REVIEW

Mediators of tubuloglomerular feedback regulation of glomerular filtration: ATP and adenosine

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Abstract

In the juxtaglomerular apparatus of the kidney the loop of Henle gets into close contact to its parent glomerulus. This anatomical link between the tubular system and the vasculature of the afferent and efferent arteriole enables specialized tubular cells, the macula densa (MD) cells, to establish an intra-nephron feedback loop designed to control preglomerular resistance and thereby single nephron glomerular filtration rate. This review focuses on the signalling mechanisms which link salt-sensing MD cells and the regulation of preglomerular resistance, a feedback loop known as tubuloglomerular feedback (TGF). Two purinergic molecules, ATP and adenosine, have emerged over the years as most likely candidates to serve as mediators of TGF. Data will be reviewed supporting a role of either ATP or adenosine as mediators of TGF. In addition, a concept will be discussed that integrates both ATP and adenosine into one signalling cascade that includes (i) release of ATP from MD cells upon increases in tubular salt concentration, (ii) extracellular degradation of ATP to form adenosine, and (iii) adenosine-mediated vasoconstriction of the afferent arteriole.

Keywords adenosine, adenosine triphosphate, glomerular filtration rate, macula densa, purinergic signaling, renal autoregulation.

The tone of the afferent arterioles of the kidney vasculature is the main determinant of renal vascular resistance. Whereas renal blood flow is set by total vascular resistance, the tone of each single afferent arteriole determines the glomerular filtration rate (GFR) of the respective nephron. The resistance of the afferent arterioles is affected by numerous systemic factors; in addition, substantial evidence supports the concept that preglomerular resistance is determined by an intrarenal regulatory pathway residing in the juxtaglomerular apparatus (JGA). In the JGA, a close anatomical link between the tubular system and the vasculature of the afferent and efferent arteriole enables specialized tubular cells of the cortical thick ascending limb of Henle, the macula densa (MD) cells, located adjacent to the extraglomerular mesangium, to establish an intra-nephron feedback loop designed to control preglomerular resistance, and by that single nephron GFR. This review will focus on the signalling mechanisms which link salt-sensing tubular MD cells and the regulation of preglomerular resistance, a feedback loop known as tubuloglomerular feedback (TGF) (Thurau & Schnermann 1965).

The initial step of the TGF is the detection of the NaCl concentration in the tubular lumen by MD cells. Transport activity of the Na, K, 2Cl co-transporter NKCC2 (BSC1) in the apical membrane of these cells is thought to be the primary mechanism by which MD cells detect tubular salt concentration (Wright & Schnermann 1974, Schlatter *et al.* 1989, Lapointe *et al.* 1990, Bell *et al.* 1991, Obermuller *et al.* 1996, Bell & Lapointe 1997). In line with this assumption, inhibition of NKCC2 transport activity by the use of loop diuretics like furosemide or bumetanide was shown to abolish

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TGF responsiveness (Wright & Schnermann 1974). As tubular NaCl concentration rises MD cells initiate a signalling process which eventually leads to a vasoconstriction of the afferent arteriole followed by a decrease of GFR of the respective nephron. Thus, TGF represents a protective mechanism against the threat of renal salt loss potentially resulting from an inappropriately high salt load to the low salt-reabsorbing capacity of the portion of the nephron downstream of the MD segment. Besides its protective role in avoiding inadequate salt loading of distal parts of the nephron, the effectiveness of the TGF was assumed also to become apparent in pathologic situations. During insults to the function of the tubular system like acute tubular necrosis, with reduced proximal reabsorption and increased salt delivery to the MD segment, TGF responsiveness was suggested to contribute to the fall in total GFR observed under these conditions. However, experimental evidence for an involvement of TGF in the decline in GFR observed under situations of reduced reabsorptive proximal tubular function has remained ambiguous (Mason *et al.* 1978, 1981, Osswald *et al.* 1980, 1996, Peterson *et al.* 1989, Braam *et al.* 1993).

