Acute renal failure from ischemia significantly contributes to cardiovascular morbidity and mortality. Extracellular adenosine has been implicated as an anti-inflammatory metabolite particularly during conditions of limited oxygen availability (e.g., ischemia). Because ecto-5′-nucleotidase (CD73) is rate limiting for extracellular adenosine generation, this study examined the contribution of CD73-dependent adenosine production to ischemic preconditioning (IP) of the kidneys. After the initial observation that murine CD73 transcript, protein, and function are induced by renal IP, its role in IP-mediated kidney protection was studied. In fact, increases in renal adenosine concentration with IP are attenuated in cd73−/− mice. Moreover, pharmacologic inhibition of CD73 or its targeted gene deletion abolished renal protection by IP as measured by clearance studies, plasma electrolytes, and renal tubular destruction, and reconstitution of cd73−/− mice with soluble 5′-nucleotidase resulted in complete restoration of renal protection by IP. Finally, renal injury after ischemia was attenuated by intraperitoneal treatment of wild-type mice with soluble 5′-nucleotidase to a similar degree as by IP. Taken together, these data reveal what is believed to be a previously unrecognized role of CD73 in renal protection from ischemia and suggest treatment with soluble 5′-nucleotidase as a novel therapeutic approach in the treatment of renal diseases that are precipitated by limited oxygen availability.

phate (APCP) found renoprotective effects by inhibition of adenosine generation (17).

On the basis of these conflicting findings, we pursued the contribution of CD73-dependent adenosine generation to renal protection by IP combining genetic and pharmacologic approaches in a novel model of in situ IP. This model uses a hanging-weight system for performing intermittent occlusion of the renal artery in mice (18). Systematic evaluation of this technique revealed highly reproducible renal injury and protection by IP, thereby minimizing the variability that is associated with previously used techniques (e.g., by applying a surgical clamp to the renal pedicle [vein and artery]) (18). In this study, we applied this model in mice that were gene-targeted for cd73. In addition, we used specific pharmacologic approaches to confirm the findings from gene-targeted mice.

Materials and Methods

Mice

Experiment protocols were approved in accordance with the German Law on the Protection of Animals. Mice that were deficient in cd73 on the C57BL/6 strain have been described previously (10). Cd73+/− littermates were used as controls and were matched in age, gender, and weight. C57BL/6j mice were obtained from Charles River (Sulzfeld, Germany).

Murine Model for Renal IP

Renal artery occlusion for ischemia and IP (four cycles of 4 min of ischemia and 4 min of reperfusion before 30 min of ischemia) was performed using a hanging-weight system, as described previously (18). At the end of surgery, mice received 0.3 ml of normal saline intraperitoneally and were allowed to recover for 2 h under a heating lamp before being placed into metabolic cages (Tecniplast Deutschland, Hohenpeissenberg, Germany) for evaluation of renal function.

Assessment of Renal Function

Plasma and urine creatinine were measured 24 h after renal ischemia using a commercially available colorimetric method (LT-SYS; Labor + Technik, Berlin, Germany). Plasma and urine concentrations of Na+ and K+ were determined with a flame-emission photometer (ELEX 6361; Eppendorf AG, Hamburg, Germany). Renal excretory and hemodynamic values were calculated using standard formulas. Kidneys were harvested after 24 h and stored at −80°C until further analysis.

Adenosine Measurements

Kidneys with and without IP were removed and immediately snap-frozen with clamps that were precooled to the temperature of liquid nitrogen within a time lag of 1 to 3 s. The frozen kidneys were pulverized under liquid nitrogen, and the tissue protein was precipitated with 0.6 N ice-cold perchloric acid. Tissue adenosine levels were determined as described previously (19).

Histology and Immunohistochemistry

Renal tissues were fixed in 4.5% buffered formalin, dehydrated, and embedded in paraffin. Sections (3 μm) were stained with hematoxylin, eosin, and periodic acid-Schiff. Examination and scoring of three representative sections of each kidney (n = 4 to 6 for each condition) were carried out by a board-certified renal pathologist who was blinded to the experimental group, as described previously (20,21).

