGF in vitro, using a technique we developed that consisted of perfusion of a microdissected Af-Art and its adherent tubular segment containing the macula densa. We induced TGF by increasing NaCl in the macula densa perfusate and measured changes in Af-Art diameter. Using this method, we showed that increased NaCl concentration at the macula densa constricts the Af-Art [10]. An accumulated body of data now stands as incontrovertible evidence that changes in NaCl concentration at the macula densa serve as the luminal signal that ultimately alters Af-Art resistance and hence SNGFR. Therefore, regulation of Af-Art resistance through TGF is well documented.

Because the Af-Art, glomerulus and Ef-Art are arranged in series, they regulate inflow and outflow of blood through the glomeruli, and thus their dynamics are closely interrelated. However, the specific response of the Ef-Art is not as well defined. In early micropuncture studies using the stop-flow technique, Schnermann and Briggs [11] reported that when the rate of perfusion through the loop of Henle was increased, SNGFR decreased while SFP remained essentially unchanged, suggesting that the change in SNGFR was primarily due to a reduction in ultrafiltration coefficient (K_f); and since SFP did not change, Af-Art and Ef-Art resistance were either unaltered or else were changed in the same direction. Likewise, Davis [12] administered a low dose of the calcium channel blocker nitrendipine and found that TGF-induced decreases in SNGFR were not accompanied by changes in glomerular capillary pressure (P_{GC}), suggesting parallel alterations in Af-Art and Ef-Art resistance. Briggs and Wright [13] further examined stellate vessel pressure and single-nephron plasma flow, as well as SNGFR and SFP (as indicators of P_{GC}), while raising the perfusion rate from 16 nl/min to 40 nl/min. They found that both SNGFR and SFP decreased but stellate vessel pressure did not change, and they concluded that the feedback response to increased flow through the loop of Henle was probably mediated primarily by Af-Art constriction. Since stellate vessel pressure did not change despite the decrease in P_{GC} , one possible explanation was that Ef-Arts dilated during TGF, decreasing resistance and improving transmission of pressure to the stellate vessels. Persson et al. [14] examined TGF responses of SNGFR and glomerular capillary pressure in control rats and in rats subjected to simultaneous blockade of angiotensin II and prostaglandin. They found that, in the control, the increasing of late proximal perfusion decreased SNGFR and P_{GC} , although the turning point for changes in pressure always appeared at higher flows. After blockade, the SNGFR response remained intact, but P_{GC} did not change. Although changes in Af-Art and Ef-Art resistance were not directly assessed in that study, the authors attributed to the Ef-Art vasodilation achieved in the control condition, after combined hormonal blockade had led to vasoconstriction of the Ef-Art, which allowed maintenance of P_{GC} . However, most of the evidence supporting participation of the Ef-Art in TGF is indirect, depending on calculated values, and direct measurements are needed in order for one to be certain. Using the isolated perfused macula densa and Ef-Art in vitro, we have obtained direct evidence that precontracted Ef-Arts relax when the NaCl concentration at the macula densa

is increased [15]. Although none of the in vivo micropuncture studies produced convincing evidence or required net Ef-Art dilatation to explain the observed TGF-induced reductions in SNGFR, based on our direct evidence a possible contribution of Ef-Art dilatation in response to large increases in the TGF signal could not absolutely ruled out. Based on our results and previous micropuncture studies, Blantz and Vallon proposed that, in basal conditions, the co-response of the Af-Art and the strength of TGF stimuli may dictate the net magnitude, and even the direction, of the Ef-Art response [16].

