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Tubuloglomerular Feedback - Mechanistic Insights from Gene-Manipulated Mice

Jurgen Schnermann[1] and **Josephine P. Briggs**[2]

1*National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD*

2*Howard Hughes Medical Institute, Chevy Chase, MD*

Abstract

Tubuloglomerular feedback (TGF) describes a causal and direct relationship between tubular NaCl concentration at the end of the ascending limb of the loop of Henle and afferent arteriolar tone. The use of genetically altered mice has led to an expansion of our understanding of the mechanisms underlying the functional coupling of epithelial, mesangial, and vascular cells in TGF. Studies in mice with deletions of the A or B isoform of NKCC2, and of ROMK indicate that NaCl uptake is required for response initiation. A role for transcellular salt transport is suggested by the inhibitory effect of ouabain in mutant mice with a ouabain-sensitive α1 Na,K-ATPase. No effect on TGF was observed in NHE2 and H/K-ATPase-deficient mice. TGF responses are abolished in A1 adenosine receptor-deficient mice, and studies in mice with null mutations in NTPDase1 or ecto-5′-nucleotidase indicate that adenosine involved in TGF is mainly derived from dephosphorylation of released ATP. Angiotensin II is a required co-factor for the elicitation of TGF responses as AT1 receptor or ACE deficiencies reduce TGF responses, mostly by reducing adenosine effectiveness. Overall, the evidence from these studies in genetically altered mice indicates that transcellular NaCl transport induces the generation of adenosine that in conjunction with angiotensin II elicits afferent arteriolar constriction.

Keywords

NaCl transport; macula densa; Na,K-ATPase; adenosine; ATP; angiotensin II

Introduction

Changes in NaCl concentration in the tubular lumen near the tubulo-vascular contact point at the distal end of the ascending loop of Henle elicit adjustments in glomerular arteriolar resistance, a phenomenon referred to as ‘tubuloglomerular feedback’ (TGF) [1]. Since increases in NaCl concentration cause increases of afferent arteriolar resistance and a fall in glomerular filtration rate, the system is constructed as a negative feedback loop that serves to keep NaCl delivery into the distal parts of the nephron within narrow boundaries. The TGF response is complex, requiring coordinated functional changes in epithelial, mesangial, and smooth muscle cells, and delineation of the cellular mechanisms responsible for linking the NaCl input with the vascular endpoints has been relatively slow. Micropuncture has proven to be the most valuable tool in establishing the relationship between luminal NaCl concentration and glomerular filtration rate or glomerular capillary pressure, but this approach has major

Send all correspondence to: Jurgen Schnermann, MD., National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 10 Center Drive-MSC 1370, Bethesda. MD 20892, USA, Tel 1-301-435-6580, Fax 1-301-435-6587, E-mail jurgens@intra.niddk.nih.gov.

limitations in resolving the intermediate steps in the transmission pathway. The use of imaging and electrophysiological techniques in isolated perfused tubule/glomerulus preparations has provided an approach for the detailed study of the changes in epithelial function that result from changes in luminal composition, but the relationship between specific changes in epithelial function and the glomerular arteriolar endpoint has been difficult to study with these preparations [2, 3].

The use of gene-manipulated mice has generated a new venue to further explore the mechanisms responsible for TGF. Since micropuncture can be relatively easily adapted to this species, the effect of targeted deletions of gene products potentially involved in TGF can be studied without some of the uncertainties associated with pharmacologic interventions. In this review we are focusing on two areas where gene-manipulated mice have facilitated major progress in understanding. Substantial experimental evidence supports the notions that luminal NaCl concentration initiates TGF responses by changes in tubular NaCl transport, and that the signal arising from changes in NaCl transport is transmitted across the juxtaglomerular interstitium by the generation of paracrine messengers. The availability of animals with defined transport deficits and with targeted deficiencies in the generation or action of potential mediators has permitted new insights in both of these areas of TGF function.

NaCl transport

Apical NaCl uptake - NKCC2

There is general agreement that the primary mechanism mediating the transduction of luminal NaCl concentration into a propagated signal across the juxtaglomerular interstitium is activation of the Na,K,2Cl-cotransporter, NKCC2, in the apical membrane of macula densa cells. This basic tenet rests on the observation that a number of loop diuretics including furosemide, bumetanide, piretanide, ethacrynic acid, triflocin, or l-ozolinone [1, 4] produce complete TGF inhibition, and on the good quantitative agreement between the inhibitor concentrations causing half-maximal inhibition of transport and TGF [5]. Furosemide also blocks TGF responses during retrograde perfusion, indicating that TGF inhibition does not depend upon a metabolic product of the thick ascending limb (TAL) transmitted to the MD cells [6]. The direct evidence of a NaCl transport-dependency of renin secretion, the other endpoint of luminal NaCl concentration changes, is not as strong, but nevertheless highly suggestive [1]. NKCC2 has been found to be expressed in MD cells, and its inhibition causes cellular hyperpolarization and reductions in cytosolic Na and Cl concentrations [2, 3].

