Integrating multiple paracrine regulators of renal microvascular dynamics

Navar, L. Gabriel. Integrating multiple paracrine regulators of renal microvascular dynamics. Am. J. Physiol. 274 (Renal Physiol. 43): F433–F444, 1998.—There has been tremendous growth in our knowledge about the multiple interacting mechanisms that regulate renal microvascular function. Paracrine signals originating from endothelial and epithelial cells exert profound influences on the basal tone and reactivity of the pre- and postglomerular arterioles. Selective responsiveness of these arterioles to various stimuli is possible because of differential activating mechanisms in vascular smooth muscle cells of afferent and efferent arterioles. Afferent arterioles rely predominantly on voltage-dependent calcium channels, while efferent arterioles utilize other mechanisms for calcium entry as well as intracellular calcium mobilization. The autoregulatory responses of pregglomerular arterioles exemplify the selectivity of these complex control mechanisms. The myogenic mechanism responds to increases in renal perfusion pressure through “stretch-activated” cation channels that lead to depolarization, calcium entry, and vascular contraction. Autoregulatory efficiency is enhanced by the tubuloglomerular feedback (TGF) mechanism which responds to flow-dependent changes in tubular fluid composition at the level of the macula densa and transmits signals to the afferent arterioles to alter the activation state of voltage-dependent calcium channels. Recent studies have implicated extracellular ATP as one paracrine factor mediating TGF and autoregulatory related signals to the afferent arterioles. Other paracrine agents including nitric oxide, angiotensin II, adenosine, and arachidonic acid metabolites modulate vascular responsiveness in order to maintain an optimal balance between the metabolically determined reabsorptive capabilities of the tubules and the hemodynamically dependent filtered load.

renal autoregulation; tubuloglomerular feedback mechanism; calcium channels; angiotensin II; extracellular adenosine 5'-triphosphate; nitric oxide; arachidonic acid metabolites

It was truly a great honor to be designated as the 1997 Gottschalk Distinguished Lecturer. Carl Gottschalk is an outstanding pioneer in the world of renal physiology, and his incisive studies have had a great impact on my own work. His landmark paper of 1959 in which he reported the osmolalities of tubular fluid samples collected from multiple sites in the nephron provided definitive evidence that early distal tubule fluid is very hypotonic and closely regulated (22). These results strongly influenced our ideas regarding the role of the macula densa in regulating afferent arteriolar tone. Indeed, I can remember discussions with my mentor, Dr. Arthur Guyton, when he was developing his concepts for renal autoregulation by feedback from the macula densa (23). He was impressed by the findings of Dr. Gottschalk and used these data as the basis for the hypothesis that the regulation of early distal tubular fluid composition is a critical function of the feedback mechanism operating from the macula densa to the glomerular arterioles.

It has been over three decades since the concepts related to the tubuloglomerular feedback (TGF) mechanism began to receive serious attention by physiologists. We have learned a great deal since that time, but we must admit that the elusive intrinsic mechanisms that regulate renal microcirculatory dynamics have continued to defy complete resolution. Nevertheless, when one considers the myriad of recently discovered paracrine agents that have been shown to influence the
The presence of calcium channel blockade, further regulatory components of the renal vascular resistance. In which also exerted vasodilatory actions on nonautoregulatory activation mechanisms (67, 73). This effect was demonstrated that Ca\(^{2+}\) channel blockers primarily vasodilate preglomerular arterioles and have minimal effects on efferent arterioles (12, 18, 52, 53). It was also demonstrated that while angiotensin II (ANG II) exerts clear vasoconstriction of both afferent and efferent arterioles, calcium channel blockers prevent only the afferent but not the efferent vasoconstrictor actions of ANG II (12, 16). Additionally, the depolarizing effects of high KCl concentrations primarily cause afferent arteriolar constriction and elicit increases in cytosolic Ca\(^{2+}\) in afferent arteriolar vascular smooth muscle cells through activation of voltage-dependent Ca\(^{2+}\) channels (9, 16, 43, 55). Recent studies by Inscho et al. (43) on isolated preglomerular vascular smooth muscle cells demonstrated that membrane depolarization increases cytosolic Ca\(^{2+}\) via entry of extracellular Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels. Loutzenhiser et al. (51) recently reported that ANG II-mediated vasoconstriction is associated with membrane depolarization in afferent arterioles, while efferent arterioles vasocostrict but do not depolarize during exposure to ANG II. Collectively, these studies demonstrate that Ca\(^{2+}\) influx through voltage-gated channels is of major importance in the regulation of afferent arteriolar tone and is the main effector of autoregulatory responses. In contrast, efferent arterioles are more dependent on other Ca\(^{2+}\) entry pathways or on mechanisms that primarily activate intracellular calcium mobilization or influence other mechanisms. Certain paracrine agents such as ATP may selectively influence only one of these mechanisms. Other agents, such as ANG II, vasoconstrict both afferent and efferent arterioles, but through distinct mechanisms. The differential effects of calcium channel blockers on afferent and efferent arteriolar diameters and on the ANG II-mediated vasoconstric-

