SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Quantification of renal nucleotides and nucleosides by HPLC

The ATP, ADP, AMP, adenosine and inosine content of the supernatant was analyzed by HPLC using a 2.1 x 220 mm C18 column with a 3.2 x 15 mm C18 guard column (Brownlee Spheri 5) and a flow rate of 0.2 ml/min. Different elution conditions were used for different nucleotides and nucleosides. ATP, ADP and AMP were analyzed as described by Bernocchi *et al.*[1] Buffer A was 100 mmol/L KH_2PO_4, 5 mmol/L tetrabutylammonium bisulfate, pH adjusted to 5.0 with 3 mol/L KOH. Buffer B was 90% acetonitrile in water. Elution was performed with buffer A for 18 min, then a gradient from 0-40% buffer B over 40 min, and detection was performed at λ=260 nm (Supplemental Figure 1). Nucleotide peaks were identified from their elution times in comparison with standards (Sigma-Aldrich Pty. Ltd, Australia), and the changes in peak areas in response to ischemia.

Adenosine was analyzed as described by Saadjian *et al.*[2] Buffer A was 50 mmol/L Na_2HPO_4, pH adjusted to 4.0 with orthophosphoric acid. Buffer B was 90% methanol in water. Elution was performed with buffer A for 12 min, then a gradient from 0-40% buffer B over 40 min, and detection was performed at λ=260 nm (Supplemental Figure 2). The adenosine peak was identified from the elution time in comparison with the adenosine standard (Sigma-Aldrich Pty. Ltd, Australia) and the change in peak area in response to ischemia. In addition, the identity of the adenosine peak was confirmed by its degradation to inosine by adenosine deaminase (prepared from calf

intestine, Roche Diagnostics Gmbh, Cat no. 10 102 105 001), as described by Delabar *et al*.[3] and Saadjian *et al*.[2] (Supplemental Figure 3).

Inosine was analyzed using buffer conditions similar to those reported by Severini and Aliberti.[4] Buffer A was 50 mmol/L NaPi, pH 6.0, and buffer B was 90% methanol in water. Elution was performed with buffer A for 12 min, then a gradient from 0-40% B over 40 min, and detection was performed at λ=249 nm. The inosine peak was identified from the elution time in comparison with the inosine standard (Sigma-Aldrich Pty. Ltd, Australia), and the change in peak area in response to ischemia (Supplemental Figure 4). The identity of the inosine peak was further confirmed by its degradation by recombinant purine nucleoside phosphorylase (produced in Escherichia coli, Sigma-Aldrich Pty. Ltd, Australia, Cat no. 53113), as described by Severini and Aliberti,[4] which can also utilize adenosine as a substrate and degrades both inosine and adenosine[5,6] (Supplemental Figure 5).

SUPPLEMENTAL RESULTS

Examination of the duration of apyrase activity in mouse kidney

There are currently no data available on the duration of apyrase activity in the extracellular tissue compartment of an *in vivo* model such as that of the kidney. To investigate the duration of apyrase activity, we examined the effect of vehicle or apyrase administration on renal nucleotide and nucleoside levels at 23.5 minutes of ischemia in mice administered vehicle or apyrase either 20 min or 8 days before ischemia. In contrast to the reduction in renal ATP, ADP and AMP levels produced by apyrase administered 20 minutes before ischemia (Table 2), apyrase administered 8 days before ischemia had no effect on renal nucleotide or nucleoside levels measured at 23.5 min ischemia (Supplemental Table 2). These data indicate that no residual apyrase activity was detectable at 8 days post treatment.