The salt-losing phenotype of mice with deficiencies in the proximal fluid transporters NHE3 or AQP1 is relatively mild despite the fact that the reabsorption of a substantial amount of filtered NaCl and water depends upon these transport pathways [7, 8]. TGF-mediated reductions of GFR and filtered NaCl have been observed in both NHE3-/- and AQP1-/- mice, and it has been surmised that reductions in filtered NaCl load by TGF are a major reason for the ability of mice with proximal transport defects to achieve Na balance [9]. In contrast, mice with complete inactivation of the NKCC2 gene display the severe salt-losing phenotype of antenatal Bartter syndrome [10]. Although TGF responses have not been directly assessed in these mice, inactivation of TGF is suggested by the apparent inability to respond to the elevated distal NaCl load with a reduction in GFR. In mice heterozygous for the NKCC2 null mutation NKCC2 protein expression was found to be normal, and mice were indistinguishable from wild type [11]. Further functional exploration of the consequences of NKCC2 deficiency has become possible with the generation of mice with targeted disruption of single NKCC2 isoforms [12, 13]. The existence of three different full length variants of NKCC2, first reported by Payne and Forbush in the rabbit kidney [14], has been confirmed in all species studied [15-17]. These isoforms, called NKCC2B, NKCC2A, and NKCC2F, are derived from differential splicing of the variable exon 4 of the *Slc12a1* gene, a short 96-bp exon that encodes for the second

transmembrane domain and parts of the adjacent intracellular loop of the transporter [14]. NKCC2 isoform expression shows a distinct distribution pattern with F found exclusively in the medullary thick ascending limb, A in both outer medulla and cortex, and B in the cortex [15, 17, 18]. Macula densa cells of the mouse have been found to express both the B- and A-isoforms of the cotransporter [12]. Marked differences in ion affinities have been identified in *in vitro* heterologous expression studies in *Xenopus laevis* oocytes: F was found to have much lower Na^+ and Cl^- affinities than the A or the B isoform [18]. To create NKCC2B and NKCC2A-deficient mice, the alternate exons 4B or 4A were modified by the introduction of in-frame stop codons resulting in the premature termination of translation. Thus, these strains of isoform-specific knockout mice lack both the full length and the corresponding truncated NKCC2 isoforms.

In vivo microperfusion of loops of Henle showed that in NKCC2B-deficient mice Cl^- reabsorption was significantly reduced at low flow rates [12], while the lack of NKCC2A resulted in reduced Cl^- absorption at high perfusion rates [13]. These *in vivo* data are in line with the notion that TAL reabsorption at low NaCl concentrations relies on the activity of the high Cl^--affinity NKCC2B isoform while NKCC2A comes into play under when higher salt concentrations are achieved by high loop perfusion flow. Assessment of TGF responses has confirmed that macula densa signaling function depends on the successive engagement of NKCC2B and NKCC2A (Fig. 1). In the low flow range NKCC2B-deficient mice were less responsive than wild type animals whereas TGF responses of NKCC2A-/- mice were reduced at high flow rates. Thus, $V_{1/2}$, the flow rate causing half maximum TGF responses, increased from 6.5 nl/min in mice expressing only NKCC2B to 15.5 nl/min in mice possessing only NKCC2A. These data suggest that the successive activation of the high Cl^- affinity NKCC2B and the lower Cl^- affinity NKCC2A is responsible for the surprisingly wide range of NaCl concentrations over which TGF operates.

Apical NaCl uptake - ROMK

Whereas the inhibitory effect of luminal barium on TGF responses was diminished by a pronounced direct vascular constrictor action [19], retrograde application of the K^+ channel blocker U37883A caused an almost complete inhibition of TGF responsiveness [20]. This effect is mediated by ROMK type K^+ channels since TGF responses were largely absent in mice with targeted ROMK deletion [21]. The finding of a significantly reduced, but not abolished TGF response has subsequently been confirmed in mice in which selective breeding of surviving animals has generated ROMK-deficient mice with less compromised kidney function and reasonably well maintained blood pressure [22]. As shown in Fig. 2, the mean TGF response of 11.3 ± 1.2 mm Hg in wild type mice was reduced to 2.2 ± 0.6 mm Hg in ROMK-deficient mice ($p<0.001$). The observation that inhibition of NKCC2 and ROMK has similar effects on TGF responses argues against a specific “sensor” function of the actual transport proteins suggesting instead a critical role of some consequence of MD NaCl transport. Since ambient distal K^+ concentrations near the MD are close to the K^+ affinity of the cotransporter variations in luminal K^+ may regulate TGF response magnitude [20].

Apical NaCl uptake - Na/H exchanger

Detailed studies in the isolated perfused JGA preparation of the rabbit have clearly documented the existence of Na/H exchange activity in both the apical and the basolateral membrane of macula densa cells. Increases in luminal NaCl concentration cause increased hydrogen efflux and subsequent alkalinization of the macula densa cell cytosol [23]. It has been estimated that about 20% of total Na entry may be mediated by Na/H exchange. Immunocytochemical data indicate that the exchanger isoform in the apical membrane is NHE2 whereas NHE4 is the isoform of the basolateral membrane [24]. Macula densa cells are thus distinct from TAL cells where NHE3 is the dominant variant of the exchanger [25]. How Na/H exchange in macula densa