**Fig. 1.** Results from the blood-perfused juxtamedullary nephron preparation demonstrating the differential effects of Ca\(^{2+}\) channel blockade with diltiazem on resting afferent (left) and efferent (right) arteriolar diameter and on ANG II-induced vasoconstriction. ANG II vasoconstricted both afferent and efferent arterioles. Ca\(^{2+}\) channel blockade caused vasodilation of afferent arterioles but not of efferent arterioles and completely prevented ANG II-induced vasoconstriction of afferent arterioles. In contrast, the ability of ANG II to constrict efferent arterioles remained intact. [Data from Carmines and Navar (12).]
Characteristics of the Renal Autoregulatory Response

It is well known that in response to changes in renal arterial perfusion pressure over a wide range from as low as 70 mmHg to over 180 mmHg, all intrarenal indices of renal microvascular function exhibit highly efficient autoregulatory behavior such that steady-state renal blood flow (RBF), GFR, glomerular pressure, proximal tubule pressure, and peritubular capillary pressure remain relatively unchanged (1, 66, 68). The close coupling of all these indices of renal function indicates that the predominant resistance changes are localized to preglomerular segments. In some cases, however, autoregulation of GFR is more efficient than autoregulation of RBF, suggesting that, in response to decreases in arterial pressure, there is a reciprocal increase in efferent arteriolar resistance combined with the decrease in afferent arteriolar resistance (32, 56, 76, 100). This secondary mechanism, when it occurs, may be due to activation of the renin-angiotensin system in response to decreases in arterial perfusion pressure allowing GFR to be preserved more efficiently than RBF, especially at arterial pressures toward the lower part of the autoregulatory range. In contrast, other studies have shown that RBF is sometimes autoregulated to lower arterial pressures than GFR (24, 49, 79). These studies suggest that as the vasodilatory signals to the afferent arterioles increase in intensity, there is a spillover effect so that the efferent arterioles also dilate slightly. When this occurs, autoregulation of RBF is maintained to lower arterial pressures than autoregulation of GFR, and there is an uncoupling of RBF and GFR autoregulation (79). Nevertheless, over the major portion of the autoregulatory range, there is close coupling of RBF and GFR autoregulation which supports the view that most or all of the autoregulatory-mediated changes in renal vascular resistance are localized at preglomerular arterioles.

Recent in vitro studies directly visualizing effenter arteriolar diameters in the juxtamedullary nephron preparation provided further evidence that the effenter arterioles do not contribute perceptibly to the vascular resistance adjustments in response to changes in perfusion pressure (28). Effenter arterioles do not appear to have a myogenic mechanism, since they did not respond to changes in perfusion pressure even when the preglomerular arterioles were dilated to allow transmission of pressures to the postglomerular network. Collectively, the findings of a selective vasoactive action on only one limb of the glomerular vasculature have raised the intriguing question regarding how there can be such a high degree of selectivity exerted by the signals mediating the autoregulatory response. This has led to consideration of unique mechanisms that are able to sense changes in perfusion pressure and selectively regulate preglomerular vascular tone (66). Studies using the juxtamedullary nephron preparation, which allows visualization of the entire preglomerular vasculature (15), have shown that the larger preglomerular arterioles as well as the afferent arterioles respond to changes in perfusion pressure (10). This may help explain why there is some contribution of interlobular arterioles to the autoregulatory related adjustments in vascular resistance (34). Nevertheless, the pressure profile along the preglomerular arteriolar tree indicates that the major fraction of the changes in vascular resistance in response to changes in arterial pressure occurs at the level of the afferent arterioles (13–15, 80).