Myeloperoxidase Activity

To quantify neutrophil infiltration, we performed myeloperoxidase measurements as described previously (18).

Real-Time PCR

Cd73 transcript levels were quantified by real-time reverse transcriptase–PCR (iCycler; Bio-Rad Laboratories, Munich, Germany) as described previously (18). Primer sets (sense sequence, antisense sequence, and transcript size, respectively) for cd73 were 5′-CAA ATC CCA CAC AAC CAC TG-3′ and 5′-TGC TCA CTT GGT CAC AGG AC-3′ (123 bp). The target sequence was amplified using increasing numbers of cycles of 94°C for 60 s, 58°C for 30 s, 72°C for 60 s. Murine β-actin mRNA (sense primer 5′-ACA TTG GCA TGG CTT TGT TT-3′ and antisense primer 5′-GTT TGC TCC AAC CAA CTG CT-3′) was amplified in identical reactions to control for the amount of starting template.

Immunoblotting Experiments

Kidney tissue was homogenized and lysed for 10 min. Renal CD73 was blotted as described previously (22).

CD73 Immunohistochemistry

Mice were killed at the indicated time points, and the kidneys were removed and fixed in Tissue-Tek (Sakura, Torrance, CA) for 24 h. Cryostat sections were mounted on glass slides, air dried, and postfixed in acetone/methanol (1:1) for 10 min at room temperature. Sections were blocked by incubation in 5% powdered skim milk and 0.1% Triton-X-100 (Sigma, Munich, Germany) in Tris-buffered saline for 30 min. Sections were incubated for 1 h at room temperature in rabbit anti-CD73 antibody (1:200). After three washes with Tris-buffered saline, sections were incubated for 45 min with Cy3 goat anti-rabbit Ig (Amersham Pharmacia, Freiburg, Germany) at room temperature. Fluorescence was visualized with a confocal laser scanning microscope (Leica, Germany).

Ecto-5′-Nucleotidase Enzyme Assays

Ecto-5′-nucleotidase enzyme activity was assayed by measurement of the conversion of [14C]IMP to [14C]inosine as described previously (10).

Statistical Analyses

Renal injury score data are given as median (range); all other data are presented as means ± SD from six to eight mice per condition. We performed statistical analyses using t test (two tailed, P < 0.05) or ANOVA to determine group differences. Renal injury was analyzed with the Kruskal-Wallis rank test.

Results

Renal CD73 Is Induced by IP

On the basis of previous studies that showed tissue protection by extracellular adenosine that was generated via hypoxia-inducible CD73 (10,23,24), we hypothesized that CD73-dependent adenosine generation may play an important role during renal IP. Therefore, we first investigated renal CD73 expression and function using a previously described model of in situ IP (18) that is composed of four cycles of intermittent renal artery occlusion and reperfusion (4 min of ischemia, 4 min of reperfusion; Figure 1A). Intermittent renal artery occlusion was performed by using a hanging-weight system for isolated renal artery occlusion. Kidney tissue from mice that were subjected
Figure 1. CD73 is induced by renal ischemic preconditioning (IP). (A) Murine model of renal IP. Age-, gender-, and weight-matched mice were subjected to right nephrectomy followed by in situ IP with a hanging-weight system for atraumatic occlusion of the left renal artery. The IP protocol consisted of four cycles of ischemia/reperfusion (4 min each), followed by the indicated times of reperfusion. (B) CD73 mRNA is induced by IP. At the indicated times, kidneys were excised, total RNA was isolated, and cd73 mRNA levels were determined by real-time reverse transcriptase–PCR. Data were calculated relative to β-actin and are expressed as fold change compared with control (no IP). (C) CD73 protein is induced by IP Western blotting. Kidneys were excised at the indicated times, flash-frozen, and lysed; proteins were resolved by SDS-PAGE and transferred to nitrocellulose. Membranes were probed with anti-CD73 antibody. A representative experiment of three is shown. The same blot was reprobed for β-actin as a control for protein loading. (D) CD73 protein is induced by IP immunohistochemistry. Wild-type (WT) mice were subjected to IP. Kidneys were harvested after the indicated times, sectioned, stained with a CD73 antibody, and visualized with confocal laser-scanning microscopy. Tissue from a perfused but unpreconditioned WT mouse served as a control. Staining of WT tissue with a secondary antibody alone is shown in the top left and labeled “negative.” (E) CD73 enzyme activity is induced by IP. Kidneys were excised after IP treatment and flash-frozen, and extracts were prepared as described in the Materials and Methods section. 5′-Nucleotidase enzyme activity was evaluated by measurement of the conversion of [14C]IMP to [14C]inosine in the presence and absence of 5′-(α,β-methylene) diphosphate (APCP). Enzyme activity is reported as nmol/h per mg protein of APCP-inhibitable IMP hydrolyzing activity and compared with unpreconditioned kidneys (C).
to IP was compared with that from unpreconditioned age-, weight-, and gender-matched littersmates. For definition of transcriptional effects of IP, renal tissue was harvested at indicated time points after IP treatment for real-time reverse transcriptase–PCR measurement. We found a strong induction of cd73 mRNA (e.g., 90 min after renal IP, 3.9 ± 1.7-fold; P < 0.01; Figure 1B). Western blot analysis (Figure 1C) and immunohisto logic staining and imaging via confocal laser scanning microscopy (Figure 1D) confirmed CD73 protein induction after IP. To assess functional induction of CD73, we measured ecto-5′-nucleotidase enzyme activity (10). As shown in Figure 1E, ecto-5′-nucleotidase activity was induced almost three-fold already after 30 min after IP treatment. Taken together, these data strongly support CD73 induction by renal IP.

