Renal interstitial adenosine metabolism during ischemia in dogs

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Received 13 March 2000; accepted in final form 10 October 2000

Nishiyama, Akira, Shoji Kimura, Hong He, Kat-suyuki Miura, Matlub Rahman, Yoshihide Fujisawa, Toshiki Fukui, and Youichi Abe. Renal interstitial adenosine metabolism during ischemia in dogs. Am J Physiol Renal Physiol 280: F231–F238, 2001.—The present study was conducted to determine the metabolism of renal interstitial adenosine under resting conditions and during ischemia. By using a microdialysis method with HPLC-fluorometric analysis, renal interstitial concentrations of adenosine, inosine, and hypoxanthine were assessed in pentobarbital-anesthetized dogs. Average basal renal interstitial concentrations of adenosine, inosine, and hypoxanthine were 0.18 ± 0.04, 0.31 ± 0.05, and 0.35 ± 0.05 μmol/l, respectively. Local inhibition of adenosine kinase with iodotubercidin (10 μmol/l in perfusate) or inhibition of adenosine deaminase with erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA; 100 μmol/l in perfusate) did not change adenosine concentrations in the nonischemic kidneys (0.18 ± 0.04 and 0.24 ± 0.05 μmol/l, respectively). On the other hand, treatment with iodotubercidin + EHNA significantly increased adenosine concentration (0.52 ± 0.07 μmol/l) with significant decreases in inosine and hypoxanthine levels (0.13 ± 0.03 and 0.19 ± 0.04 μmol/l, respectively). During 30 min of ischemia, adenosine, inosine, and hypoxanthine were significantly increased to 0.76 ± 0.29, 2.14 ± 0.45, and 21.8 ± 4.7 μmol/l, respectively. The treatment with iodotubercidin did not alter ischemia-induced increase in adenosine (0.84 ± 0.18 μmol/l); however, EHNA alone markedly enhanced adenosine accumulation (13.54 ± 2.16 μmol/l), the value of which was not augmented by an addition of iodotubercidin (15.80 ± 1.24 μmol/l). In contrast, ischemia-induced increases in inosine and hypoxanthine were inversely diminished by the treatment with iodotubercidin + EHNA (0.90 ± 0.20 and 9.86 ± 1.96 μmol/l, respectively). These results suggest that both adenosine kinase and adenosine deaminase contribute to the metabolism of renal interstitial adenosine under resting conditions, whereas adenosine produced during ischemia is mainly metabolized by adenosine deaminase and the rephosphorylation of adenosine by adenosine kinase is small.

EXTRACELLULAR ADENOSINE SERVES as an important paracrine function to regulate renal hemodynamics (23, 37, 38). Endogenously produced adenosine is proposed to be formed primarily by renal tubular epithelial cells (20, 23, 37, 38) and approaches the vascular smooth cells of renal vasculature from the extracellular space, i.e., interstitium (13, 23, 37, 38). Activation of adenosine A1 and A2 receptors, located on the surface of cell membranes, can elicit vasoconstriction (A1) and vasodilation (A2) (23, 37, 38), respectively. Arend et al. (5) have reported that renal blood flow (RBF) and glomerular filtration rate (GFR) are significantly decreased by the administration of dipyridamole, which inhibits cellular uptake of endogenous adenosine and thereby elevates the interstitial adenosine levels. Studies using chronically implanted capsules to infuse drugs directly into the renal interstitium have shown that interstitial administration of adenosine significantly decreases GFR and the nonselective adenosine receptor antagonist theophylline completely blocks the effect of adenosine (31). Agmon et al. (1) have shown that the adenosine A1 receptor agonist N6-cyclopentyladenosine in the renal interstitium at the corticomedullary junction significantly decreases cortical and medullary blood flow. The authors also observed that interstitial administration of adenosine A2-receptor agonist 2-[p-(carboxyethyl) phenethylamino]-5'-N-ethylcarboxamidoadenosine significantly increases medullary blood flow (1). Recent studies have shown that renal medullary interstitial infusion of selective adenosine A2 receptor antagonist 3,7-dimethyl-1-propargylxanthine significantly decreases medullary blood flow (40). Collectively, these observations suggest that the effects of adenosine on renal hemodynamics depend on its concentration in the interstitium.