The role of TGF in the physiology and pathophysiology of the kidney has nourished the interest in identifying the factor(s) which mediate the constriction of the afferent arteriole upon detection of increased tubular salt concentration by MD cells. A possible mediator of the TGF response is supposed to fulfil at least two key characteristics: (i) it needs to be generated in the JGA as some function of the salt concentration in the tubular lumen, and (ii) it needs to cause vasoconstriction of the afferent arteriole leading to reduced GFR of the respective nephron. The latter implies that a mediator of TGF regulation of GFR is supposed to be a vasoconstrictor agent and adequate receptors need to be present on smooth muscle cells of the distal part of the afferent arteriole within proximity of the MD segment. Though several different vasoconstrictor candidates have been discussed to serve as mediators of TGF, evidence has accumulated to suggest a key role of two purinergic molecules: ATP and adenosine. Both adenosine and ATP fulfil the prerequisites outlined above, and substantial experimental data have been gathered supporting a role of either one as the mediating factor of TGF. While ATP or adenosine are often discussed as independent mediators of TGF regulation of GFR, it is conceivable that *both* ATP and adenosine are required, serving as *primary* and *secondary* mediators. Experimental evidence will be reviewed suggesting a signalling cascade that includes (i) regulated release of ATP from MD cells, (ii) subsequent extracellular degradation of ATP leading to the formation of adenosine, and (iii) adenosine-mediated constriction of afferent arterioles.

Tubuloglomerular feedback: role of adenosine

Adenosine constricts afferent arterioles

All classes of adenosine receptors are expressed in the kidney including the vasoconstrictor A1 adenosine receptor (A1AR), the vasodilator A2 adenosine receptors (A2aAR and A2bAR), and A3 receptors, with a possible role of the latter in modulating renal vascular resistance being unclear. A1AR are present in the afferent arteriole with highest expression levels in the most distal part of the vessel (Weaver & Reppert 1992, Jackson *et al.* 2002), the region closest to the MD segment and the site of the most pronounced vasoconstriction during TGF responses (Weihprecht *et al.* 1992, Peti-Peterdi *et al.* 2002a, Peti-Peterdi 2006). A1AR are also present in various segments of the tubular system (Yamaguchi *et al.* 1995, Kreisberg *et al.* 1997) where they account for the natriuretic and diuretic action of A1AR antagonists like methylxanthines (e.g. caffeine) (Knight *et al.* 1993, Wilcox *et al.* 1999, Rieg *et al.* 2005). Vasoconstriction upon A1AR activation is mediated by G_i-dependent activation of phospholipase C and a subsequent rise of cytosolic calcium through activation of voltage-dependent calcium channels (Hansen *et al.* 2003). Thus, adenosine and angiotensin II share similar intracellular signalling pathways leading to an increase in intracellular calcium and eventually vasoconstriction. To the extent that adenosine is involved in the mediation of TGF, this mechanism may provide a basis for the finding that TGF responses are enhanced under situations of high angiotensin levels (Mitchell & Navar 1988, Schnermann & Briggs 1989, Weihprecht *et al.* 1994) and absent in AT1 angiotensin receptor-deficient mice (Schnermann *et al.* 1997). Besides vasoconstrictor A1AR, the presence of dilator A2AR in the renal vasculature was established on the basis of functional evidence and expression analysis (Holz & Steinhausen 1987, Kost & Jackson 1991, Zhang *et al.* 1994, Nishiyama *et al.* 2001, Chen *et al.* 2002, Jackson *et al.* 2002). A recent study addressing the effect of adenosine on the tone of isolated perfused afferent arterioles suggested that the net effect of adenosine on A1AR-mediated constrictor and A2-mediated dilator effects is dependent on the route of administration. In isolated perfused afferent arterioles, application of adenosine (10^{-7} M) to the bath caused sustained vasoconstriction whereas no effect was observed when adenosine was added to the perfusate (Hansen *et al.* 2005). Similarly, at the level of the whole organ, systemic infusion of adenosine caused total renal vascular resistance to fall, an effect suggested to be mediated by endothelial A2 receptors and subsequent NO release (Li *et al.* 1998, Olanrewaju & Mustafa 2000, Wyatt *et al.* 2002). Conversely, infusion of

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adenosine into the subcapsular interstitium reduced superficial renal blood flow (Hansen *et al.* 2005). Thus, the overall effect on afferent arteriolar tone appears to be side-dependent congruent with the presence of A1AR and A2AR on vascular smooth muscle and endothelial cells, respectively. During interstitial application adenosine concentrations acting on vasoconstrictor A1AR may be substantially higher than adenosine concentrations reaching the endothelium due to dilution and wash-out in the blood stream. Considering involvement of adenosine in TGF signalling one would assume that adenosine, released from MD cells or generated within the JGA, reaches its target from the interstitial side.