Renal Tissue Adenosine Concentrations during IP

To determine whether CD73 induction is important for extracellular adenosine production during IP, we next determined renal adenosine tissue levels in kidneys that were shock-frozen immediately after the last ischemia period of IP and compared with control mice without IP. As shown in Figure 2, renal adenosine concentrations were approximately 10-fold higher after renal IP as compared with baseline. To demonstrate that CD73 contributes to increased tissue-adenosine production with IP, we repeated this experiment with gene-targeted mice for cd73 (10). Whereas basal adenosine concentrations were lower in cd73−/− mice, increases in extracellular adenosine with IP were attenuated dramatically compared with littermate controls. Taken together, these studies demonstrate that CD73 plays a key role in increasing renal adenosine levels during IP.

CD73 Inhibition Attenuates Renal Protection by IP

After having shown that CD73 is induced by IP and contributes to increased renal adenosine concentrations, we next pursued a functional role in renal protection. For this purpose, C57BL/6J mice were subjected to 30 min of renal artery occlusion with or without previous IP (four cycles, 4 min of ischemia, 4 min of reperfusion) followed by 24 h of reperfusion and assessed for renal hemodynamics. All mice survived this treatment. Plasma creatinine (Figure 3A) and potassium (Figure 3B), creatinine clearance (Figure 3C), urinary flow rate (Figure 3D), urinary excretion of sodium (Figure 3E) and potassium (Figure 3F), and neutrophil infiltration (Figure 3G) were improved by IP. To evaluate the contribution of CD73 to IP-mediated renal protection, we administered mice the specific CD73 inhibitor APCP (2 mg, intraperitoneally) or vehicle before renal ischemia with or without IP. As shown in Figure 3H, APCP treatment resulted in an approximately two-fold reduction of renal CD73 enzyme activity. The actual degree of inhibition in vivo probably was much larger, because APCP is not an irreversible inhibitor of ecto-5′-nucleotidase enzyme activity and it was diluted approximately 50-fold during the preparation of the tissue extracts. APCP treatment abolished the renal protective effects of IP (Figure 3, A through G). Taken together, blockade of ecto-5′-nucleotidase enzyme activity provides pharmacologic evidence for a critical role of CD73 in renal protection by IP.

