Tubuloglomerular feedback and renin secretion in NTPDase1/CD39-deficient mice

Mona Oppermann,1 David J. Friedman,2 Robert Faulhaber-Walter,1 Diane Mizel,1 Hayo Castrop,1 Keiichi Enjoji,2 Simon C. Robson,2 and Jurgen Schnermann1

1National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland; and
2Beth Israel Deaconess Medical Center, Boston, Massachusetts

Submitted 20 January 2007; accepted in final form 5 February 2008

Oppermann M, Friedman DJ, Faulhaber-Walter R, Mizel D, Castrop H, Enjoji K, Robson SC, Schnermann J. Tubuloglomerular feedback and renin secretion in NTPDase1/CD39-deficient mice. Am J Physiol Renal Physiol 294: F965–F970, 2008. First published February 6, 2008; doi:10.1152/ajprenal.00603.2007.—Studies in mice with null mutations of adenosine 1 receptor or ecto-5′-nucleotidase genes suggest a critical role of adenosine and its precursor 5′-AMP in tubulovascular signaling. To assess whether the source of juxtaglomerular nucleotides can be traced back to ATP dephosphorylation, experiments were performed in mice with a deficiency in NTPDase1/CD39, an ecto-ATPase catalyzing the formation of AMP from ATP and ADP. Urine osmolarity and glomerular filtration rate (GFR) were indistinguishable between NTPDase1/CD39−/− and wild-type (WT) mice. Maximum tubuloglomerular feedback (TGF) responses, as determined by proximal tubular stop flow pressure measurements, were reduced in NTPDase1/CD39−/− mice compared with controls (4.2 ± 0.9 vs. 10.5 ± 1.2 mmHg, respectively; P = 0.0002). Residual TGF responses gradually diminished after repeated changes in tubular perfusion flow averaging 2.9 ± 0.9 (on response) and 3.5 ± 1.1 (off response) mmHg after the second and 2.2 ± 0.5 (on response) and 1.5 ± 0.8 (off response) mmHg after the third challenge, whereas no fading of TGF responsiveness was observed in WT mice. Macula densa-dependent and pressure-dependent inhibition of renin secretion, as assessed by acute salt loading and phenylephrine injection, respectively, were intact in NTPDase1/CD39-deficient mice. In summary, NTPDase1/CD39-deficient mice showed a markedly compromised TGF regulation of GFR. These data support the concept of an extracellular dephosphorylation cascade during tubular-vascular signal transmission in the juxtaglomerular apparatus that is initiated by a regulated release of ATP from macula densa cells and results in adenosine-mediated afferent arteriole constriction.

adenosine 5′-triphosphate; CD73; stop flow pressure; phenylephrine; adenosine

TUBULOGLOMERULAR FEEDBACK (TGF) is defined as a negative feedback loop in which the tubular NaCl concentration in the contact region between the thick ascending limb of Henle and its parent glomerulus is sensed by specialized epithelial cells [macula densa (MD) cells] and translated into a signal that directly modulates the tone of the afferent arteriole (25). An increase in tubular NaCl at the MD, for example, results in a constriction of smooth muscle cells of the afferent arteriole, leading to a fall in glomerular capillary pressure and single nephron glomerular filtration rate (GFR), and this change tends to minimize the original perturbation. Substantial experimental evidence supports the notion that the signal arising from changes in luminal NaCl concentration is transmitted across the juxtaglomerular interstitium by paracrine messengers, but the nature of these mediators has remained contentious. There is strong evidence from both pharmacological and gene deletion studies that functional A1 adenosine receptors (A1AR) are absolutely required for the expression of TGF-induced vasoconstrictor responses (2, 6, 9, 18, 19, 26–28). These observations imply that adenosine generated in response to increased luminal NaCl serves as a transducing paracrine factor in the initiation of the TGF-induced vasoconstriction. It has been proposed that adenosine is produced and released by MD cells as a by-product of increased NaCl transport and ATP utilization (19). Alternatively, adenosine may be derived from the extracellular dephosphorylation of released ATP. This possibility has gained support from studies showing that MD cells can release ATP across their basolateral membrane and that the rate of release is directly dependent upon tubular salt concentration (1, 13). Furthermore, pharmacological inhibition and genetic ablation of ecto-5′-nucleotidase/CD73, an enzyme that catalyzes the extracellular dephosphorylation of AMP, resulted in a marked impairment of TGF responsiveness (3, 8, 19, 23).