Adenosine as a mediator of TGF

Schnermann *et al.* (1977) suggested adenosine as the mediator of TGF based on their finding that unspecific adenosine receptor antagonists like theophylline and 3-isobutyl-methylxanthine reduced TGF responsiveness. Although these results were confirmed in several studies (Osswald *et al.* 1980, 1982, Bell 1985, Franco *et al.* 1989) the interpretation of these experiments is complicated by the fact that methylxanthines not only block adenosine receptors, but also inhibit cAMP-phosphodiesterases. This could lead to increased cAMP levels in vascular smooth muscle cells of the afferent arteriole and a reduced contractility to vasoconstrictors involved in the mediation of the TGF response. However, more specific antagonists of A1AR like KW-3902, CVT-124, FK838, 8-cyclopentyl-1,3-dipropylxanthine, or 1,3-dipropyl-8-cyclopentylxanthine also inhibited TGF responses (Osswald *et al.* 1982, Schnermann *et al.* 1990, Kawabata *et al.* 1998, Wilcox *et al.* 1999, Ren *et al.* 2004, Huang *et al.* 2006). Although the studies employing pharmacologic inhibition of A1AR strongly suggested a key role of adenosine acting as a vasoconstrictor during TGF responses, the concept of adenosine as a mediator of TGF was substantially strengthened by the generation of two independent mouse strains with targeted inactivation of A1AR (Brown *et al.* 2001, Sun *et al.* 2001). Both groups independently addressed the role of adenosine for TGF in A1AR-deficient mice and unanimously reported a complete loss of TGF responsiveness in A1AR knockout mice of both strains, despite normal blood pressure, angiotensin II levels and no apparent kidney malformations (Brown *et al.* 2001, Sun *et al.* 2001). The authors concluded from their findings that adenosine acting via A1AR is an indispensable prerequisite for TGF to function normally.

In summary, experimental evidence has accumulated suggesting that adenosine approaching A1AR from the interstitial side constitutes a necessary component for normal TGF function. The question whether adenosine acts as a specific mediator or merely as a necessary co-factor in linking tubular MD cells and smooth muscle cells of the afferent arteriole during TGF responses could not be resolved conclusively by the studies summarized above. However, recent experiments using an 'adenosine clamp' support the concept that adenosine does in fact act as a mediator of TGF (Thomson *et al.* 2000). The results of this study will be further addressed later.

Tubuloglomerular feedback: role of ATP

ATP constricts afferent arterioles

Adenosine triphosphate receptors of both the P2Y and P2X families are expressed in the kidney vasculature (Churchill & Ellis 1993, Eltze & Ullrich 1996, Chan *et al.* 1998, Jankowski *et al.* 2000, White *et al.* 2001, Inscho & Cook 2002). The presence of vasoconstrictor P2X (P2X1) receptors in interlobular arteries and in afferent, but not efferent arterioles has been demonstrated by radioligand binding and immunohistochemistry (Chan *et al.* 1998). In the juxtamedullary nephron preparation, superfusion with ATP caused dose-dependent vasoconstrictor responses which were most pronounced and sustained in afferent arterioles whereas efferent arterioles turned out to be unresponsive (Inscho *et al.* 1992).

ATP as a mediator of TGF

Substantial experimental evidence suggests that ATP acting via P2 receptors is involved in renal autoregulation (Inscho *et al.* 1996, Majid *et al.* 1999, Inscho 2001), the blood pressure-dependent alteration of renal vascular resistance which results from the cooperation of myogenic and TGF-related components. Thus, afferent arteriole vasoconstriction in response to increases in renal perfusion pressure was markedly attenuated after pharmacological P2X receptor blockade as well as in P2X1 receptor-deficient mice (Inscho *et al.* 1996, 2003). Shear stress has been implicated in cellular release of ATP (Milner *et al.* 1990, Wang *et al.* 1996, Bodin & Burnstock 2001), and extracellular ATP concentration as determined by microdialysis of extracellular fluid in the kidney has been shown to be directly related to renal perfusion pressure (Nishiyama *et al.* 2000). Like P2X receptor antagonism, desensitization of ATP receptors resulting from repeated exposure to ATP impaired renal autoregulatory behaviour (Inscho *et al.* 1996). Involvement of ATP in renal autoregulatory adjustments is most likely mediated by endothelium-derived ATP acting on P2X receptors. In contrast to autoregulation the effect of ATP receptor antagonism on TGF responsiveness has been studied in only a few investigations with contradictory results. Two studies that specifically