Myogenic and TGF Mechanisms as Mediators of the Autoregulatory Response

Many of the earlier controversies were oriented toward the concept that there could be only one major mediator of the renal autoregulatory mechanism. However, two mechanisms have survived the rigors of experimental testing (Fig. 2). The myogenic mechanism is similar to that existing in many other vascular beds and is based on the ability of changes in perfusion pressure to cause an active vasoconstriction in response to an increase in pressure (P) which reduces the radius (R) and partially reduces the wall tension (T) back toward control. The TGF mechanism primarily regulates tubular fluid composition (osmolality, NaCl concentration) at the level of the macula densa and adjusts filtered load by regulating afferent arteriolar tone. Solid lines indicate direct effects, and dashed lines indicate inverse effects. [TGF diagram adapted from Braam et al. (6).]
pressure to alter the transmural tension of blood vessels and thus stimulate "stretch-activated channels" (17, 25, 59, 77). This leads to membrane depolarization and Ca\(^{2+}\) entry via voltage-dependent Ca\(^{2+}\) channels. In addition, phospholipase C activation leads to increased formation of inositol trisphosphate and release of Ca\(^{2+}\) from intracellular calcium stores (64). The active vasoconstriction reduces the radius which diminishes the transmural tension leading to the maintenance of a higher vascular resistance. It is now generally agreed that the myogenic mechanism does contribute to autoregulation of the renal microvasculature, and it has been shown that preparations that do not have an intact TGF mechanism can exhibit changes in vascular diameter in response to changes in perfusion pressure (30, 54, 88). The responses seen in the hydronephrotic kidney preparation in which the tubular network has been obliterated demonstrate residual autoregulatory capability but at very low flows (88). Nevertheless, the intact renal vasculature also exhibits myogenic responses with different dynamic characteristics than the TGF mechanism (1, 2, 35).

Many renal physiologists oriented toward mechanisms unique to the kidney have championed the hypothesis that the TGF mechanism serves as the major mediator of the renal autoregulatory mechanism (23, 65, 66, 92). Indeed, it has often been considered that the responses in renal vascular resistance to changes in arterial pressure are but one manifestation of the collective action of the TGF operating in the total nephron population (65, 69). By monitoring flow-related changes in tubular fluid composition emerging from the ascending loop of Henle, the macula densa cells are critically positioned to regulate the hemodynamic forces at the glomerulus and maintain balance between the filtered load and the reabsorptive capabilities of the tubules. As shown in Fig. 2, the macula densa can respond to a wide range of disturbances, including those associated with changes in arterial perfusion pressure (6). Other disturbances include changes in plasma colloid osmotic pressure and changes in proximal tubule reabsorption rate (65). Because of the unique characteristics of the ascending loop of Henle, there is a close relationship between the flow and the degree to which the tubular fluid is diluted (5). At normal distal flow, tubular fluid is very hypotonic, as originally reported by Gottschalk and Myle (22). The ability to dilute the tubular fluid diminishes as volume delivery from the proximal nephron increases, and the tubular fluid becomes less hypotonic, approaching isotonicity at very high delivery rates. Under normal circumstances, most of the solute is NaCl so that tubular fluid osmolality and NaCl concentration are closely coupled. At present, it is still unresolved whether the macula densa luminal activation step is responsive specifically to NaCl or to total solute concentration, but strong cases have been made for both possibilities (5–7, 69).