If ATP serves as a precursor for the local production of adenosine, the presence and operation of ectoenzymes that hydrolyze ATP and ADP to generate AMP needs to be postulated. Consistent with the presence of ATPase and ADPase activity in microvillar preparations from the kidney (24), such ecto-ATPases, either of the nucleoside triphosphate diphosphohydrolase (NTPDase) or nucleotide pyrophosphatase/phosphodiesterase (NPP) type, have been reported to be widely expressed in the renal tubular epithelium (31). In particular, NTPDase1/CD39 has been shown to be localized in the glomerulus and throughout the renal vasculature (12, 31). In the renal cortex, NTPDase1/CD39 was found within the juxtaglomerular apparatus (JGA) where it localizes to mesangial cells and to the afferent arterioles (12, 31). Thus, because of its localization and its enzymatic capacity to dephosphorylate ATP and ADP, NTPDase1/CD39 may catalyze the generation of AMP within the confines of the JGA, and it may therefore be an integral part of the paracrine signaling pathway. The lack of pharmacologic agents to block ecto-NTPDase activity selectively prompted us to use NTPDase1/CD39-deficient mice to assess the role of ATP/ADP dephosphorylation in TGF signaling. NTPDase1 knockout mice have an abnormal thromboregulation and platelet function, but their overall cir-

http://www.ajprenal.org

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
culatory function seems to be relatively normal (5). Our study shows that NTPDase1/CD39-deficient mice show a marked abnormality in TGF-mediated afferent arteriolar vasomotor responses with reduced TGF magnitude and with a successive decline of TGF responsiveness after repeated changes in tubular flow. In contrast, MD-mediated stimulation and inhibition of renin release was not measurably affected in NTPDase1-deficient mice. Our data indicate that extracellular dephosphorylation of ATP by NTPDase1/CD39 in the JGA is required for the MD-dependent regulation of vascular tone, but not of renin release.

METHODS

Animals. Adult male NTPDase1/CD39-deficient mice on a C57BL/6 background and C57BL/6 wild-type mice were used in the experiments. NTPDase1 null mutant mice used in this study were transferred to the National Institutes of Health (NIH) after genotyping from the original colony generated by Enjyoji et al. (5). Ecto-5'-nucleotidase/CD73−/− mice and wild-type littermates were from the NIH colony (3). All animal studies were performed according to protocols examined and approved by the Animal Care and Use Committee of the National Institute of Diabetes and Digestive and Kidney Diseases.

Plasma renin concentration. Blood was collected from conscious mice by puncturing the submandibular vessels with a 19-gauge needle and collecting ~20 μl of the emerging blood in an EDTA-containing microhematocrit tube. Plasma renin concentration (PRC) was measured with a RIA kit (DiaSorin, Stillwater, MN) as described previously (3). To test for MD-dependent suppression of renin secretion, mice received a single intravenous (tail vein) injection of saline (5% body wt), and blood samples were collected 30 min later. To assess pressure-dependent suppression of renin secretion, phenylephrine (1.6 mg/kg) was injected subcutaneously, and blood samples were collected 30 min later.

Determination of GFR and renal blood flow. GFR was measured by fluorescein isothiocyanate (FITC) inulin clearance after a single retro-orbital injection followed by consecutive blood sampling from the tail vein. GFR was then calculated assuming a two-compartment model of two-phase exponential decay as previously described (21). For measurements of renal blood flow (RBF), the right renal artery was approached from a flank incision and carefully dissected free to permit placement of an ultrasonic flow probe (0.5PSB nanoprobe) connected to a TS402 perivascular flowmeter module (Transonic Systems, Ithaca, NY).