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investigated the effect of ATP receptor antagonism on TGF responsiveness failed to establish a direct involvement of ATP in mediating TGF (Ren *et al.* 2004, Huang *et al.* 2006). A more indirect experimental approach in which ATP infusion to peritubular capillaries was used to desensitize vasoconstrictor P2X receptors resulted in a substantially reduced TGF responsiveness even after ATP-mediated vasoconstriction had waned. From this, the authors concluded that ATP acting on P2X receptors contributes to mediating TGF responses (Mitchell & Navar 1993). In view of the rapid desensitization of P2X1 receptors upon ATP binding the long-lasting vasoconstriction caused by sustained exposure of MD cells to high NaCl concentrations seems difficult to explain. It is possible that afferent arterioles express other subtypes of the P2X receptor family which do not desensitize rapidly and could provide sustained vasoconstriction upon exposure to ATP (van der Giet *et al.* 1999). Whereas P2X1 and P2X3 receptors are known to desensitize rapidly, other members of the P2X family including various heterodimers like P2X2/P2X3 (Lewis *et al.* 1995) or P2X1/P2X5 (Le *et al.* 1999) have been demonstrated to show little desensitization upon exposure to ATP (Werner *et al.* 1996) as reviewed in detail elsewhere (North & Barnard 1997). Smooth muscle cells of various vessels are known to express several different P2X receptor subtypes in parallel (Valdecantos *et al.* 2003).

The concept of ATP being involved in the mediation of the TGF response was boosted by the finding that MD cells release ATP across their basolateral membrane in response to increased tubular salt delivery (Bell *et al.* 2003, Komlosi *et al.* 2004). In this elegant experimental approach ATP release from MD cells was demonstrated in an isolated perfused JGA preparation by the use of bio-sensor cells (Hayashi *et al.* 2004). Using a holding pipette, P2X receptor-expressing PC12 sensor cells (Arslan *et al.* 2000) loaded with the Ca^{2+}-sensitive dye Fura-2 were positioned close to the surface of MD cells. ATP release was then determined as a P2X-mediated increase in intracellular Ca^{2+} of the sensor cell. An increase in NaCl concentration of the tubular perfusate (25–148 mM NaCl) elicited an augmented release of ATP across the basolateral membrane of MD cells. Basolateral exit of ATP from MD cells was found to utilize maxi anion channels which had previously been shown to be ATP permeable (Sabirov *et al.* 2001, Dutta *et al.* 2002). Maxi anion channels of the MD were demonstrated to be fully blocked in the presence of Gd^{3+} which had also been used before to inhibit ATP release from other cell types (Taylor *et al.* 1998, Hazama *et al.* 1999, 2000). Using mesangial cells as biosensors for the detection of ATP release by MD cells (Komlosi *et al.* 2004), a linear increase of ATP release from MD cells was observed in response to stepwise changes of perfusate NaCl concentration in the (physiological) range of 20–60 mM (Schnermann *et al.* 1982). ATP release was reduced, but not abolished in the presence of the loop diuretic furosemide. The authors speculate that the latter finding may reflect either incomplete inhibition of NKCC2 or the participation of another – non-furosemide sensitive – NaCl transporter in MD cells such as NHE2 (Peti-Peterdi *et al.* 2000, 2002b).

In a recent *in vitro* study in the rabbit, Peti-Peterdi (2006) showed that the initiation of TGF responses elicits a calcium wave that spreads within the JGA and reaches proximal parts of the afferent arteriole. As the propagation of this calcium wave could be inhibited by gap junction uncoupling, this finding suggests that ATP may be released by cells located between MD cells and smooth muscle cells (e.g. mesangial cells) during TGF responses and thus contribute to the spreading of the calcium wave. Connexins are known to either couple cells via gap junction or to form hemi-channels which allow cellular release of ATP (Cotrina *et al.* 1998, Schwiebert 2000, Arcuino *et al.* 2002, Schwiebert *et al.* 2002, Yao *et al.* 2003). Thus, cells in the JGA other than MD cells may secondarily contribute to ATP release during TGF activation. Both spreading of the calcium wave and vasoconstrictor responses were diminished by pharmacological inhibition of P2X purinergic receptors suggesting a key role of ATP acting on P2X receptors for TGF signalling in this experimental setting. Conversely, A1AR blockade was without effect. The authors speculate that the latter finding may indicate species differences between A1AR signalling in rabbits vs. mice and rats (Peti-Peterdi 2006). It is also conceivable that adenosine generated in the JGA under *in vitro* conditions is a less potent vasoconstrictor compared with the *in vivo* situation. For example, afferent arteriole constriction following TGF activation *in vivo* relies on the presence of angiotensin II as a co-constrictor (Huang *et al.* 1988, Mitchell & Navar 1988, Braam & Koomans 1995, Schnermann *et al.* 1997, Turkstra *et al.* 1998, 2000) which is most likely missing under *in vitro* conditions.