cells could affect TGF has been unclear. One possibility is that the intracellular pH determines the magnitude of the response by disinhibiting macula densa nNOS and thereby augmenting the generation of nitric oxide. In fact, it has been reported that Na/H exchange inhibition with amiloride augments TGF responses by preventing cell alkalinization and thereby causing relative inhibition of nNOS [26, 27]. In contrast to these observations in the isolated JGA preparation, there is no in vivo evidence that would support a major role of Na/H exchange activity in TGF responsiveness. Micropuncture studies in NHE2-deficient mice failed to show changes in TGF responses compared to wild type animals [28], and loop of Henle perfusion with amiloride or N-(isopropyl)-amiloride (EIPA) did not elicit measurable alterations of TGF response magnitude [4]. Thus, a major TGF-modulating role of Na/H exchange-dependent variations of cytosolic pH in TAL or MD cells is not supported by in vivo observations currently available. TGF responses have also been found to be well maintained in NHE3-/- mice [7], and autoregulation of GFR and RBF was not altered in NHE3-/- mice with transgenic expression of NHE3 in the intestinal tract [29].

Basolateral NaCl extrusion - Na,K-ATPase

Whereas an effect of apical transport inhibition on TGF is generally accepted, there is still considerable uncertainty about the subsequent steps in the juxtaglomerular signaling cascade. One possibility is that one of the intracellular consequences of NKCC2-dependent NaCl uptake is directly coupled to the mediating step. Detailed and sophisticated studies in the isolated perfused rabbit JGA have identified depolarization, alkalinization, and various ionic compositional changes as results of an increased NaCl uptake, and it is therefore conceivable that one or more of these changes trigger the signaling events directly [3, 30].

A second possibility is that signal propagation is the consequence of transcellular NaCl transport, with apical uptake being the first step in the sequence. Transcellular NaCl transport across the renal tubular epithelium universally requires the support of Na,K-ATPase-dependent energy supply. Until recently, the available data about Na,K-ATPase in the macula densa made the transcellular transport hypothesis look less likely. First, cytochemical and immunological localization studies [31, 32] suggested that the expression of Na,K-ATPase in the basolateral membrane of MD cells was quite low. In addition, a microenzymatic determination of Na,K-ATPase activity in microdissected rabbit MD cells arrived at the conclusion that the activity levels were about 50fold lower than in neighboring TAL cells [33]. However, the interpretation of these studies failed to take into account the important impact of basolateral membrane folding on enzyme density. For example, in the enzyme activity studies Na,K-ATPase was normalized to unit of cell volume rather than to membrane surface area, an approach that would underestimate a membrane-bound molecule by the membrane folding factor. Whereas the basolateral membrane of MD cells is typically non-folded, extensive infolding in the neighboring TAL cells may increase its basolateral membrane by a factor of 10 to 20 [34]. Thus, the difference in enzyme activity per unit cell surface area between MD and TAL cells may actually be at least 10fold less than assumed. Species-specific differences may also play a role [24, 35]. Extensive studies in the rat using a well-defined antibody against α1 Na,K-ATPase and its co-localization with neuronal nitric oxide synthase, a macula densa cell marker, have clearly established robust presence of the enzyme in the basolateral membrane of MD cells [36, 37]. In addition, MD cells also express the β1 subunit of the enzyme and a γ subunit that may be either γa or both γa and γb [36, 38-40]. Again, staining of MD cells appeared somewhat less intense than in neighboring TAL cells, no doubt because of absence of basolateral infoldings in MD cells. Pharmacological studies examining the effect of Na,K-ATPase inhibition on TGF were also inconclusive. In rats Na,K-ATPase inhibition by ouabain, administered by luminal or peritubular microinfusion, did not elicit clear reductions of TGF responses (own unpublished data). However, the α1 subunit of Na,K-ATPase, by far the predominant isoform of the enzyme in the kidney, is largely resistant to ouabain in rats and mice. Mice homozygous for a complete

α1 Na,K-ATPase null mutation are not viable while heterozygotes are essentially normal with respect to renal function [41].

Recently however, ingenious utilization of the possibilities of gene manipulation has brought important new information suggesting an important role of Na,K-ATPase in supporting TGF [28]. TGF responses were determined in double mutant mice in which the normal ouabain-resistance pattern was reversed with α1 Na,K-ATPase mutated to be ouabain-sensitive and α2 Na,K-ATPase to be ouabain-resistant [42, 43]. Single mutant mice with the natively ouabain-resistant α1 isoform and the mutated α2 isoform served as controls. In the double mutant mice, intravenous infusion of ouabain caused inhibition of TGF that increased over the course of 1-2 hours. No such effect was seen in the ouabain-resistant control mice indicating that the ouabain effect on TGF was not due to inhibition of a glycoside-sensitive ATPase other than Na,K-ATPase. Attenuation of TGF responses in sensitive, but not resistant mice was also seen with luminal ouabain administration. Maintenance of normal constrictor responses to direct A1AR activation indicates that the effect of ouabain was not a reflection of a general loss of vascular reactivity, but that it occurred at an earlier step in the signaling pathway. The implication that TGF signaling is an energy-consuming process is fully in accord with our earlier observations that ATP depletion by inhibitors of the respiratory chain or of oxidative phosphorylation interferes with TGF responsiveness (Briggs JP, Schnermann J; abstract; Pflugers Arch [Suppl] 1981; **389**: R40). MD cells have numerous mitochondria scattered throughout all regions of the cell including the basal part. Presumably because of the absence of basolateral infoldings their membrane-associated alignment is less obvious than it is in cells of the proximal and distal convoluted tubule and TAL, and the distribution of mitochondria is more akin to that in principal and intercalated collecting duct cells. It is worth noting that involvement of Na,K-ATPase in TGF provides an immediate explanation for the inhibitory effect of furosemide. In contrast to the effect of ouabain alone, ouabain in combination with furosemide does not cause cell swelling or membrane depolarization of TAL cells indicating that as a result of the low intracellular Na concentration the Na pump becomes non-functional in the presence of the loop diuretic [44]. Overall, studies exploring the relation between NaCl transport and TGF responses indicate that afferent arteriolar vasoconstriction is paralleled by increased epithelial ATP utilization, and they suggest a link between metabolic rate and the vascular endpoint.