Renal Protection by IP Is Abolished in cd73−/− Mice

On the basis of these pharmacologic findings demonstrating that CD73 inhibition attenuates the renal protective effects of IP, we next pursued studies in cd73 gene-targeted mice (10). After confirming the absence of CD73 enzyme activity in the renal tissue of these mice (Figure 4A), we next performed IP using a hanging-weight system for atraumatic occlusion of the left renal artery. The IP protocol consisted of four cycles of ischemia and three cycles of reperfusion (4 min each). The kidneys were snap-frozen after the last cycle of ischemia (−IP). In control mice, left kidneys were snap frozen without IP (−IP). Data are means ± SD (n = 4 to 6).
Figure 3. Inhibition of CD73 by APCP abolishes renal protection by IP. Age-, weight- and gender-matched C57BL/6J mice were subjected to 30 min of ischemia with and without previous in situ IP (four cycles of 4 min of ischemia + 4 min of reperfusion) in the presence and absence of APCP (2 mg/kg intraperitoneally) or sterile saline. Renal function tests were obtained after 24 h of reperfusion (n = 6 to 8, means ± SD). (A) Plasma creatinine. (B) Plasma potassium. (C) Creatinine clearance. (D) Urinary flow rate. (E) Urinary sodium excretion. (F) Urinary potassium excretion. (G) Myeloperoxidase (MPO) tissue levels. (H) Renal CD73 enzyme activity is inhibited by APCP treatment. Kidneys were excised 30 min after treatment with APCP (2 mg/kg intraperitoneally) or saline, and enzyme activity was evaluated by measurement of the conversion of [14C]IMP to [14C]inosine. Results are expressed as nmol/h per mg protein of APCP-inhibitable IMP hydrolyzing activity.
Figure 4. Renal protection by IP is abolished in cd73−/− mice. (A) Measurement of CD73 activity in kidneys from cd73−/− mice. Ecto-5'-nucleotidase enzyme activity was assessed in kidneys of cd73−/− mice and age-, weight-, and gender-matched littermate controls by measurement of the conversion of [14C]IMP to [14C]inosine. Results are expressed as nmol/h per mg protein of APCP-inhibitable IMP hydrolyzing activity. Renal protection by IP is abolished in cd73−/− mice. Cd73−/− mice and age-, weight-, and gender-matched WT mice were subjected to 30 min of ischemia with and without previous in situ IP (four cycles of 4 min of ischemia + 4 min of reperfusion). Renal function tests were obtained after 24 h of reperfusion (n = 6 to 8, mean ± SD). (B) Plasma creatinine. (C) Plasma potassium. (D) Creatinine clearance. (E) Urinary flow rate. (F) Urinary sodium excretion. (G) Urinary potassium excretion. (H) MPO tissue levels.
Histologic Signs of Renal Protection by IP Are Attenuated in cd73<sup>−/−</sup> Mice

To confirm the absence of renal protection of IP in cd73<sup>−/−</sup> mice, we examined kidneys from wild-type and cd73<sup>−/−</sup> mice after 30 min of ischemia with or without previous IP. Thirty minutes of ischemia in wild-type or cd73<sup>−/−</sup> mice resulted in severe acute tubular necrosis, as can be seen from the loss of tubular cell nuclei in the cortex and outer medullary stripe with almost complete destruction of the proximal tubular brush border (Figure 5A). Large numbers of casts that contained brush border blebs were observed in the outer medulla. Hyaline cast formation and intraluminal necrotic cellular debris could be seen in the cortex as well as in the outer medulla. In contrast, the kidneys of wild-type mice that were subjected to IP showed only mild histologic signs of acute tubular necrosis, as can be seen by intact tubular cell morphology. The proximal tubular brush border damage was sporadic and quantitatively mild. In addition, only small numbers of hyaline casts were apparent. Semiquantitative histologic analysis demonstrated a reduction in the Jablonski index from 3 (range 3 to 4) without IP to 2 (range 1 to 3; P < 0.01; Figure 5B) with IP. Similarly, signs of acute renal inflammation were attenuated after IP, as demonstrated by reduced cytochemical staining of granulocytes for chloracetate esterase (Figure 5C). Semiquantitative analysis also