The TGF mechanism helps explain how any disturbance that causes flow-related changes in tubular fluid composition at the level of the macula densa can influence afferent arteriolar tone and thus glomerular dynamics (6). This basic hypothesis was initially developed because of the unique morphology of the macula densa-juxtaglomerular apparatus complex (20). However, physiological evidence was provided as early as 1957, when the Hungarian physiologist, Laszlo Harsing, reported the use of phlorizin to inhibit tubular glucose reabsorption and thus diminish proximal reabsorption rate. He noted the resultant decreases in RBF and GFR and postulated that this effect was mediated by a "tubuloglomerular equilibrium" and that "...with an increase in filling of the distal tubule, impulses coming from the macula densa play a role in the regulation of RBF and GFR" (31). To my knowledge this was the first time that specific reference was made to a "tubuloglomerular" mechanism, which gave rise to our commonly used term. Clearly, the TGF mechanism has been much more attractive than the myogenic mechanism as a mechanism subserving unique aspects of kidney function rather than simply autoregulating blood flow (23, 31, 65, 69, 92).

It now seems clear that while not solely responsible, an intact TGF mechanism is essential for the manifestation of highly efficient renal autoregulatory behavior that is characteristic of the renal circulation (35, 89). Many different experimental approaches have been used to evaluate the relative contributions of the myogenic and TGF mechanisms to renal autoregulation (2, 35). We have used the juxtamедullary nephron preparation, because it provides a unique way to compare the autoregulatory responses obtained during maintained flow to the macula densa with those obtained after interruption of flow to the macula densa (80, 89). Takenaka et al. (89) followed changes in afferent arteriolar diameter and single afferent arteriolar blood flow velocity during changes in perfusion pressure. With the preparation intact, afferent arteriolar diameter decreased while blood flow velocity increased during increases in perfusion pressure. As shown in Fig. 3, the resultant afferent arteriolar blood flow exhibited highly efficient autoregulation in response to increases in perfusion pressure even though velocity remained elevated due to the reduced cross-sectional area of the afferent arteriole. A unique aspect of this preparation is that the TGF mechanism can be interrupted by transection of the papilla, which contains the loops of Henle of the nephrons being studied. The effects of this procedure were compared with the effects of high doses of furosemide, which has been shown to block the ability of the macula densa to transmit TGF responses (99). As shown in Fig. 4, following papillary transection or administration of furosemide to block macula densa transmission of TGF signals, autoregulatory efficiency was diminished. However, there was still autoregulatory capability which was subsequently blocked by superfusing the tissue with the calcium channel blocker, diltilzam. During calcium channel blockade, the afferent arterioles became passive in response to changes in arterial pressure and actually increased in diameter slightly with increases in perfusion pressure. These results indicate that the myogenic mechanism is important in preventing the passive afferent arteriolar disten-
Paracrine Mediators and Modulators of TGF Signals

Continued interest in the role of the TGF mechanism as an important regulator of GFR in a variety of circumstances has led to more detailed analysis of the mechanisms of communication between the macula densa cells and the vascular smooth cells of the afferent arteriole. While many aspects of the macula densa cell sensing, activation, and signaling mechanisms remain unresolved (69), considerable progress has been made in the analysis of potential paracrine agents serving to communicate signals from the macula densa cells to the vascular smooth muscle cells. A consensus is emerging that several vasoactive agents may be released by the macula densa cells to regulate afferent arteriolar tone. Some of these possible agents are shown in Fig. 5. This has made the search for the specific mediator of TGF signals which serves as the direct regulator of afferent arteriolar resistance in response to flow associated changes in tubular fluid composition much more challenging. Many of these other paracrine agents may exert modulating influences to dampen or accentuate the magnitude of TGF-mediated vasoconstricting signals or may simply alter basal tone or reactivity of the afferent and efferent arterioles (Fig. 6).