SUPPLEMENTARY TABLES

Table S1, SDC. Taqman primer-probe sets used in the qRT-PCR assays

Gene	Taqman Probe
GAPDH	Mm99999915_g1
$A_{2B}R$	Mm00839292_m1
TGFβ	Mm01178820_m1
KIM-1	Mm00506686_m1
CD73	Mm00501910_m1
CD39	Mm00515447_m1
hCD39 transgene	Hs00969559_m1

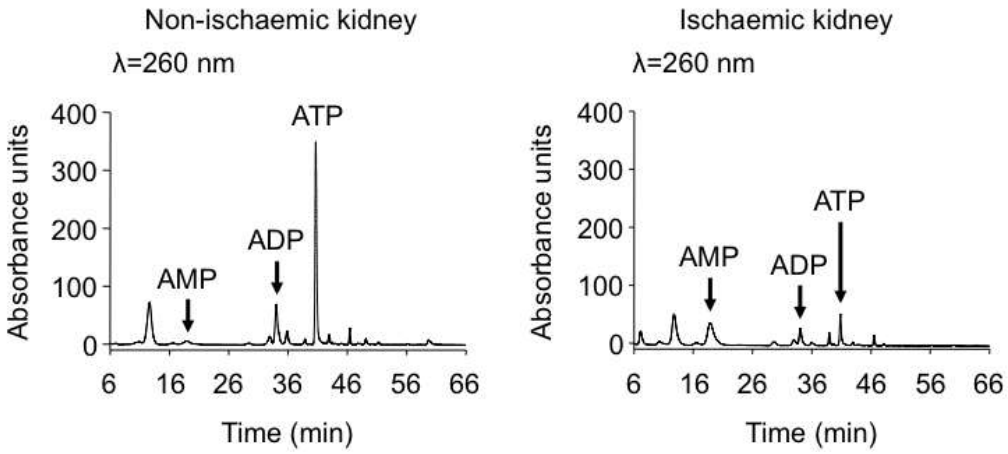
Table S2, SDC. Kidney purinergic nucleotide and nucleoside levels (nmol/g wet weight) measured at 23.5 minutes of ischemia in mice administered apyrase or vehicle, either 20 minutes or 8 days prior to induction of ischemia.

Apyrase 20 mins prior to ischemia		**Apyrase 8 days prior to ischemia**	
Vehicle (n=6)	**Apyrase (n=6)**	**Vehicle (n=6)**	**Apyrase (n=5)**
ATP (nmol/g kidney weight)			
918 ± 62	753 ± 49	918 ± 59	945 ± 44
P<0.05		*P*=ns	
ADP (nmol/g kidney weight)			
813 ± 44	686 ± 26	824 ± 45	779 ± 28
P<0.05		*P*=ns	
AMP (nmol/g kidney weight)			
1017 ± 41	843 ± 55	255 ± 28	219 ± 14
P<0.05		*P*=ns	
Adenosine (nmol/g kidney weight)			
31 ± 5.0	31 ± 6.0	54 ± 4.5	43 ± 1.8
P=ns		*P*=ns	
Inosine (nmol/g kidney weight)			
224 ± 9	197 ± 15	222 ± 20	187 ± 14
P=ns		*P*=ns	

Data for the group treated 20 min prior to induction of ischemia are as shown in Table 2. Data are expressed as means±SEM, n=5-6. (ns=non-significant)

SUPPLEMENTAL FIGURES

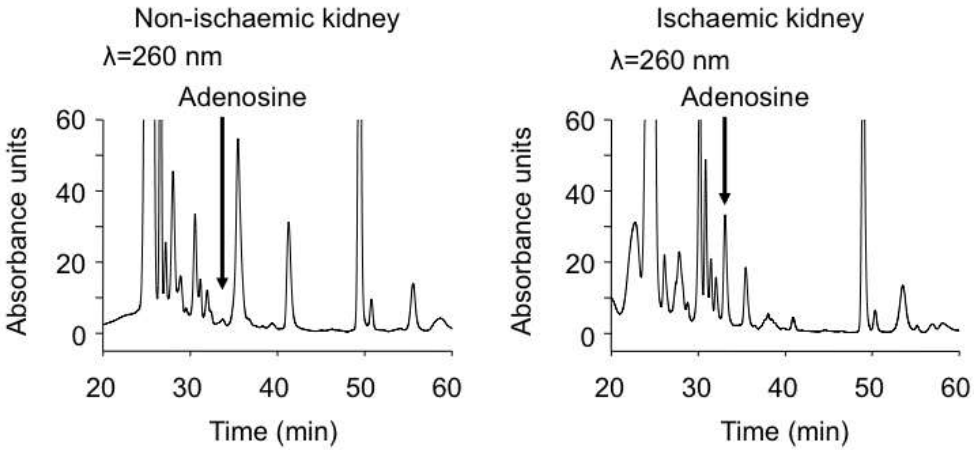
Figure S1, SDC.