Na extrusion - H(Na)/K-ATPase

An alternative model for the regulation of intracellular NaCl in macula densa cells has been suggested by the observations in the isolated rabbit JGA that the luminal administration of ouabain caused an elevation of intracellular Na and prevented the recovery of intracellular Na from the elevated levels resulting from high luminal NaCl [35]. This observation was linked to the presence of a colonic H/K-ATPase (HKα2) in the apical membrane of macula densa cells based on immunocytochemistry and the finding that an increase in luminal K^+ caused partial recovery of the cytosolic pH from the acidification resulting from luminal and basolateral NaCl removal [35, 45]. The ability of H/K-ATPase to mediate active Na extrusion has previously been demonstrated in the distal colon [46]. Nevertheless, in recent studies in H/K-ATPase-deficient mice TGF responses were found to be normal suggesting either that H/K-ATPase and its cytosolic effects do not play a major role in TGF or that H/K-ATPase in the mouse is not expressed in the cells activating the TGF pathway [28].

A word of caution may be appropriate at this point. Aside from anatomical plausibility there is no direct proof that the macula densa cells are in fact the cells responsible for the generation of the signal causing afferent arteriolar vasomotor responses. While retrograde in vivo microperfusion experiments and perfusion of dissected JGA specimen clearly point to an effect of a limited segment around the glomerulus, an influence of TAL cells within an about 50 μm segment upstream or downstream from the macula densa is entirely feasible. In fact, the clear

effect of the undisputed TAL proteins NKCC2 and Na,K-ATPase and the lack of effect of the macula densa proteins H/K-ATPase and NHE2 on TGF responses may be interpreted as supporting this possibility. It is also worth recalling that amphibian species have been shown to possess mammalian-like TGF responses although they lack cells with the typical macula densa appearance [47, 48].

Signal Mediation

Adenosine

Control of glomerular arteriolar tone by tubular NaCl concentration is the result of changes in the concentration of several paracrine mediators within the confines of the juxtaglomerular interstitium. The nucleoside adenosine has been suggested to exert metabolic control of renal blood flow and GFR via TGF-mediated vasoconstriction [49, 50]. In fact, inhibition of A1 adenosine receptors (A1AR) with receptor-specific antagonists largely prevented TGF-induced reductions of SNGFR and glomerular capillary pressure in the rat [51]. Definitive support for a critical role of A1AR in TGF responsiveness came from two studies of TGF in A1AR-deficient mice generated independently by two laboratories [52, 53]. In both experimental series TGF responses were completely abolished in the A1AR-deficient mice, indicating that adenosine, as the endogenous agonist of A1AR, is required for TGF responses to occur. In contrast to observations in wild type animals, SNGFR measured in proximal segments (with TGF interrupted) and in distal segments (with TGF intact) was not different in A1AR-/- mice indicating that the tonic suppression of GFR by ambient distal flows requires intact A1AR [54]. Furthermore, TGF-induced oscillations of proximal tubular pressure were not observed in the A1AR-deficient mice [54]. Since autoregulation of renal blood flow and GFR is mediated in part by TGF, one of the consequences of A1AR-deficiency may be a reduction in autoregulatory efficiency. In fact, steady-state autoregulation of RBF and GFR in response to a lowering of blood pressure was significantly impaired in A1AR-deficient mice [55]. In addition, the dynamic autoregulatory response of A1AR-deficient mice to a step increase in blood pressure was reduced by 50% in the time frame mediated by TGF while total autoregulation was reduced by about 30% [56].

A direct vasoconstrictor action of adenosine has been demonstrated in perfused isolated afferent arterioles of the mouse, an effect that was not seen in vessels from A1AR-deficient mice thereby establishing the role of A1AR in the action of adenosine [57, 58]. Adenosine induces vasoconstriction in mouse afferent arterioles by Gi-dependent activation of phospholipase C, release of Ca from intracellular stores, and subsequent entry of Ca^{++} through L-type Ca^{++} channels [57, 59]. Consistent with the stable vasoconstriction elicited by TGF, the constrictor effect of adenosine in isolated vessels can be maintained for extended periods of time indicating absence of rapid receptor desensitization [57]. TGF-mediated vasoconstriction by adenosine is noteworthy since in most vascular beds adenosine causes vasorelaxation through activation of Gs-coupled A2AR. In fact, the steady-state effect of administration of exogenous adenosine in the mouse kidney is vasodilatation due to an excess of the vasodilating A2AR receptor class in the entire renal vasculature. Thus, adenosine-induced vasoconstriction requires activation of A1AR without simultaneous stimulation of A2AR in other parts of the renal vasculature [60]. This requirement appears to be met by generation of adenosine in the confines of the juxtaglomerular interstitium and its exclusive delivery to afferent arterioles in which A1AR expression dominates.