The single most important criterion for a paracrine mediator of the TGF mechanism is that a change in distal flow leads to a change in the macula densa cell release of the TGF mediator such that increases in flow cause vasoconstriction, while decreases in flow lead to vasodilation. Thus there may be paracrine agents that influence afferent arteriolar tone but do not fit this criterion. For example, many studies have shown important interactions between local ANG II and the TGF mechanism (62, 82, 86). As illustrated in Fig. 6, increases in the local ANG II activity can markedly augment the sensitivity of TGF-mediated vasoconstrictor responses, and ANG II receptor blockers reduce the magnitude of TGF signals (62, 69, 82). A recent report by Schnerrmann et al. (86), demonstrating that AT1A receptor-deficient mice have marked attenuation or abolition of TGF responses, provides direct support...
Implicating the important role of ANG II in the TGF mechanism. Additionally, it was recently shown that ANG II AT1 receptors are also present in macula densa cells (29). Other recent studies demonstrating that tubular fluid ANG II concentrations are in the nanomolar range suggests that ANG II may interact at the level of the macula densa cells as well as by influencing arteriolar vascular smooth muscles directly (70). However, the macula densa mechanism for renin release and thus for enhanced local formation of ANG II exhibits operational characteristics opposite of that required for mediation of TGF signals in that reduced flow and NaCl concentration augment renin release (8). In addition, it is still unclear how macula densa cells communicate with the juxtaglomerular apparatus (JGA) cells of the afferent arterioles to regulate renin release and ANG II formation rate (81). Thus, while it is now recognized that intrarenal ANG II serves as a very important modulator of the TGF mechanism, it is not a viable candidate as the mediator. Nevertheless, it is an essential permissive factor in that normal TGF responses are markedly attenuated or cannot be elicited when ANG II is totally blocked or absent. There is growing accord for the presence of a variety of paracrine systems in macula densa cells (Fig. 5). One recent observation generating considerable interest is that the neuronal isoform of nitric oxide (NO) synthase (NOS) is present in macula densa cells (3, 63, 93, 98). Subsequent physiological studies have now shown that NO may play an important role in modulating TGF responsiveness. As shown in Fig. 6, blockade of NOS leads to enhancement of vasoconstrictor responses to increases in distal nephron perfusion rate; the afferent arteriolar vasoconstriction observed after NO blockade

![Image of Fig. 5. Postulated macula densa signaling mechanisms. Numbers in circles refer to the following sequence of events. 1) Flow-dependent changes in tubular fluid composition (osmolality, Na+, Cl−, etc.). 2) Membrane activation such as membrane depolarization and enhanced NaCl entry. 3) Intracellular Ca2+ mobilization. 4) Intracellular events: formation and release of arachidonic acid (AA) metabolites, formation and metabolism of purinergic agents, formation of NO. 5) Effects of secreted agents on membrane potential and activation of Ca2+ channels in vascular smooth muscle cells. 6) Vascular contractile responses. COX, cyclooxygenase; TX, thromboxane; Ado, adenosine; PLA2, phospholipase A2; R, receptor. [Modified from Navar et al. (69) and includes various postulated but not yet proven mechanisms.]

![Image of Fig. 6. Schematic of micropuncture procedures used to assess TGF responses (left) and representative relationships between distal nephron volume delivery and the single-nephron glomerular filtration rate (GFR) TGF response. Effects of some agents that decrease sensitivity of TGF responses and agents that increase sensitivity of TGF responses are indicated. NOS, nitric oxide synthase; HETE, 20-hydroxyeicosatetraenoic acid; PGI2, prostacyclin.](http://ajprenal.physiology.org/)
occurs primarily at high distal flows (48, 91, 98). These data suggest that increases in flow-related changes in tubular fluid NaCl or solute concentration, which are thought to increase macula densa Ca^{2+} concentration (4), elicit dual signals mediating both vasoconstriction and vasodilation. One vasodilator component is apparently due to increased NO activity, which then partially counteracts the TGF-mediated vasoconstrictor signals. When neuronal NOS is blocked, increases in flow to the macula densa segment elicit greater vasoconstriction than observed before blockade (48, 69, 91, 97). It is presumed that the status of sodium balance and degree of volume expansion modulate the magnitude of the macula densa NO-counteracting effect on TGF responsiveness (97); however, it remains unclear whether there is actually a change in NO formation rate by macula densa cells in response to increases in distal volume and solute delivery. It is also not apparent how NO formed in a small population of cells could influence overall tissue NO levels of the vascular pole which are probably determined primarily by the greater NO production rate of the adjoining endothelial cells. Blockade of NOS with nonspecific blockers such as nitro-L-arginine also increases the magnitude of TGF responses (69, 98) and has been shown to elicit constriction of efferent as well as afferent arterioles (72). With regard to overall autoregulatory capability, several laboratories have shown that nonspecific NO blockade suppresses the plateau of autoregulation but does not alter autoregulatory efficiency, although RBF can be maintained to lower arterial pressures (57, 69).