Representative HPLC profiles of non-ischemic and ischemic kidney extracts and the elution positions of ATP, ADP and AMP

Supernatants from pulverised kidney homogenized with perchloric acid were analyzed by HPLC using a 2.1 x 220 mm C18 column with a 3.2 x 15 mm guard column flow and a flow rate of 0.2 ml/min. Elution was performed with buffer A for 18 min, then a gradient from 0-40% buffer B over 40 min, and detection was performed at λ=260 nm. Buffer A: 100 mmol/L KPi, 5 mmol/L tetrabutylammonium bisulfate, pH 5.0. Buffer B: 90% acetonitrile in water.

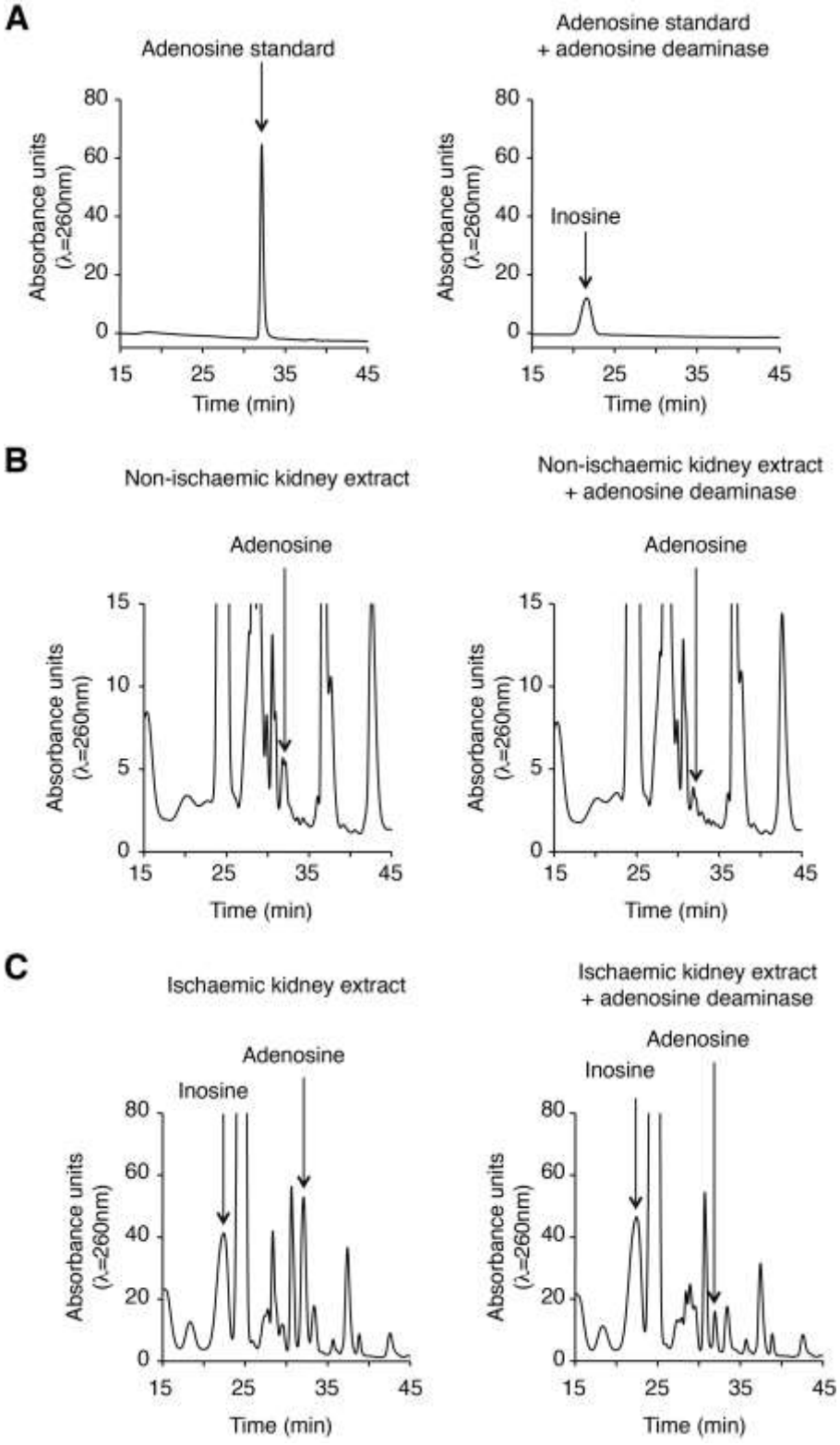
Figure S2, SDC.