Angiotensin II has been shown to act as an important co-factor of the constrictor actions of adenosine [58, 61-63]. Studies in mice with deletions of the angiotensin II receptor (AT1A) or of angiotensin-converting enzyme have shown that TGF responses are largely absent in these animals [64, 65]. Furthermore, the fall in glomerular capillary pressure caused by the administration of the A1AR agonist cyclohexyl adenosine was markedly diminished in AT1A

receptor-deficient mice suggesting that non-responsiveness to adenosine may importantly contribute to the attenuation of TGF in mice with a compromised renin-angiotensin system [66]. In mice with deletions of tissue-bound ACE TGF responses were significantly reduced [67]. This effect seems to be a result of absence of endothelial ACE since selective expression of ACE in the proximal tubule was unable to restore normal TGF responsiveness [68]. The mechanism underlying the cooperation between A1AR and angiotensin II receptors has remained unclear. One possibility is that the activation of phospholipase C by Gβγ subunits released in the course of A1AR-dependent Gi-activation is synergistically enhanced by simultaneous stimulation of Gq proteins. Alternatively, adenosine has been shown to prevent angiotensin receptor desensitization by enhancing the Ca sensitivity of myosin light chain kinase [69]. Enhanced TGF responses may also be the result of an angiotensin II-induced reduction in local levels of nitric oxide [70]. This notion agrees with the demonstration that macula densa nNOS expression is elevated in AT1 receptor-deficient mice [71].

It has been proposed that adenosine is produced and released by macula densa cells as a by-product of increased NaCl transport and ATP utilization [49]. Whereas this suggestion has not been completely excluded, the available experimental evidence suggests that a major part of the adenosine required for local A1AR activation is derived from the dephosphorylation of released ATP. In the perfused rabbit JGA an increase in luminal NaCl concentration has been shown to cause a rise of cytosolic Ca^{++} in biosensor cells positioned near the basolateral membrane of macula densa cells [72]. Inhibition of this response by suramin suggests that it was elicited by activation of P2 receptors secondary to the release of ATP.

A direct role of ATP as a vasoconstrictor in the TGF signaling pathway may be possible, but the evidence for this is currently not compelling. Mice with a deletion of the P2X1 receptor that has previously been shown to be present in afferent arterioles [73] have impaired autoregulation of afferent arteriolar tone [74], but have largely normal TGF responses (Fig. 3). Mean maximum TGF responses of 7.6 ± 1 mm Hg in wild type mice (n=18) and of 5.4 ± 0.6 mm Hg in P2X1-deficient mice were not significantly different (p=0.073). Since the autoregulation studies were performed in juxtamedullary arterioles while the TGF evidence comes from superficial nephrons, regional differences in P2X1 receptor function may be responsible for these conflicting outcomes. Nevertheless, because of their rapid desensitization, P2X1 receptors would appear unsuited as mediators of a response that can last for extended time periods so that the role of other P2 receptors needs to be further investigated.

ATP appears more likely to act predominantly through generation of adenosine in the JG interstitium by extracellular breakdown by ecto-ATPases and nucleotidases. In fact, mice with deletion of NTDPase1, an extracellular ATPase with expression at the glomerular vascular pole [75], have significantly reduced TGF responses (see prepublished paper at http://www.ncbi.nlm.nih.gov/pubmed/1826308). Since one would expect NTPDase1-deficiency to be associated with elevated ATP levels, this finding may further argue against a direct role of ATP in TGF. Inhibition of ecto-5′-nucleotidase (CD 73) with alpha-beta-methyleneadenosine 5′-diphosphate (MADP) had been shown earlier to reduce the compensatory efficiency of TGF and the slope of the TGF function [76]. Two groups of investigators using independently generated strains of ecto-5′-nucleotidase/CD73-deficient mice have reported significant attenuation of TGF responses [77, 78]. Thus, successive dephosphorylation of ATP or ADP to AMP by NTPDase 1 and from AMP to adenosine by ecto-5′-nucleotidase appears to provide most of the adenosine required for TGF responsiveness.

Nitric oxide

The high levels of expression of neuronal NOS in macula densa cells has stimulated extensive investigations of the role of nitric oxide in juxtaglomerular signaling [79, 80]. Luminal

administration of inhibitors of NO synthases enhances TGF responses to elevations of loop of Henle flow rate [1]. Nevertheless, the response of glomerular capillary pressure to changes in perfusion rate was found to be identical over the entire flow range in wild type and nNOS-deficient mice [81]. In contrast, measurements of SNGFR showed a markedly increased proximal-distal SNGFR difference, due for the most part to a significantly lower SNGFR in distal tubules of nNOS-deficient mice [81]. Thus, these data suggest that the chronic absence of a functional nNOS in macula densa cells is associated with an enhanced vasoconstrictor tone in the subnormal flow range, presumably a consequence of a proportional enhancement of pre- and postglomerular resistances. Since stimulation of NO generation by loop of Henle flow has been shown to usually occur in the supranormal flow range [1], it is conceivable that the reduced SNGFR in nNOS-/- mice is an indirect rather than direct effect of reduced NO availability. Nevertheless, it is noteworthy that direct measurements of NO concentrations in distal tubular fluid have shown an association of total NO release with reduced rather than enhanced NaCl transport [82]. TGF responses have been found to be absent in mice with concurrent deficiencies in nNOS and A1AR indicating that nNOS deficiency does not overcome the lack of A1AR signaling (Fig. 4). This observation reaffirms the primacy of A1AR signaling and a modulating role of nitric oxide in the TGF pathway.

