Angiotensin II contributes to glomerular hyperfiltration in diabetic rats independently of adenosine type I receptors

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Patinha D, Fasching A, Pinho D, Albino-Teixeira A, Morato M, Palm F. Angiotensin II contributes to glomerular hyperfiltration in diabetic rats independently of adenosine type I receptors. Am J Physiol Renal Physiol 304: F614–F622, 2013. First published January 2, 2013; doi:10.1152/ajprenal.00285.2012.—Increased angiotensin II (ANG II) or adenosine can potentiate each other in the regulation of renal hemodynamics and tubular function. Diabetes is characterized by hyperfiltration, yet the roles of ANG II and adenosine receptors on renal hemodynamics and tubular function both in control and diabetic rats. For that reason, we developed a protocol (Fig. 1B) in which the drugs were infused directly into the renal artery in a slow step-wise manner, allowing to directly investigate the effects on intrarenal hemodynamics and tubular function without confounding

ADENOSINE AND THE RENIN-ANGIOTENSIN SYSTEM (RAS) are involved in the regulation of intrarenal hemodynamics and tubular function (18, 53). It is well-known that activation of either angiotensin II (ANG II) AT1 receptors or adenosine A1 receptors alters kidney function (18, 53). ANG II, acting on AT1 receptors, reduces glomerular filtration rate (GFR) and renal blood flow (RBF) by increasing pre- and postglomerular resistances (8, 44). Indeed, the most commonly used treatment to reduce the progression of proteinuric kidney disease in diabetics includes targeting the RAS (3). Adenosine controls renin release (2, 15, 25, 48), has direct vascular effects (1, 32, 33, 38), and is a fundamental component of the tubuloglomerular feedback (TGF) mechanism (46, 53).

Diabetic nephropathy is a leading cause of morbidity and mortality. Since the initial glomerular hyperfiltration (29) is crucial for the progression of renal injury (24), it is very important to establish and target its cause yet the mechanism remains unclear. Reduced preglomerular vascular resistance has been emphasized (7) and commonly attributed to the combination of a reduced TGF (52) and myogenic response of the afferent arteriole (14). Adenosine, through activation of adenosine A1 receptors, is the main effector of the TGF-induced afferent arteriolar vasoconstriction (46, 54). However, diabetic adenosine A1 receptor knockout mice lack the TGF mechanism and still develop hyperfiltration (45), suggesting that TGF is not the only mechanism involved (39). Also, in early diabetic nephropathy there is increased proximal Na+ reabsorption (13) associated with enhanced expression of adenosine A1 receptors (37), tubular hypertrophy, and increased Na+/glucose cotransport (52), which may also contribute to hyperfiltration (39) due to the decreased pressure in the Bowman’s space that enhances the net ultrafiltration pressure by a nonvascular mechanism.

Several studies have addressed the roles of ANG II and adenosine, and their interaction, in controlling vascular tone using either isolated microvessels, in situ perfused kidneys, pathological alterations or systemic manipulation of these vasoactive systems (12, 36, 49, 56). However, the in vivo significance of tonic baseline activation of ANG II and adenosine receptors, and any interaction between the two, are presently unknown, partly due to the systemic effects of blocking these receptors. Furthermore, its relevance for the functional alterations occurring in the diabetic kidney has not previously been demonstrated. Of importance also are the effects of both ANG II and adenosine to enhanced proximal tubule reabsorption via the Na+/H+ exchanger NHE3. These might act in parallel with hyperglycemia-induced Na+-glucose reabsorption to provide a nonvascular site for interaction between the two systems that can only be studied in the intact kidneys of diabetic models. Therefore, the aim of the present study was to elucidate the role of in vivo tonic activation of ANG II and adenosine receptors on renal hemodynamics and tubular function both in control and diabetic rats. For that reason, we developed a protocol (Fig. 1B) in which the drugs were infused directly into the renal artery in a slow step-wise manner, allowing to directly investigate the effects on intrarenal hemodynamics and tubular function without confounding
systemic effects. This is important since even small changes in arterial pressure can affect proximal reabsorption and renal hemodynamics.

MATERIALS AND METHODS

All chemicals were from Sigma Aldrich (St. Louis, MO) and of highest grade available if not otherwise stated.