Regulated ATP release from MD cells is the first transport mechanism for nucleotides/nucleosides in the JGA supported by experimental evidence. Various other translocation pathways for nucleotides/nucleosides in the JGA might be possible in principle, as reviewed somewhere else in detail (Schnermann & Levine 2003) and summarized in Figure 1. They include, but are not limited to, release/uptake of nucleosides via concentration gradient-dependent equilibrative nucleoside transporters, Na^+-coupled nucleoside uptake via concentrative nucleoside transporters, and cellular export of cAMP which is readily cleaved by extracellular phosphodiesterases forming adenosine. The

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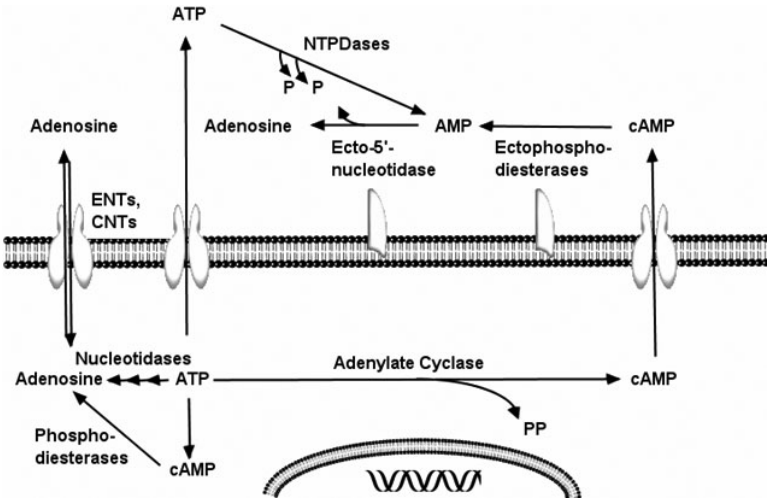

Figure 1 Schematic illustration of typical cellular transport pathways of purinergic compounds including equilibrative nucleoside transporters (ENTs), Na^+-coupled nucleoside cotransporter (CNTs), cAMP and ATP transport. ATP and cAMP are broken down to form adenosine both intra- and extracellularly.

existence and physiological relevance of these systems in the confines of the JGA, however, have not yet been established.

In summary, regulated release of ATP from the basolateral membrane of MD cells in response to modulation of tubular NaCl concentration renders ATP an ideal candidate as a mediator of TGF. Although experimental evidence for a *direct* effect of ATP on TGF-dependent vasoconstriction of afferent arterioles has remained controversial, ATP appears to be a key mediator of the myogenic, TGF-independent component of renal autoregulation.

Tubuloglomerular feedback: cooperation of ATP and adenosine

A scientific controversy has developed over the past few years as to whether ATP or adenosine eventually accounts for mediating TGF (Persson 2001, Nishiyama & Navar 2002, Schnermann 2002). Results from recent studies may offer a solution to this controversy suggesting that both ATP *and* adenosine may be involved in TGF mediation. The two key findings suggesting a role of ATP or adenosine in mediating TGF, namely the regulated release of ATP from MD cells and the complete lack of TGF responsiveness in A1AR-deficient mice, may be integrated into a single signalling pathway by assuming that ATP, released from MD cells, is the primary mediator of TGF which subsequently gets dephosphorylated within the JGA to form adenosine; adenosine may then eventually cause afferent arteriole constriction.