Figure 5. Histologic signs of renal protection by IP are absent in cd73<sup>−/−</sup> mice. Cd73<sup>−/−</sup> mice and age-, weight-, and gender-matched WT mice were subjected to 30 min of ischemia followed by 24 h of reperfusion with and without previous in situ IP (four cycles of 4 min of ischemia + 4 min of reperfusion). Renal tissue sections were prepared and evaluated as described in the Materials and Methods section. (A) Hematoxylin and eosin staining. (B) Quantification of ischemic injury with the Jablonski scale (n = 6 to 8, median and range). (C) Chloracetate esterase staining. Arrows indicate granulocytes. (D) Quantification of granulocyte infiltration (n = 6 to 8, median and range). Magnification, ×400.
showed a reduction in granulocyte infiltration, preferentially localized in peritubular areas, from 3 (range 3 to 4) without IP to 2 (range 1 to 3; \( P = 0.001 \); Figure 5D) with IP. In contrast, renal tissue protection by IP was absent in \( cd73^{-/-} \) mice. There was no reduction in the Jablonski index, and granulocyte infiltration remained elevated. Taken together, these data provide histologic evidence of an absence of renal protection by IP in \( cd73^{-/-} \) mice.

Reconstitution of \( cd73^{-/-} \) Mice with Soluble 5’-Nucleotidase

As proof of principle and to demonstrate that decrease of renal functional parameters in \( cd73^{-/-} \) mice reflects lack of ecto-5’-nucleotidase enzyme activity, we next reconstituted \( cd73^{-/-} \) mice via intraperitoneal injection with soluble 5’-nucleotidase from \( Crotalus atrox \) venom (Sigma) and subjected them to 30 min of renal ischemia with or without previous IP. As shown in Figure 6, A and B, increases in serum creatinine and serum potassium were reduced after 5 h reperfusion (Figure 5D) with IP. In contrast, renal tissue protection by IP was absent in \( cd73^{-/-} \) mice. There was no reduction in the Jablonski index, and granulocyte infiltration remained elevated. Taken together, these data provide histologic evidence of an absence of renal protection by IP in \( cd73^{-/-} \) mice.

Treatment of Renal Ischemia with Soluble 5’-Nucleotidase in Wild-Type Mice

After having shown that renal protection by IP can be restored successfully in \( cd73^{-/-} \) mice by treatment with soluble 5’-nucleotidase, we next pursued nucleotidase treatment of renal ischemia in wild-type mice. For this purpose, we treated C57BL/6J mice with \( C. atrox \) 5’-nucleotidase (10) and exposed them to 30 min of renal ischemia with or without previous IP. After 24 h of reperfusion, studies on renal function were performed. As shown in Figure 7, 5’-nucleotidase treatment provided a degree of renal protection similar to that of IP. Renal histology (Figure 8) confirmed protection from ischemia similar to what we had observed previously with IP. Taken together, these data demonstrate, for the first time, a therapeutic effect of treatment with soluble 5’-nucleotidase in renal ischemia and suggest soluble 5’-nucleotidase as a novel therapy during acute renal ischemia.

Discussion

In this study, we pursued the contribution of extracellular adenosine production to renal protection by IP. Transcriptional profiling of preconditioned renal tissue revealed a prominent induction of \( cd73 \). Similarly, increases in renal adenosine concentration with IP were attenuated in \( cd73^{-/-} \) mice. Pharmacologic inhibition or targeted gene deletion of \( cd73 \) abolished the renal protective effects of in situ IP. Moreover, soluble 5’-nucleotidase treatment mimicked renal protection by IP; it was associated with an increase in renal resistance to ischemia to a similar degree as in mice that were subjected to IP. Taken together, these studies suggest manipulation of CD73 enzyme activity to increase extracellular adenosine concentrations as a therapeutic strategy for the treatment of acute ischemic injury of the kidneys.