Animal model. Male Sprague-Dawley rats (300–350 g; Charles River) with free access to water and standard rat chow were injected with streptozotocin (50 mg/kg) or vehicle into the tail vein. Blood glucose concentration was measured using test reagent strips (MediSense, Bedford, MA). Animals were considered diabetic if blood glucose concentration was and remained >18 mmol/l within 48 h after streptozotocin injection. Experiments were conducted 14 days after induction of diabetes. All experiments were performed in accordance with the National Institutes of Health Guidelines for Use and Care of Laboratory Animals and approved by the local Animal Care and Use Committee.

Dose-response curve for candesartan and 8-cyclopentyl-1,3-dipropyl-xanthine. Normoglycemic animals were anesthetized with thiobutabarbital (120 mg/kg ip; Inactin) and surgically prepared as previously described (28). Briefly, animals were placed on a heated operating table at 38°C and tracheostomized. Polyethylene catheters were placed in the left femoral vein for infusion of Ringer solution (5 ml/kg body wt⁻¹·h⁻¹) for controls and 10 ml/kg body wt⁻¹·h⁻¹ for diabetic) and in the left femoral artery for blood pressure measurements. The bladder was catheterized for free urinary drainage of the right kidney. The left kidney was exposed by a left subcostal flank incision, immobilized in a plastic cup, and embedded in pieces of saline-soaked cotton wool, and the surface was covered with paraffin oil (Apoteksbolaget, Gothenburg, Sweden) to prevent evaporation and keep the tissue moist at body temperature. Thereafter, a catheter was advanced ~1–2 mm into the left renal artery, through a lumbar artery, for kidney-specific delivery of drugs. All infusion solutions administered into the renal artery contained lissamine green to visually verify homogenous intrarenal distribution of the vasoactive substances. The ANG II AT₁ receptor antagonist candesartan (AstraZeneca, Mölndal, Sweden) was administered in doses between 0.6 and 6.0 μg/kg and the adenosine A₁ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) at doses between 20 and 200 μg/kg to separate animals. DPCPX was dissolved in saline with a final concentration of 1% DMSO. A third group of animals received a combination of both antagonists. After 45 min of stabilization, each dose was infused every 2 min in a total volume of 100 μl (Fig. 1A).

In vivo intrarenal blockade of ANG II and adenosine receptors. Control and diabetic animals were anesthetized with thiobutabarbital (Inactin; 120 and 80 mg/kg ip for normoglycemic and hyperglycemic animals, respectively) and surgically prepared as described above. [³H]Inulin (185 kBq/kg⁻¹·h⁻¹; ARC, St. Louis, MO) and LiCl (4 mg ip bolus plus continuous infusion 2.1 mg·h⁻¹·rat⁻¹; Ref. 28) were infused in the femoral vein. After 45 min of stabilization, a 40-min period for baseline measurements was followed by an additional 40-min experimental period in which candesartan (4.2 μg/kg), DPCPX (140 μg/kg), or both were infused in the renal artery in a total volume of 700 μl. Since the half-life of candesartan is approximately 5 h (17), a single bolus dose of each drug was infused and full receptor blockade assumed for the duration of the experiments. To minimize spill-over to the systemic circulation, the infusion was stepwise during ~10 min followed by a 10-min delay before the experimental period started (Fig. 1B). Blood samples for measurement of blood gas parameters were collected at the end of each period.

In some animals, the experimental period of candesartan or DPCPX was followed by a second experimental period in which the ANG II AT₂ receptor antagonist S-(+)-1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid di(trifluoroacetate) salt hydrate (PD-123,319; 0.5 mg/kg) or the adenosine A₂ receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX; 0.5 mg/kg) was administrated, respectively. In these experiments, the blood gas parameters were not assessed due to the large volume of blood required for these measurements.

Measurements of kidney hemodynamics and tubular function. Mean arterial blood pressure (MAP) was measured using a transducer (model P23dB; Statham Laboratories, Los Angeles, CA) connected to the left femoral artery catheter. Total RBF was measured using an ultrasound probe (Transonic Systems, Ithaca, NY) placed around the
left renal artery; care was taken so that the probe remained in the same position throughout the protocol. These parameters were continuously recorded with a Power Lab instrument (AD Instruments, Hastings, UK). GFR was determined by \(^{3}H\)inulin clearance. \(^{3}H\) activities in plasma and urine were measured by liquid scintillation. Blood gas concentrations were quantified by flame spectrophotometry in a multianalyzer (model IL543; Instrumentation Lab, Milan, Italy).