Increased extracellular adenosine, produced as a consequence of the energy-deficit state during ischemia, has been suggested as a regulator of renal hemodynamics in postischemia (16–18). Lin et al. (16, 18) have reported that theophylline prevents ischemia-

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induced reductions in RBF and GFR during the initiation or maintenance phase of postischemic acute renal failure. It has also been observed that dipyridamole aggravates the hemodynamic changes in postischemic acute renal failure and theophylline reverses the effects of dipyridamole (17). Studies performed to examine adenosine production quantitatively have demonstrated that tissue levels of adenosine are actually increased in the ischemic kidney (20, 28). However, the results from these studies have shown that tissue adenosine concentrations increase only several-fold during global renal ischemia of a 10- to 30-min duration (20, 28). Because adenosine can be rapidly rephosphorylated by adenosine kinase and degraded by adenosine deaminase (4, 23, 37, 38), the possibility exists that an ischemia-induced accumulation of a large concentration of renal interstitial adenosine may be prevented by these enzymes.

The purpose of the present study is to evaluate the metabolism of renal interstitial adenosine under resting conditions and during ischemia. We hypothesized that nonischemic and ischemic kidneys exhibit differential participation of adenosine kinase- and deaminase-mediated adenosine metabolism. To test this hypothesis, we utilized an in vivo renal microdialysis method (6, 24–27, 36, 40) and assessed the effects of the adenosine kinase inhibitor idotubercidin (19, 29, 32, 34) and adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (19, 32, 34, 35) on renal interstitial concentrations of adenosine, inosine, and hypoxanthine. These inhibitors were administered locally through the dialysis membrane by inclusion of the drugs in the perfusates as previously reported (19, 25, 26, 32, 34).

METHODS

Characteristics of the Microdialysis Probe

For the determination of renal interstitial concentrations of adenosine, inosine, and hypoxanthine, we used a microdialysis probe as previously reported (24–27). The dialysis membrane is made from cuprophan fiber, measuring 15 mm in length with a 5,500-Da transmembrane diffusion cutoff (Toyobo, Otsu, Japan). Thin stainless steel tubes (outer diameter: 190 μm, inner diameter: 100 μm) were inserted into both sides of the cuprophan fiber. The inflow and outflow ends were inserted into the polyethylene tubes (PE-10) and sealed in place with glue. Preliminary results from in vitro experiments demonstrate that a negligible amount of adenosine, inosine, and hypoxanthine stick to the polyethylene tubes. The probes were connected to a CMA/100 microinfusion pump (Carnegie Medicine, Stockholm, Sweden) and perfused continuously at a rate of 10 μl/min.

The efficiency of the microdialysis probe was determined as previously described (6, 19, 26, 27, 32). Briefly, the probes were soaked in beakers containing saline solution with different concentrations (0.1–5 μmol/l) of adenosine, inosine, and hypoxanthine (n = 5, respectively). The probes were perfused with saline solution with heparin (30 U/ml) at a perfusion rate of 10 μl/min. The dialysates were collected, and the equilibrium rates for adenosine, inosine, and hypoxanthine were calculated by dividing the concentrations in the dialysates by the concentrations in the medium. At a perfusion rate of 10 μl/min, the relative equilibrium rates of adenosine, inosine, and hypoxanthine were 16 ± 2, 17 ± 2, and 25 ± 3%, respectively.

Studies were also performed to determine the effects of idotubercidin and EHNA on the relative equilibrium rates of adenosine, inosine, and hypoxanthine. Microdialysis probes were soaked in beakers containing saline solution with different concentrations (0.1–5 μmol/l) of adenosine, inosine, and hypoxanthine (n = 5, respectively). The probes were perfused at a perfusion rate of 10 μl/min with the following solutions: 1) saline solution with heparin (30 U/ml) containing idotubercidin (10 μmol/l), 2) saline solution with heparin (30 unit/ml) containing EHNA (100 μmol/l), and 3) saline solution with heparin (30 unit/ml) containing idotubercidin (10 μmol/l) plus EHNA (100 μmol/l). In cases where these solutions were used as a perfusate, calculated equilibrium rates of adenosine, inosine, and hypoxanthine were not significantly different from the relative equilibrium rates in the case of saline solutions (data not shown).