Representative HPLC profiles of non-ischemic and ischemic kidney extracts and the elution position of adenosine

Supernatants from pulverised kidney homogenized with perchloric acid were analyzed by HPLC using a 2.1 x 220 mm C18 column with a 3.2 x 15 mm guard column and a flow rate of 0.2 ml/min. Elution was performed with buffer A for 12 min, then a gradient from 0-40% buffer B over 40 min, and detection was performed at λ=260 nm. Buffer A: 50 mmol/L NaPi, pH 4.0; Buffer B: 90% methanol in water.

Supplemental Figure 3.

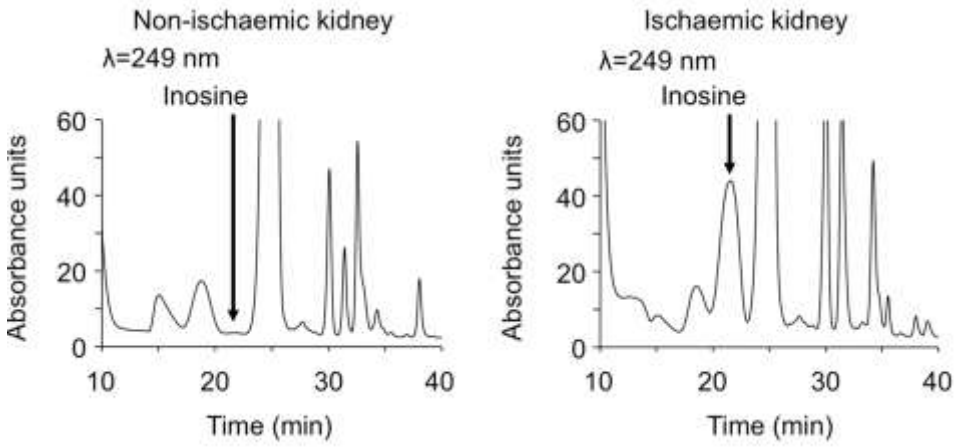


Identification of the adenosine peak was validated using adenosine deaminase.

(**A**): Adenosine deaminase converted the adenosine standard to inosine.

(**B**): Adenosine deaminase reduced the adenosine peak in non-ischemic kidney extract. The levels of inosine in non-ischemic kidney were below the detection limit. (**C**): Adenosine deaminase reduced the adenosine peak in ischemic kidney extract with slight increase in the inosine peak.