Comparison between control and diabetic rats

**Table 1. Comparison between control and diabetic rats during baseline**

<table>
<thead>
<tr>
<th></th>
<th>Control Rats</th>
<th>Diabetic Rats</th>
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<tbody>
<tr>
<td>Blood glucose, mM</td>
<td>6.5 ± 0.3</td>
<td>23.7 ± 0.5*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>377 ± 5</td>
<td>355 ± 4*</td>
</tr>
<tr>
<td>Left kidney weight, g</td>
<td>1.24 ± 0.02</td>
<td>1.73 ± 0.03*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>108 ± 2</td>
<td>107 ± 2</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>8.4 ± 0.2</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>RVR, mmHg/min * ml</td>
<td>13.2 ± 0.4</td>
<td>13.2 ± 0.4</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>1.6 ± 0.1</td>
<td>3.5 ± 0.2*</td>
</tr>
<tr>
<td>FF, %</td>
<td>10.9 ± 0.9</td>
<td>21.9 ± 1.2*</td>
</tr>
<tr>
<td>Urine flow, µl/min</td>
<td>5.4 ± 0.6</td>
<td>28.6 ± 1.8*</td>
</tr>
<tr>
<td>(T_Na), µmol/min</td>
<td>226 ± 17</td>
<td>492 ± 27*</td>
</tr>
<tr>
<td>(FE_{Na}), %</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0*</td>
</tr>
<tr>
<td>(FE_{Li}), %</td>
<td>40.9 ± 5.6</td>
<td>24.5 ± 3.0*</td>
</tr>
<tr>
<td>(Q_{(\frac{\Delta O_2}{t})}), µmol/min</td>
<td>8.8 ± 1.1</td>
<td>14.0 ± 1.4*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE for \(n = 34–44\), except for oxygen consumption (\(Q_{(\frac{\Delta O_2}{t})}\)) where \(n = 22–23\). MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; FF, filtration fraction; \(T_Na\), tubular Na\(^{+}\) transport; \(FE_{Na}\), fractional Na\(^{+}\) excretion; \(FE_{Li}\), fractional Li\(^{+}\) excretion. *\(P < 0.05\).

Calculations. GFR was calculated using the formula GFR = \(U \times V/P\), where \(U\) and \(P\) denote the quantity of \(^{3}H\)inulin in the urine and plasma samples, respectively, and \(V\) denotes the urine flow rate. Renal vascular resistance (RVR) was calculated as MAP divided by total RBF. The filtration fraction (FF) was calculated using the formula FF = GFR/total RBF * (1 – Hct). In vivo renal oxygen consumption was estimated from the arteriovenous difference in O\(_2\) content \([O_{2at} = ([Hb] \times oxygen saturation) + P_{O2} \times 0.003) \times total RBF]\). Tubular Na\(^{+}\) transport \((T_Na)\) was calculated as \(T_{Na} = [P_{Na}] = GFR \times [UNa] = urine flow, where [P\(_{Na}\)] and [UNa] are plasma and urine Na\(^{+}\) concentration, respectively. Fractional Na\(^{+}\) excretion \((FE_{Na})\) was estimated from \([UNa] \times [P_{inulin}]/[P_{Na}] \times [U_{inulin}]\), and fractional Li\(^{+}\) excretion \((FE_{Li})\) from \([ULi] \times [P_{inulin}]/[P_{Li}] \times [U_{inulin}]\), where \([P_{inulin}]\) and \([U_{inulin}]\) represent plasma and urinary inulin concentration, and \([P_{Li}]\) is plasma Li\(^{+}\) concentration.

**RESULTS**

Dose-response curves for the AT\(_1\) receptor antagonist candesartan, AT\(_2\) receptor antagonist DPCPX or the combination of the two antagonists. Intrarenal infusions of Candesartan, DPCPX and their combination dose-dependently increased RBF and decreased RVR without affecting MAP (Fig. 2). Maximal effects were obtained with 4.2 µg/kg of candesartan and 140 µg/kg of DPCPX, which were therefore chosen for the subsequent experiments.