Animal Preparation

Experiments were carried out on mongrel dogs, weighing from 10 to 15 kg, which had been maintained on standard laboratory chow. All surgical and experimental procedures were performed according to the guidelines for the care and use of animals as established by the Kagawa Medical University. The animals were anesthetized with pentobarbital sodium (30 mg/kg iv) and given additional doses as required. After a tracheotomy was performed, the dogs were artificially ventilated with room air. The right brachial veins were cannulated for administration of an isotonic saline solution (0.2 ml·g⁻¹·min⁻¹) and additional anesthetic. An adjustable clamp was placed on the renal artery to be used later as an occlusion devise.

The left kidney was exposed through a retroperitoneal flank incision and denervated by the cutting of all visible renal nerves. The renal capsule was carefully opened along the convexity to avoid damage to the cortical tissue. Four microdialysis probes were gently implanted into the renal cortex and perfused with saline solution with heparin (30 unit/ml) at a rate of 10 μl/min.

Experimental Protocols

A series of preliminary experiments was performed to determine the recovery time necessary after the implantation of the microdialysis probes. The results show that the concentrations of adenosine, inosine, and hypoxanthine in dialysates were elevated immediately after the implantation of the microdialysis probe. These concentrations dropped rapidly within the first 30 min and remained stable after 60 min (data not shown). Therefore, the following experiments were started 90 min after the implantation of the microdialysis probe.

The initial series of experiments was designed to determine the metabolism of interstitial adenosine in the nonischemic kidneys (n = 7). The experimental protocol was started with dialysate collections for two consecutive 10-min periods, with all four probes perfused with saline solution with heparin (30 units/ml). Then, the perfusion medium of the probes was replaced with the following solutions: probe 1: saline solution with heparin (30 units/ml); probe 2: saline solution containing idotubercidin (10 μmol/l); probe 3: saline solution containing EHNA (100 μmol/l); and probe 4: saline solution containing idotubercidin (10 μmol/l) plus EHNA (100 μmol/l). After 10 min of infusion, two consecutive 10-min dialysate samples were collected. The doses of EHNA (100...
μmol/l) and iodotubercidin (10 μmol/l) were chosen on the basis of results from the previous microdialysis studies (19, 25, 26, 34). The local administrations of iodotubercidin (10 μmol/l) plus EHNA (100 μmol/l) via renal microdialysis membrane did not influence renal hemodynamics, as previously reported (25, 26). In these experiments, samples from all four probes were collected to be used for measurements of adenosine, inosine, and hypoxanthine.

The second series of experiments was designed to determine the metabolism of renal interstitial adenosine during ischemia and after recirculation in a separate group of animals (n = 7). Four probes were perfused with the four solutions as described above for the first series of experiments. After two consecutive 10-min periods, the renal pedicle was occluded by using an adjustable renal arterial clamp for 35 min. Five minutes were allowed for stabilization before three consecutive 10-min dialysate samples were collected during renal ischemia. Two additional 10-min collection periods were performed 10 and 60 min after release of the renal arterial clamp (recovery period). In these experiments, samples from all four probes were collected to be used for measurements of adenosine, inosine, and hypoxanthine.

In a separate group of animals, the effect of a higher dose of iodotubercidin (100 μmol/l) on renal interstitial concentrations of adenosine were examined under resting conditions and during ischemia (n = 3, respectively). The protocols used in these studies were identical to the protocols described above, except that iodotubercidin was used at a dose of 100 μmol/l instead of 10 μmol/l (probe 2).