Conclusions

Taken together, evidence from studies in genetically modified mice has resulted in a fairly robust mechanistic framework of TGF operation. Metabolic activation of epithelial cells resulting from increased transcellular NaCl transport mediated by NKCC2, ROMK and Na,K-ATPase is coupled to ATP release and extracellular adenosine formation; this in turn leads to activation of A1AR and vasoconstriction (Fig. 5). Several other paracrine factors interact with this pathway to set vascular sensitivity. Since this scheme identifies a considerable number of testable (and still untested) hypotheses, we expect that the further utilization of gene-manipulated mice will remain a useful strategy to enhance the understanding of juxtaglomerular signaling mechanisms.

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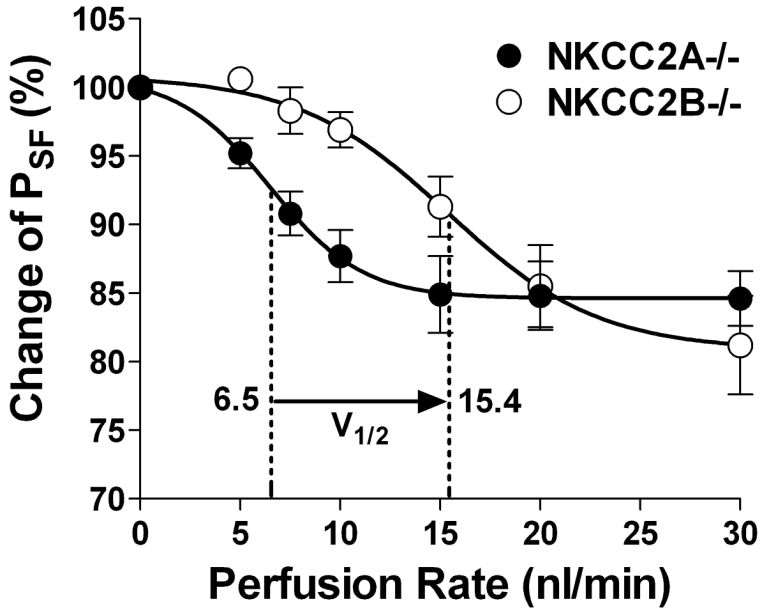

Fig. 1.

Relationship between loop of Henle perfusion rate and the percentage reduction of stop flow pressure (± SEM), an expression of TGF responsiveness, in mice lacking NKCC2B (circles) or NKCC2A (dots). Dashed lines indicate position of $V_{1/2}$, the flow rates causing half maximum reduction of P_{SF}. Data are redrawn from references [45] and [46].

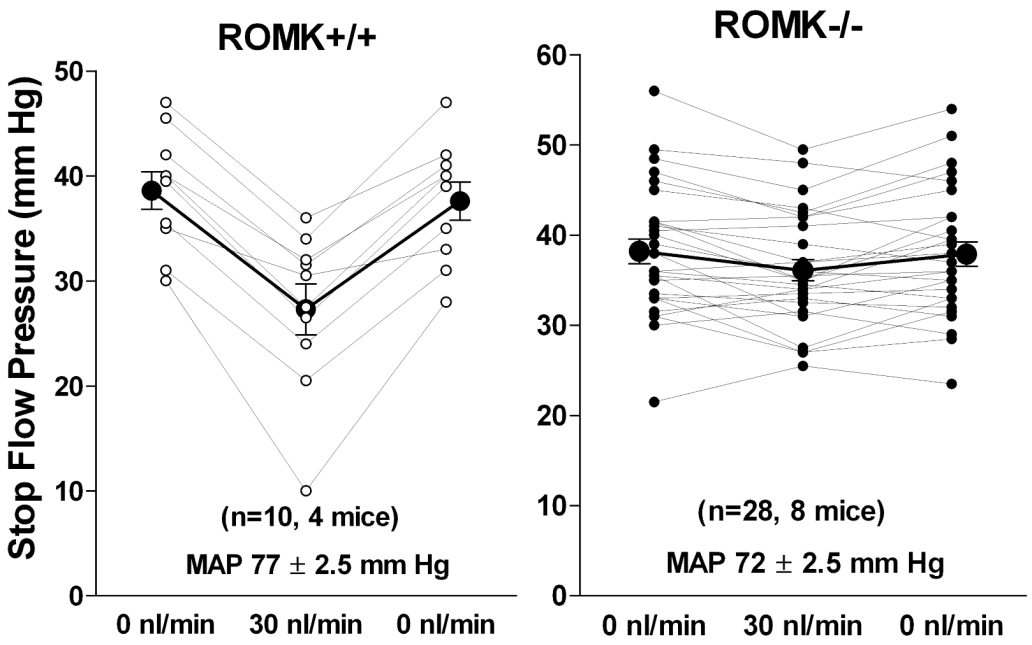

Fig. 2.