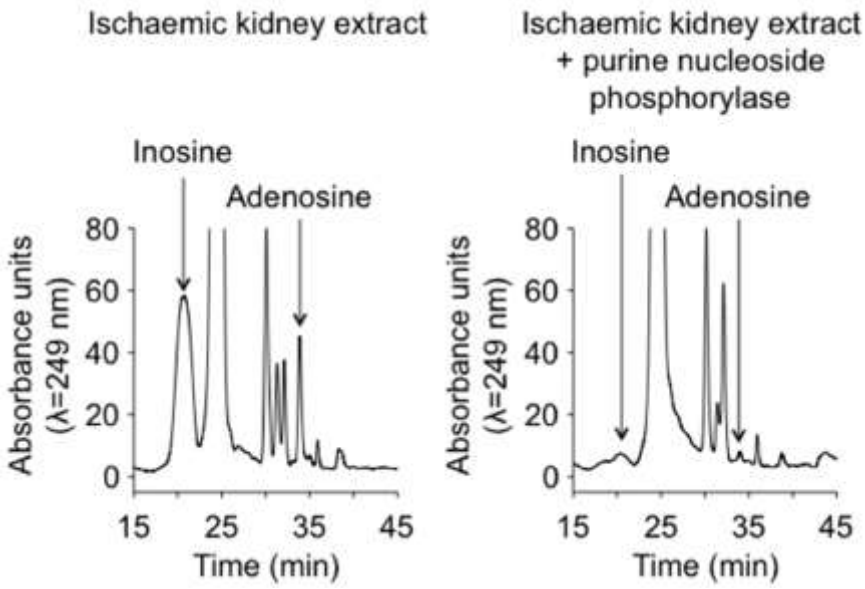
Supplemental Figure 4.



Representative HPLC profiles of non-ischemic and ischemic kidney extracts and the elution position of inosine

Supernatants from pulverised kidney homogenized with perchloric acid were analyzed by HPLC using a 2.1 x 220 mm C18 column with a 3.2 x 15 mm guard column and a flow rate of 0.2 ml/min. Elution was performed with buffer A for 12 min, then a gradient from 0-40% buffer B over 40 min, and detection was performed at λ=249 nm. Buffer A: 50 mmol/L NaPi, pH 6.0; Buffer B: 90% methanol in water.

Supplemental Figure 5.



Identification of the inosine peak from ischemic kidney was confirmed by its degradation with purine nucleoside phosphorylase.

Inosine and adenosine peaks from ischemic kidney extracts obtained by the HPLC method were degraded by recombinant purine nucleoside phosphorylase (produced in Escherichia coli).

SUPPLEMENTAL REFERENCES

1. Bernocchi P, Ceconi C, Cargnoni A, Pedersini P, Curello S, Ferrari R. Extraction and assay of creatine phosphate, purine, and pyridine nucleotides in cardiac tissue by reversed-phase high-performance liquid chromatography. *Anal Biochem.* 1994;222(2):374-379.
2. Saadjian AY, Levy S, Franceschi F, Zouher I, Paganelli F, Guieu RP. Role of endogenous adenosine as a modulator of syncope induced during tilt testing. *Circulation.* 2002;106(5):569-574.
3. Delabar U, Luippold G, Kloor D, Mühlbauer B. Simultaneous determination of adenosine, S-adenosylhomocysteine and S-adenosylmethionine in biological samples using solid-phase extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl.* 1999;724(2):231-238.
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5. Bzowska A, Kulikowska E, Poopeiko NE, Shugar D. Kinetics of phosphorolysis of 3-(beta-D-ribofuranosyl)adenine and 3-(beta-D-ribofuranosyl)hypoxanthine, non-conventional substrates of purine-nucleoside phosphorylase. *Eur J Biochem.* 1996;239(1):229-234.
6. Bzowska A, Kulikowska E, Shugar D. Purine nucleoside phosphorylases: properties, functions, and clinical aspects. *Pharmacol Ther.* 2000;88(3):349-425.