In every nephron the glomerular filtration rate is adapted to changes in the salt concentration of early distal tubular fluid through the mechanism of tubuloglomerular feedback. Recent studies indicate that adenosine and possibly ATP mediate this mechanism and demonstrate its role in glomerular hemodynamic alterations in the early diabetic kidney.

The functional unit of the kidney, the single nephron, shows a particular organization: the tubular system that originates from every glomerulus makes along its run a second contact with its own glomerulum. More precisely, the end of the thick ascending limb of the loop of Henle runs through an angle generated by the afferent and efferent glomerular arterioles and thus makes contact with the vascular pole of its own glomerulum. The juxtaglomerular apparatus comprises all structures that form this site of contact and represents a functional and structural link between 1) the macula densa cells, which represent specialized tubular cells at the end of the thick ascending limb of Henle's loop, 2) the cells of the extraglomerular mesangium, which fill the angle between the afferent and the efferent glomerular arteriole, and 3) the vascular smooth muscle cells and renin-secreting cells in the media of the afferent glomerular arteriole.

The functional relevance of this particular tubuloglomerular contact site is related to the large amounts of fluid and electrolytes that are filtered from the glomeruli into the tubular system, namely ~180 l of fluid and 25 mol of sodium every day in healthy adults. This “glomerular leak” poses a permanent danger for excessive body fluid and electrolyte loss, and on a standard Western diet only ~0.5% of the fluid or sodium filtered in the glomeruli is actually excreted in the urine. The remaining 99–99.5% is reabsorbed along the tubular and collecting duct system. This implies a fine coordination of glomerular filtration and subsequent reabsorption of fluid and electrolytes to adjust the urinary excretion according to bodily needs. The latter may be appreciated from the fact that a disparity of as little as 5% between glomerular filtration and subsequent reabsorption would lead to a net loss of about one-third of total extracellular fluid volume in one day, a situation that inevitably would lead to vascular collapse. Conversely, renal sodium retention exceeding the body’s needs is thought to play an important role in the development of arterial hypertension. Besides glomerulotubular balance, i.e., the normal flow dependence of tubular reabsorption in every nephron segment, the juxtaglomerular apparatus significantly contributes to the fine coordination between glomerular filtration and tubular reabsorption through the mechanism of tubuloglomerular feedback (TGF).

The TGF mechanism refers to a series of events whereby changes in the Na⁺, Cl⁻, and K⁺ concentrations in the tubular fluid are sensed by the macula densa via the Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2) in its luminal membrane. An increase or decrease in glomerular filtration rate (GFR) by altering the vascular tone predominately of the afferent arteriole (for review on TGF, see Ref. 10). NKCC2 is inhibited by loop diuretics like furosemide and therefore loop diuretics do not lower GFR even though they increase the salt concentration at the macula densa, which contributes to their potent diuretic effect. As a consequence of TGF, the fluid and electrolyte delivery to the distal nephron is kept within certain limits, which facilitates the fine adjustments in reabsorption or excretion in the distal nephron under the control of aldosterone and vasopressin. In this regard, the TGF mechanism serves to establish an appropriate balance between GFR and tubular reabsorption upstream from the macula densa. In the absence of primary changes in reabsorption upstream from the macula densa, by adjusting GFR to keep early distal tubular fluid and electrolyte delivery constant, the TGF mechanism also contributes to autoregulation of GFR, which is a hallmark of kidney function. These two issues, namely the role of TGF in renal autoregulation as well as in stabilization of distal fluid and electrolyte delivery, are outlined below in the special context of the diabetic kidney. But first, some new insights on the signal transmission in the juxtaglomerular apparatus are discussed.

What is the mediator of TGF in the juxtaglomerular apparatus?