Maximum reductions of stop flow pressure (P_{SF}) in ROMK+/+ (left) and ROMK-/- mice (right). Lines connect values from individual tubules taken at saturating flow rate (30 nl/min) bracketed by two measurements of P_{SF} at zero loop flow. Mice were supplied by Tong Wang and Steve Hebert (Yale University, New Haven CT), and some of the characteristics of the new ROMK strain have been previously published [41].

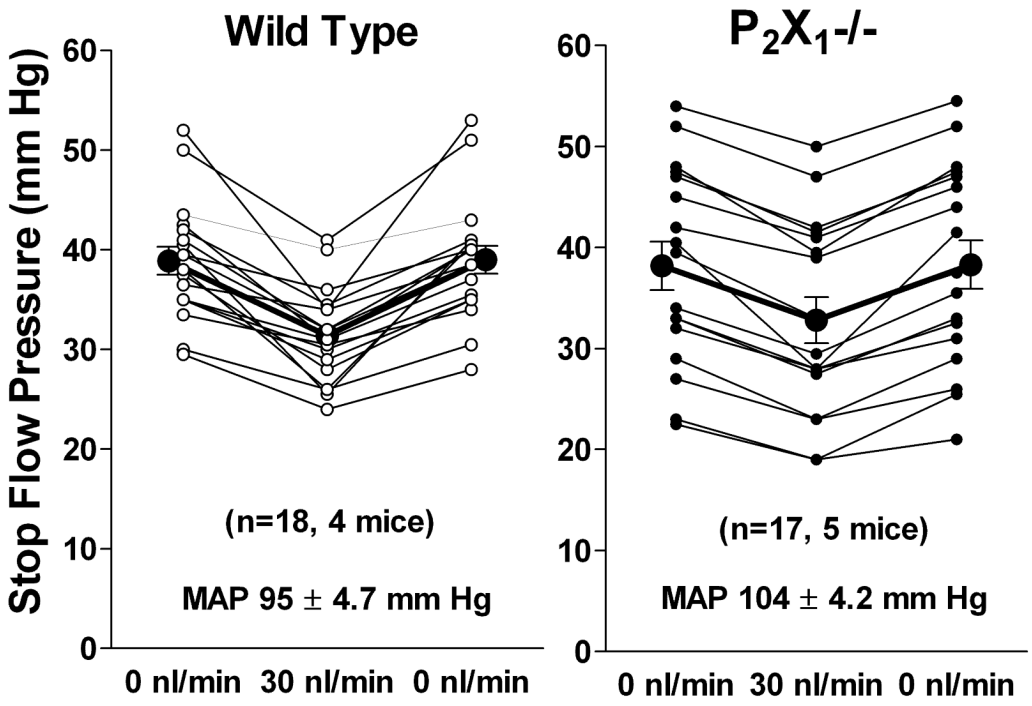

Fig. 3.

Maximum reductions of stop flow pressure (P_{SF}) in wild type mice (left) and in P2X1-deficient mice (right). Lines connect values from individual tubules measured at saturating flow rate (30 nl/min) and bracketed by two measurements of P_{SF} at zero loop flow. Mice originally generated by R. Evans (University of Leicester, UK) were supplied by E. Inscho (Medical College of Georgia, Atlanta, GA).

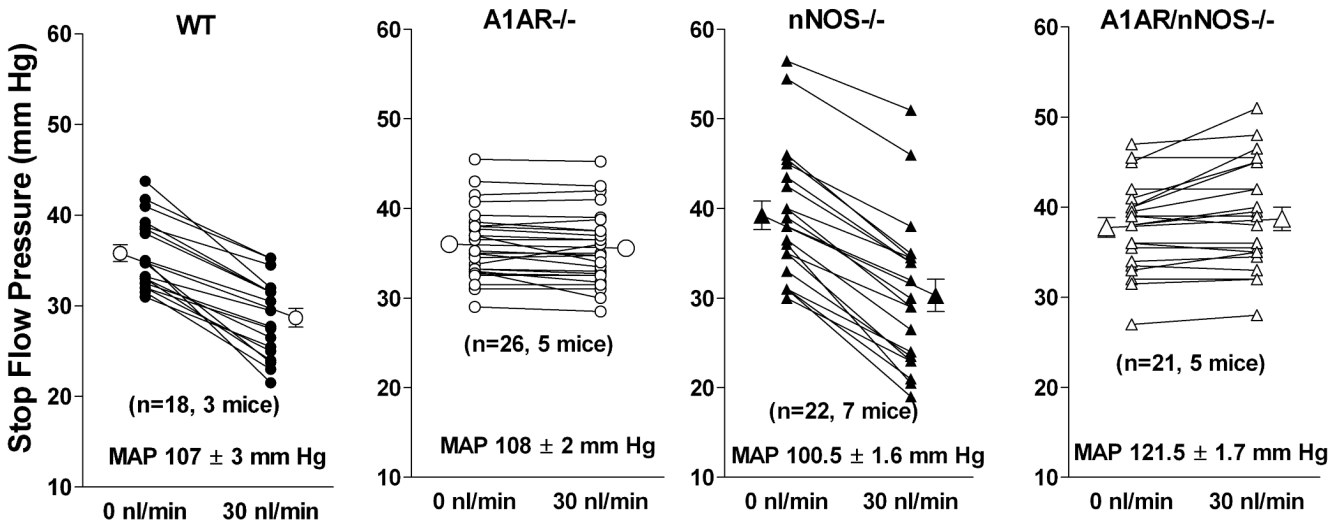

Fig. 4.