Analytical Procedures of Nucleosides in Dialysate

Adenosine. Adenosine in the dialysate was measured according to the method developed by Miura et al. (21). The procedure is briefly described as follows. One hundred microliters of dialysate were transferred into a microcentrifuge tube, and 20 μl of 1 μmol/l acetate buffer (pH = 4.5) and 4.5 μl of 40% chloroacetaldehyde were added. The preparation was incubated at 60°C for 4 h for conversion of adenosine to a fluorescent reagent, 6-etheno-adenosine. After the incubation, the sample was mixed with twice the volume of alumina-freon reagent and centrifuged at 1,000 g for 3 min, and the upper aqueous phase was collected. For HPLC, a reverse-phase HPLC column (Nucleosil 7C18, 4.6 × 250 mm, Nagel) was maintained at 30°C with a column oven (655A-52, Hitachi). An isocratic elution with 7.5% acetonitrile in 20 mmol/l potassium phosphate buffer (pH = 5.7) was performed with an HPLC pump (L-600, Hitachi). Fifty microliters of the sample were injected with an autosampler (655A-40, Hitachi), and the elution was monitored by using a fluorescence spectrometer (F-1000, Hitachi) at an excitation wavelength of 280 nm and an emission wavelength of 340 nm. A chromatointegrator (D-2000, Hitachi) was used for recording. In every 10 samples, a standard sample was injected. Adenosine was quantified by comparing the peak height with that for standard adenosine.

Inosine and hypoxanthine. One hundred microliters of dialysate were directly applied onto the reverse-phase HPLC column (Nucleosil 7C18, 4.6 × 250 mm, Nagel). The column was developed isocratically with 3.5% methanol in 10 mmol/l KH2PO4 at a flow rate of 1 ml/min. The effluent was monitored at a wavelength of 254 nm by an ultraviolet monitor. In every 10 samples, a standard sample was also injected.

Statistical Analysis

One-way ANOVA for repeated measures combined with the Newman-Keuls post hoc test was used for within-group analyses. Between-group analyses were performed with a two-way ANOVA for repeated measures combined with the Newman-Keuls post hoc test. Data are presented as means ± SE.

RESULTS

Effects of Iodotubercidin and EHNA on Renal Interstitial Concentrations of Adenosine, Inosine, and Hypoxanthine Under Resting Conditions

Figure 1 shows the effects of iodotubercidin and EHNA on renal interstitial concentrations of adenosine in the nonischemic kidneys. When the probe equilibrium rate of 16% (see METHODS) was taken into account, the basal average adenosine concentration from probe 1 was estimated to be 0.17 ± 0.04 μmol/l (n = 7), which was not significantly different from the average control value from other probes (probes 2–4, n = 7, respectively). In probe 1, replacement of the same solution (saline solution) did not change the adenosine concentration (0.17 ± 0.03 μmol/l). Similarly, neither iodotubercidin (probe 2) nor EHNA (probe 3) caused any significant changes in adenosine concentrations (from 0.15 ± 0.03 to 0.18 ± 0.04 μmol/l and from 0.17 ± 0.05 to 0.24 ± 0.05 μmol/l, n = 7, respectively). In contrast, treatment with iodotubercidin plus EHNA (probe 4) significantly increased the adenosine concentration from 0.24 ± 0.05 to 0.52 ± 0.07 μmol/l (P < 0.05, n = 7). When the probe equilibrium rates of 17 and 25% (see METHODS) were taken into account, basal renal interstitial concentrations of inosine and hypoxanthine were esti-
mated to be $0.31 \pm 0.05$ and $0.35 \pm 0.05 \mu mol/l$, respectively, in probe 1, which were not significantly different from the average control value from other probes (probes 2–4, $n = 7$, respectively). Iodotubercidin (probe 2) did not alter renal interstitial concentrations of inosine ($0.27 \pm 0.03 \mu mol/l$) or hypoxanthine ($0.32 \pm 0.07 \mu mol/l$). On the other hand, EHNA significantly decreased inosine and hypoxanthine to $0.17 \pm 0.07$ and $0.23 \pm 0.09 \mu mol/l$ (probe 3; $P < 0.05$, respectively). As shown in Fig. 2, iodotubercidin plus EHNA (probe 4) significantly decreased inosine and hypoxanthine levels to $0.13 \pm 0.02$ and $0.19 \pm 0.04 \mu mol/l$ ($P < 0.05$, respectively), which were not different from those during treatment with EHNA alone (probe 3).