Urine osmolality. Urine osmolarities under ambient conditions were determined in spot urine samples. Urine osmolarity was determined by the freezing-point suppression method (Advanced Instruments, Norwood CA).

Micropuncture experiments. Male mice were anesthetized with 100 mg/kg thiobutabarbital (inactin) intraperitoneally and 100 mg/kg ketamine subcutaneously. Body temperature was maintained at 38.0°C by placing the animals on an operating table with a servo-controlled heating plate. The trachea was cannulated, and a stream of 100% oxygen was blown toward the tracheal tube throughout the experiment. The left femoral artery was catheterized with hand-drawn polyethylene tubing for continuous measurement of arterial blood pressure. A catheter was also inserted in the right jugular vein for an intravenous maintenance infusion of saline at a rate of 12 μl/g body wt−1·h−1. The bladder was catheterized for urine collections. The left kidney was approached from a flank incision, freed from adherent fat and connective tissue, placed in a Lucite cup, and covered with mineral oil. Measurements of stop flow pressure (Psrf) during loop of Henle perfusion were done as described (32). When Psrf had stabilized, loop of Henle perfusion rate was increased to 30 nl/min, and maximum Psrf responses were determined. In a second set of experiments, perfusion rates were changed repeatedly between 0 and 30 nl/min and maintained until steady states of Psrf were achieved at each flow rate. The perfusion fluid contained (in mmol/l): 136 NaCl, 4 NaHCO3, 4 KCl, 2 CaCl2, 7.5 urea, and 100 mg/100 ml FD & C green (Keystone, Bellefonte, PA).

Statistical analysis. Unpaired t-test was used for comparisons between different conditions. Multiple groups were analyzed with ANOVA followed by Bonferroni posttest. A P value <0.05 was considered significant.

RESULTS

Response to ADP. To confirm the absence of NTPDase1/CD39 in a functional assay, we determined the heart rate response to an intravenous bolus injection of ADP in anesthetized mice. As shown in Fig. 1, injections of 0.5, 1, 2, 5, and 10 ng ADP reduced the heart rate of wild-type mice by 17 ± 7, 26 ± 3, 25 ± 4, 27 ± 3, and 44 ± 9 beats/min, respectively (P = 0.06, 0.001, 0.002, 0.0003, and 0.002). Heart rate decreased by only 11 ± 3, 12 ± 2, 9 ± 2, 10 ± 2, and 9 ± 1 beats/min in NTPDase1/CD39−/−, not significantly different from vehicle injections but significantly less than in the wild type at all except the lowest dose. Thus the bradycardic effect of ADP appears to be largely dependent on ADP breakdown catalyzed by NTPDase1/CD39.

Urine osmolality and GFR. Ambient urine osmolality determined in spot urine samples was similar between both genotypes, averaging 1,645 ± 108 mosmol/l (n = 25) in NTPDase1/CD39−/− and 1,738 ± 146 mosmol/l (n = 11) in wild-type (WT) mice (P = 0.63). GFR as determined by FITC inulin clearance in conscious mice was not different between NTPDase1/CD39-deficient and WT animals (287 ± 18 vs. 282 ± 18 μl/min; n = 6 vs. n = 11; P = 0.85). Similarly, RBF of the left kidneys of anesthetized mice was identical between genotypes (NTPDase1/CD39−/−: 1.37 ± 0.06 ml/min, n = 9; WT: 1.36 ± 0.03 ml/min, n = 8). Arterial blood pressure, body and kidney weights, and urine flow were not different between genotypes in this experimental series.