The mechanism(s) by which the macula densa cells transduce the luminal signal, i.e., the luminal Na⁺, Cl⁻, and K⁺ concentrations at the macula densa, into one or more mediators that alter afferent arteriolar tone is still incompletely understood. What are the requirements for a mediator of the TGF response? First, within seconds the factor must induce an afferent arteriolar vasoconstriction that persists in the presence of the mediator but rapidly vanishes when the mediator is withdrawn. Second, the factor must be generated or released locally, depending on the luminal salt concentration at the macula densa. Because a rise in the salt concentration at the macula densa is in addition associated with an inhibition of renin secretion, the factor should also have an inhibitory action on renin release if the factor mediates both responses.
In 1980, Osswald and colleagues (7) proposed that adenosine may act as a mediator of TGF and that the concept of an adenosine-mediated metabolic control of organ function may also apply to the kidney. In contrast to other organs, blood flow in the kidney cortex primarily determines glomerular filtration and consequently energy-consuming transport work. Thus, unlike other organs such as heart or muscle, a metabolic control of kidney function requires a renal cortical vasoconstrictor. In fact, adenosine is a vasoconstrictor of the afferent arteriole, and the sustained afferent arteriolar vasoconstriction during continuous application of adenosine into the renal artery induces a sustained reduction in GFR of the whole kidney as well as of superficial single nephrons accessible to micropuncture (8). In addition, the vasoinhibition is rapid in onset and short in duration when adenosine is withdrawn. Importantly, with regard to TGF, local pharmacological blockade of the adenosine A\textsubscript{1} receptor by 8-cyclopentyl-1,3-dipropylxanthine was found to completely abolish the TGF response (12). Furthermore, local application of the 5'-nucleotidase inhibitor α,β-methylene adenosine 5'-diphosphate (MADP), i.e., a substance that inhibits the conversion of AMP to adenosine, exerted a similar response (14). More recently, two groups have investigated the role of adenosine A\textsubscript{1} receptors in TGF response by studying respective knockout mice. Sun et al. (13) reported that the TGF response assessed as the fall in nephron filtration rate was completely inhibited in adenosine A\textsubscript{1} receptor knockout mice. Similar results were reported by Brown et al. (3), and this group in addition found significantly greater plasma renin activity in the adenosine A\textsubscript{1} receptor knockout mice. The latter finding confirms previous pharmacological studies using adenosine A\textsubscript{1} receptor inhibitors and is consistent with adenosine-mediated inhibition of renin release.

The above findings propose that adenosine is involved in TGF-mediated control of GFR. The findings do not address, however, how adenosine contributes to this control. Local adenosine could establish a relative constant vasoconstrictor tone that establishes a necessary background for another mediator to elicit the TGF response, and thus adenosine may act as a modulator of TGF rather than a mediator. Angiotensin II serves such a modulating role as outlined below.

Alternatively, intact TGF may require local adenosine concentrations in the juxtaglomerular apparatus to vary directly with luminal NaCl concentration at the macula densa, which would imply a role for adenosine as a mediator of TGF. To further elucidate the role of adenosine as a mediator or modulator, we studied whether activation of adenosine A\textsubscript{1} receptors in the juxtaglomerular apparatus fluctuates as a function of the NaCl concentration at the macula densa for a normal TGF response to occur. To address this issue, we performed micropuncture experiments in rat kidney in which the local adenosine A\textsubscript{1} receptor activity was clamped by inhibiting 5'-nucleotidase with MADP and adding back an adenosine A\textsubscript{1} receptor agonist, namely cyclohexyladenosine, to mimic a constant local adenosine A\textsubscript{1} receptor activation (14). If local adenosine would just induce a relative constant adenosine A\textsubscript{1} receptor-mediated vasoconstrictor tone to establish a necessary background for another mediator to elicit the TGF response, then the above maneuver should not inhibit the TGF response. It was observed, however, that this maneuver significantly reduced the slope of the TGF curve, which experimentally is determined as the dependence of nephron filtration rate on late proximal tubular flow rate. An alteration in the latter changes the fluid and salt load to the loop of Henle, thus the salt concentration at the end of the loop of Henle, at the macula densa.