It is widely assumed that an increase in luminal NaCl concentration causes changes in the interstitium of the JGA that alter the function of vascular smooth muscle cells. Because of the cellular discontinuity, the most common notion is that these changes affect both the appearance and interstitial concentration of a paracrine mediator. A number of such mediators, including adenosine triphosphate (ATP), prostaglandin E₂, and nitric oxide have been invoked on the basis of pharmacological inhibitor studies. Rather solid pharmacological evidence derived from various in vivo and in vitro preparations has implicated adenosine as a major mediator of the TGF response [17–19]. In vitro, using an isolated double-perfused Af-Art and distal tubule containing the macula densa, we found that the adenosine A₁ receptor antagonist FK838 fully prevented Af-Art constriction in response to an increase in the luminal NaCl concentration of the macula densa [10]. TGF responses of SFP or SNGFR were found to be entirely absent in adenosine A₁ knockout mice [20]. The exact source of adenosine still remains to be identified. Reportedly, ATP is released from macula densa cells into the interstitium of the extraglomerular mesangium, where it is broken down by nucleoside triphosphate diphosphohydrolases to adenosine monophosphate (AMP) [21,22] and is further hydrolyzed to adenosine by the ecto-5'-nucleotidase [10]. In ecto-5'-nucleotidase/CD73-deficient mice [23] as well as in rats or rabbits, when the e-5'-NT/D73 inhibitor β,γ -methylene adenosine diphosphate was used, TGF response was found to be markedly impaired [18,24]. Huang et al. found that adenosine generated by both ecto-5'-nucleotidase-dependent and -independent mechanisms participated in mediation of TGF in vivo [25]. We and others have shown that adenosine constricts the Af-Art and dilates the Ef-Art via activation of the A₁ and A₂ receptor, respectively [26–29]. In vitro, using an isolated double-perfused Ef-Art and distal tubule containing the macula densa, we have shown that decreasing adenosine formation by blocking 5'-nucleotidase inhibits Ef-Art TGF, while increasing adenosine formation by enhancing ATP hydrolysis augments Ef-Art TGF. We also showed that increasing NaCl at the macula densa induced Ef-Art dilatation, which was blocked by the adenosine A₂ antagonist [15]. The ecto-5'-nucleotidase that hydrolyzes AMP is widely distributed in the kidney and is expressed in all nephron segments, including the glomerular tuft and the macula densa cells [30–32]. Based on these findings, it is reasonable to propose that Af-Art and Ef-Art TGF share the same mediator, namely, adenosine, which activates the adenosine A₁ receptor in the Af-Art, causing it to constrict [24,33], and also activates the adenosine A₂ receptor in the Ef-Art, causing it to dilate [15,34].

In humans and all other mammals studied to date, there is a transitional region of varying length between the distal convoluted tubule (DCT) and the cortical collecting duct, called the connecting tubule (CNT). In the rabbit this segment is well demarcated and consists of three specific cell types: CNT cells, and intercalated cells, type A (light) and type B (dark) [35]. Faarup [36] in 1965 examined serial sections of the rat kidney and commented that the “intercalated part” of the distal tubule returned to the vascular pole of the glomerulus and accompanied the Af-Art for varying distances. Based on our immunohistochemical studies of kallikrein (located in the CNT) and renin (located mainly in the Af-Art), we concluded that in rats the CNT is consistently in close proximity to the Af-Art [37]. Vio et al. [38] reported that this association also occurs in humans. In the renal outer cortex it reportedly occurs in 66% to 100% of nephrons, while in the middle and inner cortex, it occurs less frequently [37,39].