Another rapidly growing area of interest is related to the numerous vasoactive arachidonic acid metabolites (eicosanoids). It is recognized that several of the products derived via the various enzymatic pathways can exert powerful actions on the renal microvasculature (40, 41, 58, 69, 78). In addition, the recent demonstration of the presence of cyclooxygenase-2 in the cells of the macula densa and surrounding thick ascending loop of Henle cells provides evidence for the generation of cyclooxygenase metabolites in the macula densa cells (26, 27). These findings support the concept that a variety of eicosanoids are produced by the epithelial cells of the nephron including macula densa cells (Fig. 5). Earlier studies focused on the possible role of cyclooxygenase-derived products in the control of GFR and in the mediation of renal autoregulatory or TGF responses (83). Important actions have been attributed to both vasodilator and vasoconstrictor products of the cyclooxygenase pathway (83, 94, 96). With continued studies, it became apparent that cyclooxygenase inhibition did not interfere with the autoregulatory mechanism, although it could reset the plateau of autoregulation (69). In addition, it was shown that tromboxane can serve a potent modulating role by enhancing overall TGF sensitivity in a manner similar to that shown for ANG II (94–96). It was suggested that the ability of ANG II to enhance TGF sensitivity is due, in part, to increased tromboxane production (95). Thus cyclooxygenase metabolites can either decrease or increase the sensitivity of the TGF mechanism, depending on which specific metabolite predominates.

The potential interactions among the various eicosanoids are complex, and it is not known if the rate of formation and release of eicosanoids is altered by changes in flow past the macula densa. In studies where arachidonic acid was perfused in a retrograde manner from the distal tubule toward the macula densa to allow the predominant metabolic pathway to be expressed, a vasoconstrictor response simulating a TGF signal was noted (19). It was suggested that the arachidonic acid was metabolized by the tubular cells to release predominantly vasoconstrictor substances. In experiments using the juxtamedullary nephron preparation, arachidonic acid was added to either the superfusion solution bathing the tissues from the interstitial side or the blood perfusing the vasculature (38). It was noted that arachidonic acid added from either side exerted a vasoconstrictor response primarily of the pregglomerular arterioles. Efferent arteriolar responses were minor, again demonstrating the differential responsiveness of pre- and postglomerular arterioles. An important finding of this study is that the responses to arachidonic acid are relatively slow, reaching a maximum only after ~2–3 min (38). If the responses resulting from arachidonic acid released from macula densa cells subsequent to activation of phospholipaseA2 have the same slow onset, then it seems unlikely that vasoactive eicosanoids could be directly responsible for the mediation of TGF signals which occur within seconds following the stimulating event.

Several inhibitors of the major arachidonic acid metabolism pathways have been used in efforts to delineate the nature of the eicosanoids responsible for the vasoconstrictor and vasodilator responses during treatment with arachidonic acid (38). Cyclooxygenase inhibition blocked the vasoconstrictor response caused by arachidonic acid in the blood but only slightly reduced the vasoconstrictor response to arachidonic acid added to the superfusate. In contrast, cytochrome P-450 inhibitors markedly attenuated the vasoconstrictor responses to superfused arachidonic acid suggesting important vasoactive responses of the cytochrome P-450 metabolites. The results of these experiments suggest that eicosanoids of luminal or endothelial origin include cyclooxygenase vasoconstrictors and cytochrome P-450 vasodilatory metabolites. In contrast, arachidonic acid metabolites of interstitial origin are dominated by vasoconstrictor cytochromeP-450 metabolites.