PRC. PRC under baseline conditions determined in blood collected from the mandibular venous plexus did not differ between NTPDase1/CD39−/− and WT mice. PRC averaged 574 ± 7, 26 ± 3, 25 ± 4, 27 ± 3, and 44 ± 9 beats/min, respectively (P = 0.06, 0.001, 0.002, 0.0003, and 0.002). To test for the absence of MD-dependent regulation of vascular tone, but not of renin release.
isotonic saline was injected in the tail vein, and PRC was determined 30 min later (Fig. 2). This maneuver has been shown to increase luminal NaCl concentration at the MD and to suppress renin secretion (10, 11, 15). In WT mice (n = 7), PRC fell from 509 ± 67 to 316 ± 42 ng ANG I·ml⁻¹·h⁻¹ (P = 0.008). Suppression of PRC was preserved in NTPDase1/CD39⁻/⁻ mice (n = 14), with PRC falling from 741 ± 92 to 291 ± 35 ng ANG I·ml⁻¹·h⁻¹ (P < 0.001). To assess pressure-dependent regulation of renin secretion, phenylephrine was injected (1.6 mg/kg sc), and PRC was determined after 30 min. As shown in Fig. 2, phenylephrine administration reduced PRC to a comparable extent in WT (from 646 ± 86 to 302 ± 42 ng ANG I·ml⁻¹·h⁻¹; P = 0.004; n = 13) and NTPDase1/CD39⁻/⁻ mice (from 666 ± 85 to 320 ± 20 ng ANG I·ml⁻¹·h⁻¹; P < 0.001; n = 18). In view of the preserved reduction of PRC in NTPDase1/CD39-deficient mice, we assessed the response of PRC to phenylephrine in ecto-5'‐nucleotidase/CD73 mice in which the dephosphorylation cascade is disrupted further downstream. After injection of phenylephrine, PRC fell from 432 ± 69 to 269 ± 50 ng ANG I·ml⁻¹·h⁻¹ in WT mice (n = 12; P = 0.006). In ecto-5'‐nucleotidase/CD73-deficient mice, PRC decreased from 533 ± 108 to 352 ± 58 ng ANG I·ml⁻¹·h⁻¹, but this change did not reach statistical significance due to a greater heterogeneity in the responses of individual animals (n = 11; P = 0.19; Fig. 4).

**Micropuncture studies.** TGF responses were determined by measuring proximal tubular Pₛᵣ during loop perfusion rates of 0 and 30 nl/min. Nephrons of six male WT (n = 18 nephrons) and 9 male NTPDase1/CD39-deficient mice (n = 21 nephrons) were investigated. Body weights of the examined mice were similar, averaging 29.3 ± 1.3 g in WT and 26.9 ± 0.8 g in NTPDase1/CD39⁻/⁻ mice (P = 0.11). Mean arterial pressure under micropuncture conditions was not different between NTPDase1/CD39⁻/⁻ and NTPDase1/CD39⁻/⁻ mice (94.2 ± 2.9 and 95.5 ± 2.5 mmHg, respectively; P = 0.72). Pₛᵣ at zero loop perfusion averaged 44.5 ± 1.1 mmHg in WT and 41.3 ± 1.2 mmHg in NTPDase1/CD39⁻/⁻ mice (P = 0.058). At a saturating perfusion rate of 30 nl/min, Pₛᵣ decreased to steady-state levels of 34.0 ± 1.5 mmHg in WT and 37.1 ± 1.7 mmHg in NTPDase1/CD39⁻/⁻ mice (P = 0.0002) (Figs. 5 and 6). Subsequent reduction of perfusion rate from 30 to 0 nl/min (off response) was associated with a Pₛᵣ increase by 11.1 ± 1.6 mmHg in WT and by 4.4 ± 1.1 mmHg in NTPDase1/CD39⁻/⁻ mice (P = 0.001). As shown in Fig. 7, repeated changes in tubular perfusion flow between 0 and 30 nl/min did not significantly affect on or off responses in nephrons from WT mice. In NTPDase1/CD39⁻/⁻ mice, on the other hand, there was a further decrease of both TGF on responses (second response: 2.9 ± 0.9 mmHg; third response: 2.2 ± 0.5 mmHg) and off responses (second response: 3.5 ± 1.1 mmHg; third response: 1.5 ± 0.8 mmHg). Thus the magnitude of maximum TGF responses was significantly reduced in NTPDase1/CD39⁻/⁻ compared with WT mice, and the remaining TGF responses faded further after repeated changes in tubular perfusion flow.