The induction of renal CD73 with IP is consistent with previous studies that found that exposure of intestinal epithelia to ambient hypoxia (2% oxygen) resulted in a robust induction of \( cd73 \) transcript, protein, and function (22,23). Similarly, studies in \( cd73^{-/-} \) mice demonstrated increased vascular leak syndrome and increased pulmonary edema upon hypoxia exposure (8% oxygen over 4 h), confirming a role of CD73-dependent adenosine production in vascular barrier function during hypoxia. Studies that examined the \( cd73 \) gene promoter found a binding site for hypoxia-inducible factor-1α (HIF-1α), and inhibition of HIF-1α expression resulted in attenuation of hypoxia-inducible \( cd73 \) expression. Similarly, studies that used luciferase reporter constructs showed that site-directed mutagenesis of the HIF-1α binding site was associated with a loss of hypoxia inducibility, thereby confirming a HIF-1α-dependent pathway for \( cd73 \) induction by hypoxia (22). Therefore, it is tempting to speculate that the observed renal protective effects of CD73-dependent adenosine production during renal IP are transcriptionally coordinated by HIF-1α. Previous studies have shown a critical role of HIF-1α in the transcriptional adaptation of the proximal tubular epithelial cell response to hypoxia (25). Moreover, hypoxic preconditioning results in HIF-1α-dependent erythropoietin production in the kidneys, leading to protection of the myocardium from ischemia (“second organ protection”) (26). However, a direct role of HIF-1α in renal protection during ischemia and reperfusion has yet to be confirmed experimentally.

Which AR mediate renal protection by IP remains unclear from our data. Other studies have found tissue protection during hypoxia or inflammation through signaling pathways that involve the A2AAR (9,10,27). For example, extensive tissue damage, prolonged and higher levels of proinflammatory cytokines, and death were observed in \( A2AAR^{-/-} \) mice that were exposed to an inflammatory stimulus that caused minimal tissue damage in control animals. Similar observations were made using other models of inflammation, liver damage, or bacterial endotoxin-induced septic shock (28). Moreover, other studies of renal ischemia and reperfusion in chimeric mice found that bone marrow–derived cells play an important role in \( A2AAR \)–mediated tissue protection (15). Further work using adoptive transfers into \( Rag^{-/-} \) mice revealed that IFN-γ that is produced by CD4+ T cells seems to be an important mediator for this complex interplay (14). Other studies have indicated a critical role of signaling through the \( A2B \) AR in rescuing of endothelia during transendothelial migration of neutrophils (29), particularly during conditions of limited oxygen availability (23). Moreover, pharmacologic inhibition of the \( A2B \) AR during hypoxia exposure was associated with increased pulmonary edema and vascular leakage (10). Although these studies strongly suggest adenosine-mediated tissue protection by activation of AR during renal IP, further studies with mice that exhibit tissue-specific deletion of \( cd73 \) will be required to identify the source of most of the extracellular adenosine (e.g., renal vascular endothelia, tubular epithelia). In addition, the mechanism(s) of how extracellular adenosine signaling contributes to
renal protection from ischemia remains unclear. A widely accepted view is that ambient levels of ATP in the ischemic kidney determine the level of renal injury (30). As such, a previous in vitro study on ischemic preconditioning in cultured human proximal tubule (HK-2) cells revealed attenuated ATP depletion associated with specific activation of

![Graphs showing changes in plasma creatinine, plasma K+, creatinine clearance, urinary flow rate, urinary Na+ excretion, and urinary K+ excretion after treatment with soluble 5'-nucleotidase.](image)

Figure 6. Treatment with soluble 5'-nucleotidase improves renal hemodynamics in cd73−/− mice that are subjected to ischemia. Cd73−/− mice were subjected to 30 min of ischemia with and without previous in situ IP (four cycles of 4 min of ischemia + 4 min of reperfusion) and with (+5'-NT) and without (-5'-NT) treatment with 1 U of soluble 5'-nucleotidase IP from Crotalus atrox venom 30 min before the induction of ischemia. Renal function tests were obtained after 24 h of reperfusion (n = 6 to 8, mean ± SD). (A) Plasma creatinine. (B) Plasma potassium. (C) Creatinine clearance. (D) Urinary flow rate. (E) Urinary sodium excretion. (F) Urinary potassium excretion.
A<sub>1</sub>AR or A<sub>2A</sub>AR (31). Consistent with such studies, one could speculate that renal ATP depletion would be more severe during renal ischemia after genetic or pharmacologic ablation of CD73 function.