**Effects of Iodotubercidin and EHNA on Renal Interstitial Concentrations of Adenosine, Inosine, and Hypoxanthine During Ischemia and After Recirculation**

Figure 3 shows the changes in renal interstitial concentrations of adenosine during ischemia and after recirculation. After clamping of the renal artery, adenosine concentration significantly increased from $0.17 \pm 0.03$ to $0.55 \pm 0.1$ (5–15 min), $0.65 \pm 0.10$ (15–25 min), and $0.76 \pm 0.19 \mu mol/l$ (25–35 min) ($P < 0.05$, $n = 7$, respectively) in the case where saline solution was used as a perfusate (probe 1). During ischemia, the changes in adenosine levels under treatment with iodotubercidin (probe 2) were not different from the case without any inhibitors ($0.47 \pm 0.06 \mu mol/l$ in 5–15 min, $0.71 \pm 0.15 \mu mol/l$ in 15–25 min, $0.84 \pm 0.19 \mu mol/l$ in 25–35 min, $P < 0.05$, $n = 7$, respectively). In contrast, EHNA (probe 3) markedly augmented the changes in adenosine levels ($7.41 \pm 1.79 \mu mol/l$ in 5–15 min, $12.33 \pm 1.92 \mu mol/l$ in 15–25 min, $13.49 \pm 2.24 \mu mol/l$ in 25–35 min, $P < 0.05$, $n = 7$, respectively). These changes induced by EHNA were not augmented by an addition of iodotubercidin (probe 4: $7.06 \pm 0.80 \mu mol/l$ in 5–15 min, $13.81 \pm 1.18 \mu mol/l$ in 15–25 min, $15.80 \pm 1.24 \mu mol/l$ in 25–35 min, $n = 7$, respectively). Sixty minutes after the release of the clamp, adenosine concentrations returned to their respective preischemic levels in all cases (Fig. 3).

Figure 4 shows the changes in renal interstitial concentrations of inosine and hypoxanthine during ischemia. In the case where saline solution was used as a perfusate (probe 1), inosine and hypoxanthine concentrations significantly increased to $2.38 \pm 0.55$ and $14.22 \pm 3.01$ (5–15 min), $2.34 \pm 0.38$ and $21.77 \pm 4.62$ (15–25 min), and $2.14 \pm 0.45$ and $21.8 \pm 4.7 \mu mol/l$ (25–35 min, $P < 0.05$, $n = 7$, respectively).
(25–35 min) after clamping of the renal artery ($P < 0.05$, $n = 7$, respectively). In iodotubercidin-treated kidneys (probe 2), inosine and hypoxanthine levels were also significantly increased during ischemia ($2.02 \pm 0.21$ and $17.84 \pm 1.06 \mu$mol/l in 25–35 min, respectively), which were not different from those without the inhibitors (probe 1). In contrast, ischemia-induced increases in inosine and hypoxanthine levels were significantly diminished by treatment with iodotubercidin plus EHNA (probe 4: $0.90 \pm 0.20$ and $9.86 \pm 1.96 \mu$mol/l in 25–35 min; $P < 0.05$, $n = 7$, respectively; Fig. 4), but these changes were not different from those during treatment with EHNA alone (probe 3: $0.86 \pm 0.14$ and $8.78 \pm 0.77 \mu$mol/l, in 25–35 min, respectively). Sixty minutes after the release of the clamp, inosine and hypoxanthine concentrations returned to their respective preischemic levels in all cases (data not shown).

To ensure that the lack of response of iodotubercidin to renal interstitial adenosine levels was not solely dose related, the protocols were repeated with a higher dose of iodotubercidin (100 $\mu$mol/l) under resting conditions ($n = 3$) and during ischemia ($n = 3$). Similar to the results obtained using 10 $\mu$mol/l iodotubercidin (probe 2), 100 $\mu$mol/l iodotubercidin did not alter renal interstitial adenosine concentrations (from $0.18 \pm 0.05$ to $0.20 \pm 0.07 \mu$mol/l). Furthermore, the ischemia-induced increase in adenosine levels during treatment with 100 $\mu$mol/l iodotubercidin (0.84 $\pm 0.22 \mu$mol/l in 25–35 min) was similar to the increase without the inhibitors (probe 1) or during treatment with 10 $\mu$mol/l iodotubercidin (probe 2).