Another approach to study TGF is to perfuse the macula densa in a retrograde fashion from the early distal tubule, which, because of the proximity to the macula densa, allows for better control of the applied drug concentrations at the juxtaglomerular apparatus. Using this approach, we found that clamping of adenosine A\textsubscript{1} receptor activation as described completely inhibited the fall in nephron filtration rate in response to an increase in NaCl concentration in the perfusate (14). Thus TGF is attenuated by adenosine A\textsubscript{1} receptor blockade or inhibiting 5'-nucleotidase-mediated generation of adenosine and cannot be restored by establishing a constant adenosine A\textsubscript{1} receptor activation. These data suggest that adenosine activity must fluctuate for normal TGF to occur, indicating that adenosine is a mediator of TGF.

The concept proposed by Osswald and colleagues (7) that the juxtaglomerular apparatus adenosine couples energy metabolism (ATP hydrolysis for tubular electrolyte transport) to the control of GFR (and renin secretion), i.e., adenosine acts as a mediator of TGF, could be realized in the following manner (Fig. 1): cotransport-dependent hydrolysis of ATP in the macula densa cells (or in the tubular cells in close proximity to the juxtaglomerular apparatus) would lead to enhanced generation of AMP. The generated AMP is dephosphorylated in the cell to adenosine by cytosolic 5'-nucleotidase or plasma membrane-bound endo-5'-nucleotidase, and the generated adenosine could be released through a nucleoside transporter into the interstitium of the extraglomerular mesangium. Alternatively, AMP may leave the cell and plasma membrane-bound ecto-5'-nucleotidase may convert it to adenosine in the interstitium. The 5'-nucleotidase inhibitor MADP that inhibited TGF in the above-mentioned micropuncture studies (14) is supposed to inhibit plasma membrane-bound 5'-nucleotidase but not AMP-specific cytosolic 5'-nucleotidase. Thus plasma membrane-bound ecto- or endo-5'-nucleotidase may serve to generate the adenosine mediating the TGF. Extracellular adenosine then binds to adenosine A\textsubscript{1} receptors at the surface of extraglomerular mesangial cells and increases cytosolic Ca\textsuperscript{2+} concentrations (6). Gap junctions transmit the Ca\textsuperscript{2+} transients to the target cells in the afferent arteriole, which results in inhibition of renin release and afferent arteriolar vasoconstriction.

Alternatively (or in addition), the source of ATP being used for local adenosine formation may not contribute to macula

"...adenosine is involved in TGF-mediated control of GFR."
densa transport: a recent in vitro study suggests that ATP could be released across the basolateral side of macula densa cells through a large-conductance anion channel dependent on changes in the salt concentration in the luminal fluid at the macula densa (2). It was further proposed that the released ATP itself through activation of P2 receptors triggers an increase in cytosolic Ca\(^{2+}\) in the extraglomerular mesangial cells and/or the smooth muscle cells of the afferent arteriole, and thus ATP acts as the principal mediator of TGF (1, 5).

However, no convincing data on a direct role of a P2 receptor in the TGF response, such as studies with selective receptor antagonists or knockout mice, have been published. Considering the outlined existing evidence for adenosine and adenosine A\(_1\) receptors in mediating TGF, it seems possible that ATP being released from the macula densa is converted in the interstitium by ecto-ATPase and ecto-5’-nucleotidase to adenosine. In this situation, ATP and adenosine would both be considered mediators of TGF since both are generated or released depending on the NaCl concentration at the macula densa and both are part of a signaling cascade that finally triggers afferent arteriolar vasoconstriction. It even seems possible that concomitant activation of both adenosine A\(_1\) receptors and P2-type purinergic receptors may be required for a normal TGF response to occur. This thought is derived from the observation that concomitant angiotensin II AT\(_1\) receptor activation is also a prerequisite for adenosine-mediated afferent arteriolar vasoconstriction and TGF response (for review, see Refs. 10 and 16). Clearly, to further delineate the relationship between adenosine and ATP, studies on the role in TGF of ecto-ATPase, ecto- and endo-5’-nucleotidase, as well as P2-type purinergic receptors, including studies in knockout mice, are required (11).