ATP degrading enzymes in the JGA

The stepwise extracellular dephosphorylation of ATP requires ATP-degrading enzymes which may functionally be divided into two classes: enzymes that hydrolyse ATP and ADP with various selectivity for either ATP or ADP, and enzymes that specifically hydrolyse 5′-AMP (Resta *et al.* 1993, Wang & Guidotti 1996, Kegel *et al.* 1997). ATP (and ADP)-degrading enzymes which comprise the families of ectonucleotide triphosphate diphosphohydrolases (NTPDase, NTPDase1-8) and ectonucleotide pyrophosphatase phosphodiesterases (NPPs, NPP1-3) are widely expressed in many tissues including the kidney (Chadwick & Frischauf 1997, 1998, Kegel *et al.* 1997, Vekaria *et al.* 2006). NTPDase1 was shown to be localized in the brush border of the proximal tubule, throughout the renal vasculature and in mesangial cells (Sandoval *et al.* 1996, Kishore *et al.* 2005, Vekaria *et al.* 2006). Immunohistochemistry also revealed high expression of ectonucleotide pyrophosphatase phosphodiesterases (NPP3) in the glomerulus (Vekaria *et al.* 2006). The investigation of the role of these ecto-NTPDases in TGF signalling is somewhat hampered by the lack of pharmacologic agents to specifically block ecto-ATPase activity. Known inhibitors of ecto-NTPDases also block P2 receptors (e.g. suramin or reactive blue 2) or exhibit substantial general toxicity (e.g. sodium azide). However, NTPDase1 knockout mice have been generated (Enjyoji *et al.* 1999), and TGF responsiveness in this mouse model awaits investigation. Dephosphorylation of 5′-AMP producing adenosine is the final step in the successive degradation of ATP. Enzymes capable of dephosphorylating 5′-AMP in the extracellular space include the ubiquitously expressed alkaline phosphatase and ecto-5′-nucleotidase. Ecto-5′-nucleotidase is a membrane-bound enzyme that exerts its catalytic activity in the extracellular space. This enzyme is identical with the lymphocyte differentiation protein CD73 found on the surface of a subset of T cells (Resta & Thompson 1997). Ecto-5′-nucleotidase is found in many organs and tissues with particularly high expression levels in the kidney where it is localized in the brush border of the

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proximal tubules, cortical interstitial cells (peritubular fibroblasts) and in the intra- and extra-glomerular mesangium (Ardaillou *et al.* 1992, Bachmann *et al.* 1993, Le Hir & Kaissling 1993, Resta *et al.* 1993, Wu *et al.* 1999, Castrop *et al.* 2004, Vekaria *et al.* 2006). Ecto-5′-nucleotidase enzyme activity was observed in freshly isolated glomeruli from the rat kidney (Satriano *et al.* 2006). Thus, the localization of ecto-5′-nucleotidase in the glomerular mesangium and in peritubular fibroblasts often surrounding the afferent arteriole, is compatible with the concept of an ATP-adenosine axis in JGA function. ATP may be released in a regulated fashion from the basolateral membrane of MD cells – and potentially secondarily from mesangial cells (Peti-Peterdi 2006) – and may after degradation to 5′-AMP lead to ecto-5′-nucleotidase-dependent formation of adenosine during diffusion in the extracellular compartment of the JGA.

Ecto-5′-nucleotidase and TGF

Activity of ecto-5′-nucleotidase catalysing the final step of the successive degradation of ATP would play a key role in adenosine formation after degradation of ATP released by MD cells. Osswald *et al.* (1982) first suggested a role of ecto-5′-nucleotidase in the TGF signalling cascade based on their finding that TGF responses were diminished by the ecto-5′-nucleotidase inhibitor α,β-methylene adenosine diphosphate (MADP). In a more recent study in an isolated perfused JGA preparation, the ecto-5′-nucleotidase inhibitor MADP was shown to abolish the constriction of afferent arterioles caused by increases in tubular NaCl concentration (Ren *et al.* 2004). An impairment of TGF responsiveness during ecto-5′-nucleotidase inhibition, however, does not rule out the possibility that adenosine generated via ecto-5′-nucleotidase is a necessary co-factor rather than a final mediator of a signalling cascade which may originate from ATP released by MD cells. As mentioned before, this issue was addressed in a recent elegant study by Thomson *et al.* (2000) who investigated TGF responses under conditions of a pharmacologic adenosine clamp. They first demonstrated that TGF responses were markedly reduced during ecto-5′-nucleotidase inhibition with MADP. They then added the A1AR agonist cyclohexyladenosine (CHA) to achieve constant activation of A1A receptors. If adenosine generated by ecto-5′-nucleotidase (or some other source) was a necessary co-factor for TGF to function normally one would expect that TGF responsiveness would be – at least partially – restored after application of CHA. However, CHA further reduced TGF responses. The authors concluded that constant activation of A1AR is not sufficient to cause afferent arteriole vasoconstriction in response to increases in tubular NaCl concentration, but that adenosine concentrations in the JGA compartment need to fluctuate depending on tubular salt concentration.