The results from our study stand in contrast to a previous study in which intramuscular treatment of rats with the CD73 inhibitor APCP before a 45-min period of ischemia was associated with an increased nucleotide pool and improved ATP recovery. Similarly,

Figure 7. Treatment with soluble 5′-nucleotidase mimics the protective effect of IP in WT mice. Cd73<sup>+/+</sup> mice were subjected to 30 min of ischemia with and without previous in situ IP (four cycles of 4 min of ischemia + 4 min of reperfusion) and with (+5′-NT) and without (−5′-NT) treatment with 1 U of soluble 5′-nucleotidase IP from C atrox venom 30 min before the induction of ischemia. Renal function tests were obtained after 24 h of reperfusion (n = 6 to 8, mean ± SD). (A) Plasma creatinine. (B) Plasma potassium. (C) Creatinine clearance. (D) Urinary flow rate. (E) Urinary sodium excretion. (F) Urinary potassium excretion.
clearance studies showed improved renal function parameters in APCP-treated animals (17). Why the results of this study disagree with our own is not understood but may be related to different methods for performing renal IP/ischemia. In addition, there may be distinctive differences regarding resistance to hypoxia or ischemia between rats and mice. In fact, a recent study showed that Brown-Norway rats are virtually resistant to renal ischemic injury, thereby providing a model for studying mechanisms of protection from acute renal failure (32).

The exact source of extracellular adenosine that is generated during conditions of hypoxia or ischemia remains unknown. ATP can be released by polymorphonuclear leukocytes, platelets, or endothelia during conditions of inflammation or hypoxia (23,24,33). Extracellular ATP either may signal directly to renal ATP receptors or may function as a metabolic substrate for conversion to adenosine via CD39 (ecto-apyrase, conversion of ATP/ADP to AMP) and ecto-5′-nucleotidase (34,35). Therefore, it seems likely that in addition to CD73, CD39 contributes to renal protection during ischemia. CD39 can influence extracellular adenosine generation in two ways. First, CD39-dependent conversion of extracellular ATP/ADP to AMP provides the metabolic substrate for CD73-dependent adenosine generation. Second, CD39 is responsible for decreasing extracellular ADP levels, thereby eliminating ADP as feed-forward inhibitor of CD73. However, studies to address the role of CD39 in renal ischemia have yet to be performed.

Figure 8. Histologic signs of renal protection by treatment of WT mice with soluble 5′-nucleotidase. WT mice were subjected to 30 min of ischemia with and without previous in situ IP (four cycles of 4 min of ischemia + 4 min of reperfusion) and with (+5′-NT) and without (−5′-NT) treatment with 1 U of soluble 5′-nucleotidase IP from C atrox venom 30 min before the induction of ischemia. Renal tissue sections were prepared after 24 h of reperfusion and evaluated as described in the Materials and Methods section. (A) Hematoxylin and eosin staining. (B) Quantification of ischemic injury with the Jablonski scale (n = 6 to 8, median and range). (C) Chloracetate esterase staining. Arrows indicate granulocytes. (D) Quantification of granulocyte infiltration (n = 6 to 8, median and range). Magnification, ×400.
Conclusion
This study demonstrates protection during renal ischemia via CD73-dependent generation of extracellular adenosine. Gene-targeted mice that lack the major extracellular pathway of adenosine generation (CD73) are not protected from renal ischemia by IP. The significant attenuation of renal injury after ischemia by soluble 5′-nucleotidase treatment suggests possible new strategies to ameliorate the consequences of renal hypoxia. Future challenges include the identification of cell types that are responsible for adenosine generation, thereby allowing the delivery of adenosine therapeutics to specific anatomic sites.

Acknowledgments
This work was supported by Fortune grant 1416-0-0, Interdisciplinary Centre for Clinical Research (IZKF) Verbundprojekt 1597-0-0 from the University of Tübingen and German Research Foundation grant EL274/2-2 to H.K.E., and IZKF Nachwuchsgruppe 1605-0-0 to T.E., as well as National Institutes of Health grant AI18220 to L.F.T. L.F.T. holds the Putnam City Schools Distinguished Chair in Cancer Research.

Disclosures
A patent for the use of soluble 5′-nucleotidase in the treatment of renal ischemia is being processed by the Tübingen University Hospital.

References