In contrast to the mediator adenosine, angiotensin II and nitric oxide (NO) are considered to be modulators of TGF. Studies in rats, rabbits, or respective knockout mice have shown that inhibition or deficiency of angiotensin-converting enzyme (ACE) activity or angiotensin II AT\(_1\) receptors blunts the TGF response as well as adenosine-mediated afferent arteriolar vasoconstriction (for review, see Refs. 10 and 16). The experiments, however, also suggested that angiotensin II is not a mediator but a modulator of TGF: it was demonstrated that constant systemic infusion of angiotensin II is sufficient to restore attenuated TGF activity at least in part during pharmacological ACE inhibition and completely during volume expansion, which is known to suppress angiotensin II activity. Accordingly, constant systemic infusion of angiotensin II restored the TGF response in ACE knockout mice. These findings show that an intact TGF response does not require local angiotensin II concentrations to vary with the luminal TGF signal, indicating that angiotensin II is not a mediator of TGF. Angiotensin II through activation of angiotensin AT\(_1\) receptors rather modulates and adapts the TGF response to the salt and volume status of the body. How this modulation or adaptation works on the cellular or molecular level remains to be determined.

The renal expression of neuronal NO synthase (NOS I) is predominantly localized in macula densa cells, suggesting a
role for NO in TGF. Studies in rats, rabbits, and NOS I knockout mice have shown that inhibition or deficiency of macula densa NOS I enhances the TGF response to a given change in the salt concentration at the macula densa (for review, see Refs. 10, 11, and 16). Inhibition of a TGF mediator, however, is not expected to enhance the TGF response. Thus macula densa-generated NO does not act as a mediator of vasodilation but as a modulator of TGF. These results suggest further that macula densa-derived NO tonically attenuates the GFR-reducing influence of the TGF-mediating vasoconstrictor. As a net effect and consistent with a role in TGF resetting, macula densa-derived NO appears to increase the fluid and electrolyte load to the more distal nephron segments, which would be advantageous under conditions of volume expansion.

TGF-mediated dynamic and steady-state control of nephron filtration rate in diabetes mellitus

The physiological role and the pathophysiological importance of TGF can be illustrated in diabetes mellitus, which today is a leading cause of end-stage renal disease. The pathogenesis of diabetic nephropathy is poorly understood, but an important role can be ascribed to glomerular hyperfiltration, which is associated with reduced afferent arteriolar resistance and which occurs early in the course of diabetes. The afferent arteriolar resistance is important for both the dynamic autoregulation as well as for the steady-state control of renal function, and thus abnormalities of the afferent arteriole can be reflected 1) by greater minute-to-minute variability in GFR and glomerular capillary pressure as well as 2) by time-averaged increases in these parameters, e.g., glomerular hyperfiltration. The following outlines the dual effect of diabetes on TGF, namely 1) impaired TGF-mediated dynamic autoregulation and 2) TGF-mediated steady-state glomerular hyperfiltration.