Based on these data, we and Morsing et al. speculated that this morphology is compatible with the existence of cross-talk between the CNT and the Af-Art [37,39,40], and we have obtained direct evidence of this by simultaneously perfusing a microdissected Af-Art and adherent CNT (Fig. 1) [41]. Increasing the concentration of NaCl in the CNT from 10 mM to 80 mM markedly dilated the precontracted Af-Art. We called this effect “connecting tubule glomerular feedback” (CTGF). We found that Na entry into the CNT via epithelial sodium channels (ENaCs) is required in order to induce CTGF, and that inhibiting nitric oxide (NO) production in the CNT potentiates CTGF [41]. There is in vivo evidence that the distal tubule regulates Af-Art tone. Morsing et al. [40] performed in vivo micropuncture studies of TGF with and without interrupting distal tubule flow and found that interruption of flow significantly raised both the maximum decrease in SFP and the perfusion rate that elicited a half-maximal decrease in SFP (V1/2). Their results support the notion of a role for the distal nephron in the control of TGF. Distal blockade affects the TGF system in at least two ways: it diminishes the sensitivity of the TGF sensing step to a given signal, and it enhances the final constrictor effect on the Af-Art. This suggests that in addition to TGF, there is also crosstalk between the Af-Art and either the DCT, CNT or cortical collecting duct. Schnermann et al. [42] showed that to induce a maximum decrease in early proximal flow rate (an indicator of TGF) during orthograde perfusion of the macula densa (from the late proximal tubule) they needed to use 40 nl/min, double the rate employed for retrograde perfusion (from the early distal tubule). This can be interpreted in two ways: (a) during orthograde perfusion, fluid reaches the CNT and causes CTGF, which partially antagonizes TGF; thus, to cause maximum TGF, the rate of perfusion needs to be higher. On the other hand, retrograde perfusion from the early distal tubule will not cause CTGF, and thus the perfusion rate needs to be lower to cause maximum TGF response; or (b) in orthograde perfusion from the late proximal tubule there is greater Na reabsorption by the late proximal tubule and loop of Henle than during retrograde perfusion of the early distal tubule. Nevertheless, these results raise the possibility that modifications in the flow rate or composition of tubular fluid downstream from the macula densa affect GFR by modifying the TGF response. Our studies are critical to solve the question of whether or not the anatomical relationship between the CNT and Af-Art plays a role in the regulation of renal vascular resistance.

An obvious question would be why the kidney needs two cross-talk mechanisms between the tubular segment and the glomerular structure. There are similarities and differences between TGF and CTGF. TGF operates via the macula densa, whereas CTGF operates via the CNT. Both sense changes in Na⁺ concentration; however, Na⁺ enters the macula densa cells via the Na⁺K⁺2Cl⁻ cotransporter, whereas in the CNT it enters via the ENaC. Both TGF and CTGF are potentiated by inhibition of NO synthesis in the tubule; however, TGF causes constriction of the Af-Art, while CTGF causes dilatation, so that, while renal blood flow and GFR decrease in response to TGF, they probably increase with CTGF. CTGF is a novel regulatory mechanism of the renal microcirculation that may result in the Af-Art dilatation and increased GFR observed during high salt intake, perhaps by antagonizing or resetting TGF. In pathological situations, as for example in rats with saltsensitive hypertension, where sodium reabsorption is upregulated in the aldosterone-sensitive segment in response to high sodium intake, CTGF may increase intraglomerular pressure and renal damage by dilating the Af-Art. The CNT is at the end of the DCT; these two nephron segments are aldosterone-sensitive and play a pivotal role in the regulation of Na and K excretion. We believe cross-talk between the arterioles (Af-Art and Ef-Art) and tubule (macula densa and CNT) is a novel mechanism that contribute to regulation of renal blood flow and GFR.

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Fig. 1.

Cross-talk between tubule and arteriole. **a** Schematic representation of the relationship of nephron and vasculature. **b** *Left* simultaneous perfusion of a microdissected rabbit Af-Art and attached MD. *Right* effect of perfusing the macula densa (MD) with low or high concentration of NaCl on Af-Arts or Ef-Arts. **c** *Left* simultaneous perfusion of a microdissected rabbit Af-Art and attached CNT. *Right* effect of perfusing the CNT with low or high concentration of NaCl on precontracted Af-Arts. CNT connecting tubule, Af-Art afferent arteriole, Ef-Art efferent arteriole, DCT distal convoluted tubule, TAL thick ascending limb, TGF tubuloglomerular feedback, CTGF connecting tubule glomerular feedback