![Fig. 2. Dose-response curves for the AT\(_1\) receptor antagonist candesartan (A), the AT\(_2\) receptor antagonist DPCPX (B), or the combination of the two (C) on mean arterial pressure, total renal blood flow and renal vascular resistance. Results expressed as means ± SE for \(n = 5–9\)/group. *\(P < 0.05\) vs. time 0.](http://ajprenal.physiology.org/)

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**In vivo intrarenal blockade of ANG II and adenosine receptors: vascular effects.** Streptozotocin increased blood glucose, decreased body weight, and increased kidney weight compared with controls (Table 1). Remarkably, although baseline MAP, RBF, and RVR were similar, the GFR of diabetic rats was doubled compared with the controls, resulting in a doubling of the filtration fraction (FF) (Table 1). Candesartan reduced MAP in control (~4 mmHg) and diabetic (~5 mmHg) animals, as did the combination of the two antagonists in controls (~6 mmHg; Fig. 3). DPCPX had no effect on MAP in either group (Fig. 3). Candesartan, DPCPX, or the combination increased RBF by ~33%.

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**Fig. 3.** Vascular effects of the in vivo intrarenal blockade of ANG II and adenosine receptors. Mean arterial pressure, total renal blood flow, renal vascular resistance, glomerular filtration rate, and filtration fraction of control and diabetic animals before and after blockade of AT1 receptors by candesartan (4.2 μg/kg), A1 receptors by DPCPX (140 μg/kg), or the combined blockade of AT1 and A1 receptors. Results expressed as means ± SE for n = 8–16. *P < 0.05 vs. baseline within the same group. #P < 0.05 vs. corresponding control.
resulting in a reduction of RVR by ~27% in both controls and diabetics (Fig. 3).

In controls, DPCPX and combination of the two antagonists, but not candesartan alone, increased GFR, whereas FF remained unaffected (Fig. 3). Although the absolute decrease in GFR was similar when inhibiting AT1 and A1 receptors in diabetic rats (−0.71 ± 0.29 ml/min; n = 14; P = 0.028 vs. −0.79 ± 0.40 ml/min; n = 13; P = 0.073), only candesartan resulted in a statistically significant decrease due to the more heterogeneous response in the DPCPX group. Furthermore, also the combination of candesartan and DPCPX failed to statistically reduce GFR (−0.53 ± 0.37 ml/min; n = 11; P = 0.178) due to a large variability in the response. However, FF was reduced by all three treatments administered to diabetics but only normalized following candesartan or the combination of candesartan and DPCPX (Fig. 3).

Superimposed infusion of PD-123,319 to that of candesartan or DMPX to that of DPCPX caused no relevant alterations to the effects observed with candesartan or DPCPX alone (Table 2 and 3).

In vivo intrarenal blockade of ANG II and adenosine receptors: tubular effects. Diabetics had higher baseline urine flow and FE\textsubscript{Na} doubled the rate of net tubular Na\textsuperscript{+} transport. This remarkable increase in T\textsubscript{Na} must reflect increased Na\textsuperscript{+} filtration and enhanced tubular Na\textsuperscript{+} reabsorption. This can be ascribed predominantly to the proximal tubule since the clearance of lithium (primarily regulated by proximal reabsorption) was reduced significantly in the diabetic rats (Table 1). All three treatments increased urine flow and FE\textsubscript{Na} in controls (Fig. 4). In diabetics, candesartan decreased urine flow and T\textsubscript{Na}, whereas DPCPX and the combination of the two antagonists had no effect, except for increasing FE\textsubscript{Na} (Fig. 4). Baseline FE\textsubscript{ELi} was lower and Q\textsubscript{o2} higher in diabetics compared with controls (Table 1), indicating higher proximal tubule reabsorption in diabetics. Candesartan and the combination increased FE\textsubscript{ELi} in diabetics (Fig. 4), indicating decreased proximal tubule reabsorption. This was associated with decreased GFR and FF. Neither candesartan nor DPCPX alters oxygen consumption, but the combination of the two increased Q\textsubscript{o2} in both controls and diabetics (Fig. 4).

Superimposed treatment with PD-123,319 to that of candesartan normalized urine flow in controls (Table 2). Superimposed treatment with DMPX to that of DPCPX normalized urine flow both in control and diabetic animals and attenuated the increase in FE\textsubscript{Na} in controls (Table 3). T\textsubscript{Na} was not altered by any treatment either in controls or in diabetics (Tables 2 and 3).