Autoregulation of renal function is effected at the afferent arteriole by pressure-induced vasomotion and by TGF. We used a noninvasive optical technique in anesthetized rats to measure tubular flow rate in the proximal tubule under freeflowing conditions. Tubular flow rate was measured just upstream from a microperfusion pipette that was introduced into the late proximal tubule to add or subtract small amounts of tubular fluid. The response of tubular flow rate upstream to the perturbation is a measure of the ability of the TGF system to stabilize tubular flow rate. In addition, an insulin-dependent diabetes mellitus was induced by application of streptozotocin, which destroys insulin-secreting pancreatic β-cells. With this approach, we determined that the ability of the TGF system to compensate for small perturbations in proximal tubular flow is reduced in rats with early diabetes mellitus and that the defect is localized within the juxtaglomerular apparatus (17). Since the ability of the TGF system to stabilize proximal tubular flow predicts its ability to stabilize nephron filtration rate (17), these findings imply that the diabetic kidney is less able to autoregulate GFR. As a consequence, TGF buffers any given disturbance in renal perfusion pressure less efficiently in the diabetic kidney, which results in a greater variability in GFR and glomerular capillary pressure. Notably, blood pressure lability is known to be associated with longstanding diabetes in humans, and cardiovascular autonomic nerve dysfunction is relatively common, even in young patients newly diagnosed with insulin-dependent diabetes mellitus. Furthermore, cyclical mechanical stretch-relaxation of glomerular mesangial cells was shown to induce these cells to grow and to elaborate extracellular matrix proteins in vitro (9). Together these findings suggest that diabetes decreases the homeostatic efficiency of TGF, which contribute to the development of diabetic glomerular sclerosis by exposing the glomerular capillaries and mesangium to fluctuating physical stress. The mechanism by which diabetes reduces the efficiency of TGF within the juxtaglomerular apparatus remains to be determined.

It is evident that, besides its role in dynamic renal autoregulation, the TGF mechanism can mediate time-averaged changes in GFR: a TGF-mediated reduction in GFR can result from a primary inhibition of proximal reabsorption, e.g., from a response to a renal toxic drug or in mice deficient for transport proteins involved in proximal reabsorption (for review, see Ref. 16). In these cases, TGF-mediated reduction in GFR closes the “glomerular leak” and serves to prevent excessive renal fluid and electrolyte loss. But how can the TGF mechanism contribute to time-averaged increases in GFR in diabetes? One possibility is that, because of the reduced TGF efficiency, temporal rises in renal perfusion pressure cause temporal increases in GFR, which can enhance time-averaged GFR. More importantly, however, and in analogy to the above-given concept for TGF-mediated reductions in GFR, there is now convincing evidence for a primary increase in fluid and electrolyte reabsorption in the proximal tubule of early insulin-dependent diabetes mellitus in humans as well as in rats with streptozotocin-induced experimental diabetes mellitus. The term “primary” indicates that the rise in reabsorption is not the consequence of glomerular hyperfiltration but due to glomerulotubular balance but that it is maintained by controlling for differences in GFR. The primary increase in tubular transport in early diabetes mellitus is the combined result of an increased Na+-glucose cotransport (as a consequence of the hyperglycemia-induced rise in filtered glucose and enhanced expression of Na+-glucose cotransporter SGLT) and of tubular growth (15, 20). The increase in tubular length and reabsorption, however, lowers the concentration of Na+, Cl−, and K+ in the tubular fluid at the macula densa (20). The latter is expected to elicit a TGF-dependent increase in nephron filtration rate to restore the fluid and electrolyte load to the distal tubule. Simply stated, glomerular hyperfiltration in early diabetes is caused by primary tubular hyperreabsorption and the normal physiological action of the TGF system (15, 20) (Fig. 2A). The fact that TGF efficiency is somewhat decreased in diabetes mellitus may to a certain extent mitigate the hyperreabsorption-induced increase of nephron filtration rate.

"The pathogenesis of diabetic nephropathy is poorly understood, but an important role can be ascribed to glomerular hyperfiltration...."
TGF and the salt paradox of the diabetic kidney

In addition to causing basal glomerular hyperfiltration, diabetes also alters the renal hemodynamic response to changes in dietary NaCl intake. Whereas a low NaCl intake causes renal vasodilation and an increase in GFR, a high NaCl intake elicits opposite responses. Although we discovered the phenomenon in diabetic rats in 1995 (for review, see Ref. 18), Miller confirmed the phenomenon in 1997 in young, type I diabetic patients who responded to a low-salt diet with renal vasodilation and a rise in GFR (4). It is clear that the kidney can adjust NaCl excretion to accommodate a wide range of dietary NaCl intakes in the absence of changes in renal blood flow and GFR, implying that the kidney mainly adjusts NaCl excretion by changing tubular reabsorption. However, there are other circumstances in which enhanced dietary NaCl intake increases GFR. If all else remains equal, increasing GFR will increase NaCl excretion, and, therefore, an increase in GFR can be part of the kidney response to increased NaCl excretion. The negative impact of dietary NaCl on GFR in diabetes, however, is counterintuitive with regard to NaCl balance, and we therefore refer to it as the “salt paradox” of the diabetic kidney.