**DISCUSSION**

The specific goal of the present experiments was to pursue the concept that TGF-dependent vasoconstriction is the end-point of a cascade that includes release of ATP by MD cells, dephosphorylation of the nucleotides by ectonucleotidases, and interaction of the generated adenosine with A1AR on afferent arterioles. Support of this concept comes from experiments that have demonstrated NaCl concentration-dependent release of ATP across the basolateral membrane of MD cells (1, 13). Furthermore, genetic deletion and pharmacological inhibition of ecto-5'‐nucleotidase/CD73 are associated with a markedly impaired TGF responsiveness (3, 8, 30). Finally, mice deficient in vasoconstrictor A1AR lack TGF regulation of GFR entirely, indicating that adenosine is a required component of normal TGF function (2, 28).

In the present study, we used NTPDase1/CD39-deficient mice to further explore the extracellular signaling pathway involved in the mediation of TGF. Even though NTPDase1 deficiency has been noted to cause coagulation defects as well as fibrin deposition in various organs, including the kidneys, no abnormalities in overall renal function could be detected (5).
The main observation of our study is that the fall of PSF or increase of afferent arteriolar tone caused by an elevation of tubular NaCl concentration is markedly reduced in superficial nephrons of NTPDase1/CD39-deficient compared with WT mice. On average, the PSF response to a single saturating increase in perfusion flow was reduced by 60% in the absence of a functional NTPDase1 enzyme. Considering the evidence summarized above, a compromised ability to generate AMP and subsequently adenosine in the extracellular space of the JGA seems to be the most likely reason for the impaired TGF responsiveness. The presence of NTPDase1/CD39 as well as of the AMP-degrading enzyme ecto-5′-nucleotidase/CD73 in the confines of the JGA makes an extracellular ATP-ADP-AMP-adenosine signaling cascade a feasible hypothesis. In support of this notion, it has been reported that renal adenosine levels are significantly reduced in NTPDase1/CD39-deficient mice (7). Functional evidence for a role of ATP degradation in TGF has been obtained in the perfused tubule/vessel preparation in (7). Functional evidence for a role of ATP degradation in TGF responsiveness despite normal vasoconstrictor function of the afferent arteriole (22). Furthermore, the reduced autoregulation of GFR in Thy1−1 nephritis could be partially restored by exogenous application of ecto-5′-nucleotidase (29). These observations may be interpreted as supporting a role of mesangial cell-bound nucleotidases in ATP degradation and adenosine formation. It is conceivable that the reduced TGF activity during reductions of JGA adenosine production is a reflection of a diminished adenosine-angiotensin interaction. As has been shown in isolated perfused afferent arterioles, the constrictor response to a fixed angiotensin concentration was strongly dependent on adenosine levels (14).

Because TGF responses in mice lacking NTPDase1/CD39 are reduced, but not fully absent, additional ecto-ATPases may contribute to the dephosphorylation of ATP. The family of ecto-NTPDases and ecto-NPPases comprises at least seven members with broad tissue distribution. NTPDase1/CD39 and NPP3 were found in the glomerulus of the rat (31), and NTPDase2 was detected in the afferent arteriole of the mouse (12). Thus NPP3 and NTPDase2 as well as other ecto-ATPases may account for remnant TGF responses in NTPDase1/CD39−/− mice. For the extracellular dephosphorylation of AMP, only two enzymes are known, ecto-5′-nucleotidase/CD73 and alkaline phosphatase. Thus a compensation by alternative enzymes may be less effective after elimination of NTPDase1/CD39 and NPP3, resulting in a more severe impairment of TGF responsiveness in ecto-5′-nucleotidase/CD73 compared with NTPDase1/CD39-deficient mice. This expectation agrees with our finding that 75% of all examined nephrons of ecto-5′-nucleotidase/CD73-deficient mice showed TGF responses that were fully absent or reduced by at least 80% (3). Renal adenosine levels in ecto-5′-nucleotidase/CD73-deficient mice have been reported to be reduced by 76%, suggesting a substantial contribution of the extracellular AMP dephosphorylation pathway to renal adenosine formation (20).