Decrease of stop flow pressure in response to a flow elevation to 30 nl/min in wild type mice (WT), A1AR-deficient mice (A1AR-/-), nNOS-deficient mice (nNOS-/-) and in mice without both A1AR and nNOS (A1AR/nNOS-/-). A1AR/nNOS double knockout mice were generated in the Schnermann/Briggs laboratory.

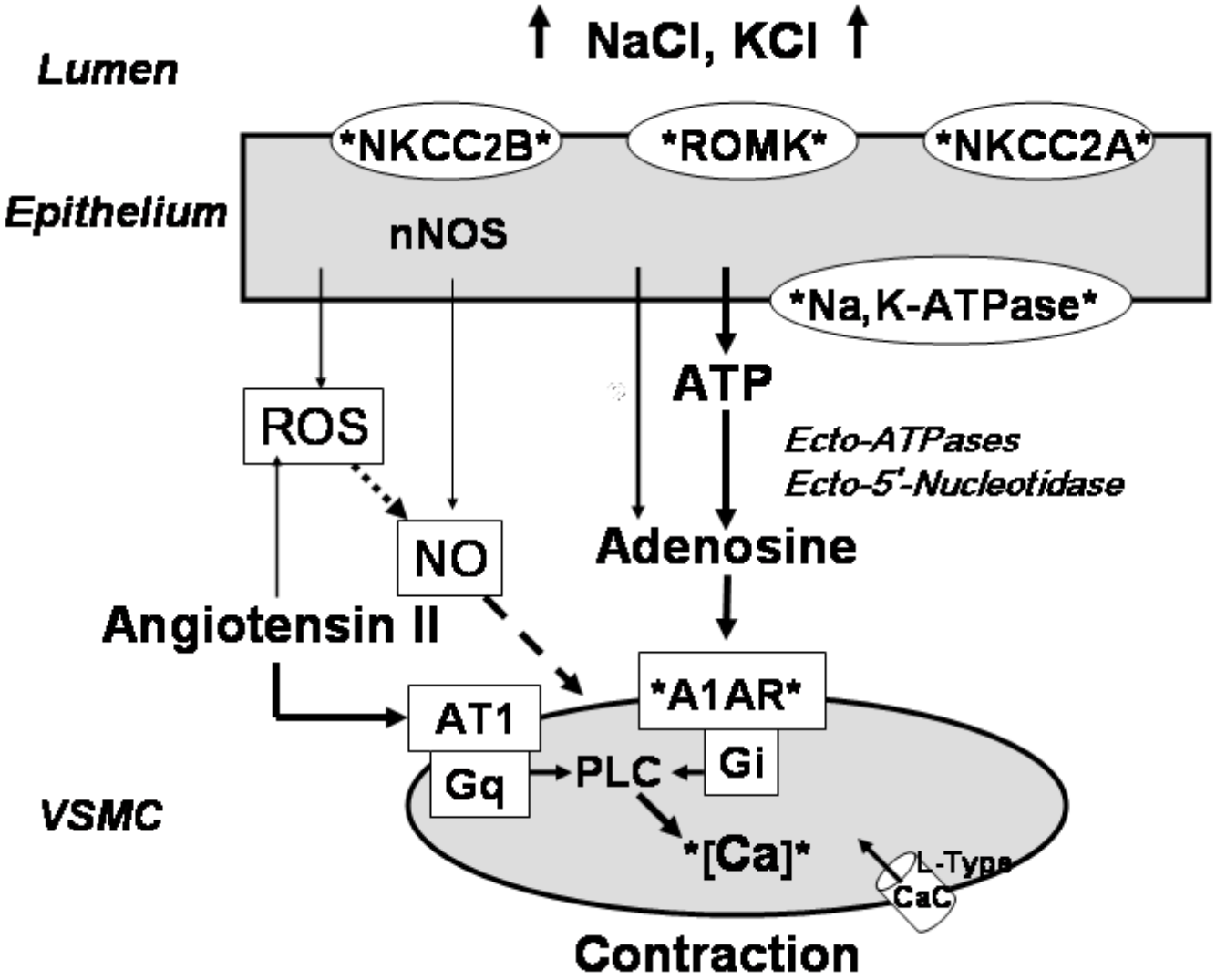

Fig. 5.

Scheme of TGF operation as supported by evidence mostly derived from studies in gene-manipulated mice. Solid arrows indicate positive/stimulatory, and broken arrows negative/inhibitory relationships. The depiction of cellular components is not meant to be complete, but to indicate those proteins for which a role in TGF is suggested by experimental evidence from gene-manipulated mice.