The role of ecto-5′-nucleotidase in a potential ATP-degrading cascade during TGF responses was further addressed by the use of mice with targeted deletion of the ecto-5′-nucleotidase/CD73 gene (Castrop *et al.* 2004, Huang *et al.* 2006). Three independent mouse lines with inactivation of ecto-5′-nucleotidase have been generated (Castrop *et al.* 2004, Koszalka *et al.* 2004, Thompson *et al.* 2004), two of which have been used to study TGF (Castrop *et al.* 2004, Huang *et al.* 2006). Although minor strain-dependent differences exist, ecto-5′-nucleotidase/CD73-deficient mice of all three strains are viable and show no gross abnormalities. In the first ecto-5′-nucleotidase/CD73 knockout mouse model reported, TGF responses of superficial nephrons were shown to be substantially impaired (Castrop *et al.* 2004). In 75% of the nephrons examined TGF was either absent or reduced by 80% despite normal constrictor responsiveness of isolated perfused afferent arterioles to adenosine. In the remaining 25% of nephrons an abnormal TGF responsiveness was observed with an initial constrictor response to saturating increases in tubular perfusion being normal, but with repeated changes in tubular perfusion causing progressively diminished responses. Also, residual TGF responses disappeared after prolonged tubular perfusion at high flow rates, i.e. after prolonged activation of TGF. This ‘exhaustion-phenomenon’ of TGF was not observed in nephrons from wild type mice. The reason for this finding was not addressed experimentally, but the authors speculated that under conditions of compromised adenosine generation ATP may reach the afferent arteriole causing transient vasoconstriction. As mentioned before, vasoconstrictor P2X1 receptors which are present in the afferent arteriole (Chan *et al.* 1998) are known to desensitize rapidly (Surprenant *et al.* 1995, Werner *et al.* 1996, Schwiebert & Kishore 2001). As TGF function was completely abolished in A1AR knockout mice (Brown *et al.* 2001, Sun *et al.* 2001), an initial transient vasoconstriction in response to increases in tubular perfusion flow may indicate increased ATP concentrations under conditions of compromised ATP degradation resulting from absence of ecto-5′-nucleotidase. Another explanation for partial TGF responsiveness in a subset of nephrons could be that 5′-AMP may be hydrolysed by other adenosine-forming enzymes like alkaline phosphatase (Hardonk & Koudstaal 1968, Briere *et al.* 1984) which was found to be upregulated in ecto-5′-nucleotidase knockout mice (Castrop *et al.* 2004). Thus, impairment of TGF in ecto-5′-nucleotidase-deficient mice supports the assumption that extracellular adenosine generation via ecto-5′-nucleotidase in the JGA may be a key step in the

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signalling mechanism linking MD cells and vascular cells of the afferent arteriole. Most recently, the role of ecto-5′-nucleotidase in TGF signalling was confirmed by a study in an independently generated strain of ecto-5′-nucleotidase-deficient mice (Huang *et al.* 2006). TGF responsiveness was substantially reduced in ecto-5′-nucleotidase-deficient mice compared with wild type controls, with remaining TGF responsiveness being fully abolished after A1AR blockade. In contrast, P2X receptor antagonism with the suramin-analogue NF279 (Damer *et al.* 1998, Lambrecht *et al.* 1999, Klapperstuck *et al.* 2000) did not significantly affect the change in single nephron GFR caused by variations of tubular perfusion flow. Nevertheless, NF279 tended to reduce TGF responsiveness in CD73-deficient mice whereas it had no obvious effect in wild types again suggesting that in situations of compromised adenosine generation (and presumably concomitant increased ATP availability) TGF signalling might become more dependent on ATP causing afferent arteriole constriction directly. It remains unclear if ATP may act as a mediator of TGF or rather a vasoconstrictor co-factor under conditions of compromised adenosine generation. In the latter case, ATP would function as a co-constrictor of the afferent arteriole similar to angiotensin II, strengthening the reduced A1AR-mediated vasoconstriction.