DISCUSSION

The main novel finding of the present study is that ANG II, acting on AT\textsubscript{1} receptors, and adenosine, acting on A\textsubscript{1} receptors, strongly influence renal hemodynamics during baseline conditions in both control and diabetic kidneys via a unifying pathway that requires both receptors to affect vascular tone. The present study also suggests increased AT\textsubscript{1} receptor signaling as a crucial mechanism for the elevated FF and glomerular hyperfiltration commonly observed in the diabetic kidney. To the best of our knowledge, this is the first time that the interaction between ANG II AT\textsubscript{1} and adenosine A\textsubscript{1} receptors in regulating intrarenal hemodynamics is demonstrated in vivo in diabetic rats.
Moreover, these data also propose that endogenous ANG II and adenosine have different roles in regulating tubular sodium handling in diabetics compared with controls. Concomitant blockade of ANG II AT1 and adenosine A1 receptors caused a similar effect to the observed when only adenosine A1 receptors were blocked. Several studies have addressed and demonstrated an interaction between ANG II AT1 and adenosine A1 receptors in the regulation of renal vascular tone (26, 36, 56). Indeed, inhibition of ANG II formation or ANG II AT1 receptor signaling reduces adenosine.
A1 receptor-mediated vasoconstriction of the renal vasculature (26, 56). Conversely, ANG II AT1 receptor-mediated vasoconstriction is also reduced by adenosine A1 receptor blockade (21). Previous studies have been performed ex vivo or in vivo with systemic administration of the vasoactive substances. Other studies evaluated the responses to increasing doses of exogenous ANG II or adenosine, which may activate other receptor subtypes, counteracting vasoactive mechanisms (22, 27), or nonreceptor-dependent mechanisms (20). However, the present study is, to the best of our knowledge, the first report demonstrating a tonic baseline interaction between these two systems without having any confounding systemic effects or manipulation to increase receptor signaling. Importantly, concomitant blockade of both ANG II AT1 and adenosine A1 receptors resulted in increased RBF, which was of the same magnitude as with either drug alone. These results add further support to a unifying vasodilatory pathway in which both receptor types need to be stimulated to affect vascular tonus.

Unaltered GFR in conjunction with decreased FF suggests that these two systems regulate both afferent and efferent arterioles. However, blockade of ANG II AT1 receptors reduced the diabetes-induced glomerular hyperfiltration, indicating increased AT1 receptor-mediated efferent arteriolar vasoconstriction. Augmented GFR in early diabetic nephropathy has been attributed to afferent arteriolar dilatation and an associated increase in RBF (7). However, we observed no differences in RBF between control and diabetic animals. This divergence of results has previously been attributed to insulin replacement (7, 42). Unaltered RBF has been reported not only in insulin-treated diabetic animals but also in response to salt load (10, 23). Diabetic hyperfiltration is associated with increased Na+/glucose cotransport (52). In the present study, diabetics had increased TNa as reported previously (55), which is associated with increased Qo2 (19). Interestingly, adenosine A1 receptor blockade had the same effect observed in controls, which also suggests a more distal regulation of TNa in diabetics. As opposed to controls, where ANG II seems to influence TNa in different parts of the nephron, in diabetics it mainly increased Na+ transport in more proximal segments of the tubule, since FELi increased after infusion of candesartan or the combination of both antagonists. Further analyses are needed to accurately determine the major site of ANG II AT1 receptor-mediated TNa in diabetics, although it seems that in diabetics it is shifted to the proximal tubule or/and Loop of Henle. It might be speculated that increased proximal tubular reabsorption in diabetics, via enhanced Na+/glucose cotransport and enhanced peritubular capillary uptake force due to the increased FF, may reduce the hydrostatic pressure in the Bowman’s capsule. This would directly increase the net filtration pressure and thus favor glomerular hyperfiltration (39). If the objective of therapy in early type 1 diabetes is to combat hyperfiltration, and to reduce arterial pressure and oxidative stress, the use of an adenosine A1 receptor antagonist alone, or in combination with ANG II AT1 receptor blockade, does not add anything to the standard-of-care therapy with an ANG II AT1 receptor blockade because only candesartan reduced GFR and this was not augmented by adding DPCPX. The present study also demonstrates that tonic activation of ANG II AT2 or adenosine A2 receptors has no major roles in diabetes.