Other studies have evaluated possible participation of specific cytochrome P-450 vasoconstrictor metabolites in the TGF mechanism. Zou et al. (101) found that cytochrome P-450 inhibitors blocked TGF-mediated vasoconstrictor responses. Furthermore, perfusion of the macula densa segment with 20-hydroxyeicosatetraenoic acid, an important hydroxy/prostaglandin product, restored TGF responses, suggesting an important permissive role for this agent. It has also been shown that cytochrome P-450 inhibition markedly attenuates the afferent arteriolar changes in vascular diameter observed.
during increases in perfusion pressure (40). Other recent studies have implicated roles for both vasodilator and vasoconstrictor effects of epoxygenase metabolites with several of these being formed by the endothelial cells (39). These representative studies demonstrate that eicosanoids can exert major influences on the vascular responsiveness of the afferent arteriole. Some of these are likely to be produced by macula densa cells to influence the intensity of the TGF mechanism. In particular, the cytochrome P-450 metabolites can strongly influence both autoregulatory and TGF mechanisms. Nevertheless, their precise role in these processes appears to be primarily modulatory. Because of the relatively long time required for arachidonic acid metabolites to exert their effects, it seems unlikely that they specifically mediate the afferent arteriolar vasoconstrictor responses that occur either with increases in arterial pressure or with macula densa-derived TGF vasoconstrictor stimuli.

The general concept that an important role of the TGF mechanism is to maintain balance between the hemodynamically determined filtered load and the metabolically dependent reabsorptive capabilities has suggested some type of linkage between metabolic end products and the TGF mechanism. Because adenosine is a major metabolic end product and is known to elicit vasoactive effects, it has often been postulated as the TGF mediator (74, 85, 87). Activation of A1 receptors leads to vasoconstriction, while activation of A2 receptors causes vasodilation. In contrast to other vascular beds, A1 receptors appear to predominate in the kidney. However, the adenosine-mediated renal vasoconstrictor often wanes with time or at higher doses and may even be reversed to vasodilation (69, 90). Studies have shown that adenosine or a precursor of adenosine is released from the macula densa cells in response to increases in distal NaCl delivery (47). Thus adenosine has been viewed as one possible paracrine candidate communicating signals from the macula densa to alter renin release from the cells of the JGA and/or mediate TGF responses (7, 8). Nevertheless, adenosine is only one limb of the purinergic system, and other studies have demonstrated that ATP may also serve a paracrine role in regulating hemodynamics (42, 44, 69). The vasoconstrictor actions of adenosine are due to activation of the inhibitory G protein which reduces cAMP levels (87). The vasoconstrictor effects of ATP are due primarily to activation of a ligand-gated ion channel that can lead to membrane depolarization and activation of voltage-dependent calcium entry (43, 69). Although A1 receptor-mediated responses have also been linked to activation of L-type Ca\(^{2+}\) channels, studies in cultured renal arterial vascular smooth muscle cells failed to show an increase in cytosolic Ca\(^{2+}\) similar to that seen upon addition of ATP (69). These different mechanisms for eliciting vasoconstriction suggest that the actions of ATP more closely fit the requirements for a mediator of the TGF mechanism than the actions of adenosine. When added to the superfusion solution bathing juxtamedullary nephrons, ATP exhibited much faster responses than adenosine, which were more in line with the response time of the TGF mechanism (44). In addition, adenosine receptor blockers do not have any perceptible effect on the ability of the kidney to autoregulate RBF and GFR (37, 69).