A key role of extracellular adenosine generation in the JGA for TGF to function normally was also suggested in a study addressing the impact of mesangial cell damage induced by Thy 1-1 antibody and complement treatment on TGF function *in vitro* (Ren *et al.* 2002). Thy 1-1/complement treatment of the JGA fully abolished TGF responsiveness despite preserved contractility of the afferent arteriole. As mesangial cells express ecto-5′-nucleotidase these data might indicate an interruption of the signalling pathway between MD cells and the afferent arteriole. Interestingly, in this experimental setting disruption of gap junctions by heptanol (Takens-Kwak *et al.* 1992, Bastiaanse *et al.* 1993) also eliminated TGF responsiveness (Ren *et al.* 2002). This may indicate that intact cell-to-cell communication within the JGA is necessary for the propagation of a signal from the MD cells to vascular smooth muscle cells of the afferent arteriole for TGF to function normally. Alternatively, destruction of gap junctions by heptanol treatment may have compromised JGA cell function in general. Similarly, in another recent *in vitro* study, heptanol compromised spreading of a calcium wave originating from MD cells and reduced proximal afferent arteriole contractility upon TGF activation (Peti-Peterdi 2006). Assuming that mesangial cells also release ATP upon TGF activation (Peti-Peterdi 2006), elimination of mesangial cell function by Thy 1-1/complement treatment (Ren *et al.* 2002) would lead to both diminished availability of ATP in the JGA and a compromised 5′-AMP degradation via ecto-5′-nucleotidase. A study in Thy-1 nephritic rats also suggested ecto-5′-nucleotidase to be involved in renal autoregulatory capacity. Thy-1 nephritic rat showed an unpaired autoregulatory behaviour that was partially restored after infusion of exogenous ecto-5′-nucleotidase (Takenaka *et al.* 2006).

Temporal regulation of TGF responsiveness by modulation of ecto-5′-nucleotidase activity?

Assuming a critical role of ecto-5′-nucleotidase in TGF signalling this enzyme would also be suited to serve as a regulator of temporal adaptations of TGF responsiveness. An inhibitory effect of NO donors on ecto-5′-nucleotidase enzyme activity was shown both in cell lines (Siegfried *et al.* 1996) and in freshly isolated glomeruli from rat kidney (Satriano *et al.* 2006). Inhibition of ecto-5′-nucleotidase activity by NO and, by inference, reduced adenosine generation in the JGA, would be compatible with the findings that maximum TGF responses are enhanced during NOS inhibition independent of direct effects of NO on vascular tone (Wilcox *et al.* 1992, Ito & Ren 1993, Thorup & Persson 1994, 1996, Braam & Koomans 1995), and that NO generation contributes to the TGF resetting mechanism (Thomson *et al.* 1999, Deng *et al.* 2002). Conversely, pre-treatment of rats with a high salt diet increased ecto-5′-nucleotidase activity in isolated glomeruli by about 28% compared with rats on a low salt diet (Satriano *et al.* 2006). Enhanced glomerular ecto-5′-nucleotidase activity during high oral salt intake may, at least in part, contribute to increased renal cortical interstitial adenosine levels observed during high salt intake (Siragy & Linden 1996, Zou *et al.* 1999). As NO formation of the MD also increases with tubular salt concentration (Liu *et al.* 2002, Kovacs *et al.* 2003), increased ecto-5′-nucleotidase activity during high salt intake *in vivo* may be counteracted by NO generation leading to a fine-tuning of ecto-5′-nucleotidase enzyme activity and eventually adenosine generation in the JGA.

In summary, a signalling cascade originating from ATP released by MD cells in responses to changes in tubular luminal salt concentration, with subsequent adenosine formation through extracellular ATP degradation, and A1AR-mediated afferent arteriole constriction may offer a concept to form a coherent picture to integrate both ATP and adenosine into a signalling sequence that mediates TGF. However, further investigations are required to establish whether this proposed pathway exclusively accounts for the mediation of TGF regulation of GFR or whether it rather constitutes one of several critical components. It appears not far-fetched that species differences and different methods

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to assess TGF responses may account for the sometimes contradictory findings regarding the mechanisms of TGF signalling. Also, contributions of single signalling components which are necessary for full TGF responsiveness may vary between more superficial or deeper portions of the renal cortex (Moore & Casellas 1990), a possibility that awaits further systematic investigation.

Conflict of interest

None.

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