Hyperglycemia has been shown to increase intrarenal ANG II levels in rats (31), and ANG II AT1 receptor activation is a key mediator of the increased oxidative stress in the diabetic kidney by direct induction of superoxide radical production by the NADPH oxidase (30). Nitric oxide (NO) bioavailability is decreased already in early stages of diabetic nephropathy (4, 34) and ANG II AT1 receptor blockade restores NO levels in the diabetic kidney (4). It is therefore possible that the observed vasodilatation after ANG II AT1 receptor blockade is at least partly due to increased NO bioavailability. On the other hand, in control animals, the renal vasculature is more sensitive to adenosine-mediated vasoconstriction when NO production is inhibited (5). Similarly, the streptozotocin-diabetic rat kidney is more prone to adenosine A1 receptor-mediated vasoconstriction (41), which has been ascribed to decreased NO-dependent vasodilatation of the afferent arteriole (40).

AT1 receptor blockade increased urine flow and FENa, but did not alter FEli and TNa in controls. In fact, activation of ANG II AT1 receptors increases TNa in both proximal and distal tubular segments and in collecting ducts (18). ANG II AT2 receptors have been shown to contribute to proximal tubule Na+ reabsorption (11), and the superimposed antagonism of ANG II AT2 receptors to that of ANG II AT1 receptors resulted in unaffected FENa and TNa, which suggests that ANG II-mediated tubular Na+ transport might occur in distal parts of the nephron. Blockade of adenosine A1 receptors in controls increased urine flow, FENa, and TNa, which is in good agreement with published results (53). Surprisingly, FEli was not significantly elevated by DPCPX (57). Although Li+ has been widely used as an indicator of proximal tubule reabsorption in cases of low FENa, as observed in the present report, Li+ may also be reabsorbed in more distal parts of the nephron (16), possibly in the loop of Henle (9, 47). Moreover, most of the previous studies investigating tubular function utilize state of the art micropuncture techniques (26, 43, 57). The nature of this technique only allows investigation of single superficial nephrons (51). Although further studies are needed to fully explain the discrepancies, our results suggest that tonic baseline activation of adenosine A1 receptors regulates TNa in distal parts of the nephron. Superimposed adenosine A2 receptor antagonism to that of adenosine A1 receptor blockade reduced urine flow and decreased FENa. This finding supports a role for in vivo tonic activation of adenosine A2 receptors in mediating diuresis and natriuresis (58), at least when adenosine A1 receptor function is reduced. Simultaneous blockade of ANG II AT1 and adenosine A1 receptors in controls also increased urine flow and FENa, although TNa and Qo2 increased. The most likely explanation for these seemingly contradictory results is that increased tubular Na+ load is due to the increased GFR. Increased Na+ in the macula densa activates TGF by stimulating adenosine production, which activates A1 receptors in the afferent arteriole causing vasoconstriction and normalization of single nephron GFR (46, 53). In the present study, A1 receptor blockade elicited a greater increase in FENa compared with that after blockade of ANG II AT1 receptors. The concomitant increase in GFR together with increased FENa after adenosine A1 receptor blockade implies TGF inactivation.

In conclusion, both ANG II AT1 receptors and adenosine A1 receptors are tonically active in control and diabetic animals during baseline and significantly influence intrarenal hemodynamics and tubular function. The hemodynamic effects mediated by simultaneous blockade of both receptors are not additive, indicating that ANG II and adenosine regulate intrarenal
hemodynamics via a unifying pathway that requires activation of both receptor types. However, this study demonstrates that increased AT1 receptor signaling, independent of adenosine A1 receptors, is a mechanistic explanation for diabetes-induced glomerular hyperfiltration. Finally, both ANG II AT1 receptors and adenosine A1 receptors tonically influence tubular Na+ reabsorption in both control and diabetic animals, but the ANG II-mediated effects seem to be altered in the diabetic kidney. The clinical relevance derives from the finding that adenosine A1 receptor blockade alone, or in combination with ANG II AT1 receptor blockade, does not add anything to the standard-of-care therapy with an ANG II AT1 receptor blockade if the manuscript.

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