Nevertheless, it is possible that the reduction of adenosine levels caused by inhibition of ATP/ADP/AMP hydrolysis results in elevated adenosine efflux through equilibrative nucleoside transporters and that this tends to maintain interstitial adenosine concentrations.
Although the scenario outlined above provides the best synthesis of published evidence, alternative explanations for the reduced TGF responsiveness in NTPDase1-deficient mice need to be considered. One may expect that, in the absence of extracellular ATP degradation by NTPDase1, basal interstitial ATP levels may increase. This may provide an alternative explanation for the reduced TGF responses in NTPDase1-deficient mice, since chronic elevation of extracellular ATP may diminish TGF responsiveness. In fact, peritubular infusion of high concentrations of ATP for 2–5 min largely eliminated NaCl-induced vasoconstriction, suggesting that raising basal ATP levels interferes with subsequent TGF signaling (16). The mechanisms responsible for this effect are unclear, but one possibility would be homologous desensitization of P2 receptors, especially of the rapidly desensitizing P2X1 receptor (4). Progressive reductions of TGF responsiveness by elevated ATP could also be the reason for the gradual fading of TGF during repeated elevations of tubular flow, since in the absence of ATP degradation one may expect interstitial ATP to increase above basal levels with successive NaCl-induced stimulations of ATP degradation. In view of the fact that plasma ATP and ADP levels have been found normal in NTPDase1-deficient mice, it seems unlikely that alterations in ATP levels outside the kidney could affect renal function (5).

In addition to influencing the tone of the afferent arteriole, MD cells also modulate renin secretion from granular juxtaglomerular cells. Basal PRC and, by inference, renin secretion did not differ between NTPDase1/CD39–/– mice and WT. Similar results were observed in ecto-5–nucleotidase/CD73–/– and in one strain of A1AR–/– mice (3, 10). These data suggest that the relevance of purinergic signaling in the long-term control of renin secretion may be limited or compensated for by other factors. Our current data show that the acute suppression of renin secretion in response to salt loading was preserved in NTPDase1/CD39–/– mice. Similarly, the reduction of PRC following a phenylephrine-induced increase in blood pressure was not altered in NTPDase1/CD39–/– compared with WT. Previous results have shown that mice lacking A1A receptors lack the renin inhibitory response to increased salt concentrations at the MD (10). Similarly, mice deficient in the MD salt sensor Na-K-2Cl cotransporter are not able to suppress renin secretion after acute salt loading (17). We suggest that a remnant capacity to form AMP in NTPDase1/CD39-deficient mice by alternative pathways, which may also account for partial TGF function, may be sufficient to reduce PRC in our experimental setting. In contrast to NTPDase1/CD39-deficient mice, the reduction of renin secretion after injection of phenylephrine was largely blunted in ecto-5–nucleotidase/CD73-deficient mice. Assuming that both MD-dependent constriction of the afferent arteriole and acute suppression of renin secretion are mediated by the same signaling steps, this finding confirms the conclusion that MD signaling is more severely compromised by disruption of ecto-5–nucleotidase/CD73 activity than by inactivation of NTPDase1/CD39.

In summary, NTPDase1/CD39-deficient mice showed a markedly compromised TGF regulation of GFR. These data support the concept of an extracellular dephosphorylation cascade during tubular-vascular signal transmission that is initiated by a regulated release of ATP from MD cells and eventually results in adenosine-mediated afferent arteriole constriction.

GRANTS

This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and by NIDDK Grant K06-DK-076688-01A1 (D. J. Friedman).

REFERENCES