Salt balance normally is maintained by adaptation of NaCl reabsorption in the distal nephron downstream from the macula densa under the control of aldosterone. There is, however, a potential effect of dietary NaCl on tubular reabsorption upstream from the macula densa, and, based on the normal operation of TGF, this arrangement confers a negative influence of NaCl intake on GFR. Hence, the salt paradox may result from an enhanced NaCl sensitivity of transport upstream from the macula densa through the normal action of TGF. We found that normal rats on various NaCl intakes were actually able to manage NaCl balance with no significant primary effect on reabsorption upstream to the macula densa. Thus adaptation of NaCl reabsorption took place in the further distal nephron segments, and an inverse effect of dietary NaCl on GFR mediated through TGF did not occur (Fig. 2B). In comparison, we observed a prominent negative impact of dietary NaCl on reabsorption upstream from the macula densa in diabetic rats (19). Furthermore, by measuring concentrations of Na⁺, Cl⁻, and K⁺ in early distal tubular fluid in rats on high- and low-NaCl diets, we confirmed that this primary effect of dietary NaCl on tubular reabsorption strongly links the TGF signal and the consequent adaptation in nephron filtration rate to dietary NaCl in diabetes (19). Thus the salt paradox occurs because diabetes causes proximal tubular reabsorption to become more sensitive to changes in dietary NaCl. This results in a strong influence of dietary NaCl on the TGF signal such that consuming more NaCl leads to greater activation of TGF and vice versa (Fig. 2C). The mechanism that makes the diabetic proximal tubule more sensitive to dietary NaCl remains to be determined.

Conclusions

Recent studies showed an absence of TGF responses in adenosine A₁ receptor knockout mice and demonstrated that a normal TGF response requires adenosine A₁ receptor activation to fluctuate depending on the luminal TGF signal at the macula densa. These findings indicate that adenosine acts as a mediator of TGF. Further studies are required to elucidate the role of ATP and its interaction with adenosine in TGF signaling. In early diabetes mellitus, the TGF-mediated control of both the dynamic autoregulation as well as the steady-state renal function is affected. The presented concepts of glomerular hyperfiltration in early diabetes mellitus. Hyperglycemia causes a primary increase in proximal tubular NaCl reabsorption through enhanced Na⁺-glucose cotransport and tubular growth (1). The enhanced reabsorption rates reduce the TGF signal at the macula densa ([Na,Cl,K]MD) (2) and, via TGF, increase the single-nephron glomerular filtration rate (SNGFR) (3). The resulting glomerular hyperfiltration serves to partly restore the fluid and electrolyte load to the distal nephron, but at the same time it initiates and/or maintains development of diabetic nephropathy. B: the normal kidney adjusts NaCl transport to dietary NaCl intake primarily downstream of the macula densa, and thus [Na,Cl,K]MD or SNGFR are not altered. C: in contrast, diabetes renders reabsorption in the proximal tubule sensitive to dietary NaCl (1) with subsequent effects on the luminal TGF signal (2) and SNGFR (3). This explains the paradoxical effect of dietary NaCl on glomerular filtration rate in early diabetes.
lar hyperfiltration and of the salt paradox in early diabetes mellitus illustrate the principle role of TGF in stabilizing the fluid and electrolyte delivery to the distal nephron as well as its potential impact on the control of renal hemodynamics, making nephron filtration rate a “slave” to tubular function when the latter exhibits primary changes. These concepts may help to identify new targets for the prevention of early renal hemodynamic changes in diabetes that might avert later damage to the kidney (18).

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