The direct effects of ATP on vascular smooth muscle are mediated by P2 receptors, of which there are several. Autoradiographic studies have demonstrated localization of P2x receptors in the preglomerular arterioles (69). ATP causes membrane depolarization and increases in intracellular calcium concentration associated with rapid contractile responses (43, 45). The vasoconstrictive effects of ATP are much more rapid than those of adenosine, and the steady-state effects are blocked by L-type Ca\(^{2+}\) channel blockers (45, 46). In addition, the vasoconstrictor effects of ATP are selective for the afferent arteriole and do not exert perceptible effects on efferent arterioles (46). The ATP hypothesis can be reconciled with the metabolic hypothesis when one considers that, in contrast to the surrounding cells of the ascending loop of Henle, the macula densa cells, while having a high density of mitochondria, apparently have relatively low Na\(^+\)-K\(^+\)-ATPase activity (84). Thus these cells can produce ATP but may have limited use for it as a source of energy. These considerations support the feasibility for the hypothesis that the macula densa cells secrete ATP into the surrounding interstitium where it functions as a paracrine regulator of afferent arteriolar tone (42, 44, 61). After its release and action on P2 receptors on vascular smooth muscle cells of the afferent arteriole, ATP would be rapidly metabolized to ADP, AMP, and ultimately adenosine by cell surface ectonucleotidase activity so that its effects would not be prolonged once secretion was terminated (21, 87). The mechanism for the exocytotic release of ATP from macula densa cells remains unknown but could be similar to the Ca\(^{2+}\)-dependent regulation of secretion seen in other cell types (69). This response could be triggered by an increase in macula densa cytosolic Ca\(^{2+}\) caused by an increase in distal tubular fluid solute concentration.
Recent data reported by Inscho et al. (42), evaluating the effects of blockade of ATP receptors on the afferent arteriolar responses to changes in perfusion pressure in the juxtaglomerular nephron preparation, provide further support to the ATP hypothesis. Three different approaches were used to interfere with transmission of signals mediated by P2 purinoceptors. Desensitization of P2 receptors was elicited by pulsatile exposure with a stable ATP analog until the afferent arteriole no longer contracted. In another series of experiments, the P2 receptors were saturated by exposing the preparation to high concentrations of slowly metabolizable agonists. In additional experiments, P2 receptor antagonists, suramin and pyridoxal-phosphate-6 azaphenyl-2′,4′-disulfonic acid (PPADS), were used to block the activation step. In each case, the ability of the afferent arteriole to vasoconstrict in response to increases in perfusion pressure was either blocked or markedly attenuated. The effects of PPADS on the afferent arteriolar responses to changes in perfusion pressure are shown in Fig. 7. Although it is recognized that the receptor antagonists available are not highly specific, these results are consistent with earlier micropuncture experiments demonstrating that peritubular infusion of saturating doses of ATP attenuate the TGF-mediated vasoconstriction responses obtained during increases in distal nephron flow (61). Collectively, the evidence generated up to now supports the hypothesis that P2 purinoceptor activation plays an important role in the mediation of autoregulatory and TGF-dependent adjustments in afferent arteriolar tone (69).

This brief consideration of the myriad of potential paracrine signals that regulate renal microvascular tone emphasizes the complexity of renal vascular control mechanisms. The available data are consistent with the following conclusions. Afferent and efferent arterioles have distinct activation mechanisms which allow differential responses to various stimuli. Afferent arteriolar vasoconstriction is mediated primarily via voltage-dependent Ca2+ channels, but efferent constriction involves other Ca2+ entry pathways as well as intracellular Ca2+ mobilization. While both TGF and myogenic mechanisms contribute to autoregulation, the TGF mechanism is essential for the highly efficient autoregulation that is characteristic of the renal circulation. Most if not all autoregulatory responsiveness is vested in preglomerular arterioles. NO exerts vasodilatory influences on both afferent and efferent arterioles, and neuronal NO may have a unique role in counteracting TGF-mediated afferent arteriolar constriction. Intrarenal ANG II is a potent vasoconstrictor of both afferent and efferent arterioles and is a powerful modulator, but not mediator, of TGF sensitivity. Several arachidonic acid metabolites arising from the cyclooxygenase and cytochrome P-450 pathways exert actions on afferent arterioles through both direct and indirect mechanisms. ATP, as well as adenosine, exerts paracrine influences on renal microcirculation. Blockade or saturation of ATP receptors interferes with autoregulatory behavior and TGF responses, suggesting a pivotal role for ATP in the mechanism of renal autoregulation.

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REFERENCES