**DISCUSSION**

The primary objective of this study is to evaluate the metabolism of renal interstitial adenosine under resting conditions and during ischemia. Adenosine metabolism may occur mainly via two pathways: 1) the phosphorylation to AMP by adenosine kinase and 2) the degradation to inosine by adenosine deaminase (4, 23, 37, 38). By use of a renal microdialysis method, we measured renal interstitial concentrations of adenosine in the presence and absence of a selective pharmacological blockade of adenosine kinase with iodotubercidin (19, 29, 32, 34) and adenosine deaminase with EHNA (19, 32, 34, 35).

Recent studies have demonstrated that the microdialysis technique is suited to measure adenosine concentrations in the renal interstitum (6, 24–27, 36, 40). The present estimated concentration of renal interstitial adenosine in the dog cortex was $-0.2 \mu$mol/l under resting conditions, which is similar to that observed in previous renal microdialysis studies of the dog (24) and rat (6, 36) cortex. In the present study, we observed that local administration of iodotubercidin or EHNA did not alter adenosine concentrations in nonischemic kidneys. In contrast, treatment with iodotubercidin plus EHNA significantly increased adenosine levels to $-0.5 \mu$mol/l. These results suggest that, in the nonischemic kidneys, adenosine release into the renal interstitium may have been very limited ($<1 \mu$mol/l). Furthermore, it is also possible that under resting conditions, both adenosine kinase and adenosine deaminase have a sufficiently high enzyme activity to maintain the renal interstitial concentration of adenosine, despite the fact that one metabolic pathway was blocked. In support of this possibility, studies measuring adenosine kinase and adenosine deaminase activities have demonstrated a wide distribution of these enzymes in the renal cortex (30).

Metabolism of renal interstitial adenosine was also evaluated during ischemia. In the present study, we observed that the elevation of interstitial adenosine levels remained only 3- to 4-fold during 30-min global renal ischemia. These data are consistent with previous observations in dogs (20) and rats (28) that tissue levels of adenosine increase only several-fold during global renal ischemia of a 10- to 30-min duration. In the present study, ischemia-induced increases in adenosine levels during treatment with iodotubercidin were not significantly different from those without the inhibitors. On the other hand, EHNA alone markedly enhanced ischemia-induced adenosine accumulation by 50- to 60-fold, levels that were not augmented by an addition of iodotubercidin. These data suggest that the accumulated interstitial adenosine during ischemia...
was mainly degraded by adenosine deaminase and the rephosphorylation of adenosine via adenosine kinase is very small. Pawelczyk et al. (30) have demonstrated that, under resting conditions, the activity of adenosine deaminase is much higher than that of adenosine kinase. Another possibility is that ischemic conditions could change the activities of adenosine kinase and/or adenosine deaminase. Studies performed in rats subjected to experimental myocardial infarction have demonstrated that adenosine deaminase activity is significantly increased in infarcted tissue (33). It has also been shown that global ischemia for 10 min significantly elevates adenosine deaminase activity in the rat heart (8). Furthermore, Decking et al. (11) have reported that hypoxia decreases adenosine kinase activity in perfused guinea pig heart. These results suggest the possibility that, under ischemic conditions, the activity of adenosine deaminase is elevated but adenosine kinase activity is reduced in the kidney.

Under resting conditions, the present estimates of renal interstitial concentrations of inosine (0.31 ± 0.05 µmol/l) and hypoxanthine (0.35 ± 0.05 µmol/l) in the dog cortex are similar to those obtained from the rat cortex (6, 12). We anticipated that inosine and hypoxanthine levels would be increased by inhibition of adenosine kinase with iodotubercidin and decreased by inhibition of adenosine deaminase with EHNA. We observed, however, that in the nonischemic kidneys iodotubercidin did not alter renal interstitial concentrations of inosine and hypoxanthine, whereas EHNA significantly decreased these levels. These data are consistent with the results from previous microdialysis studies performed in the heart (19) in which 10 µmol/l iodotubercidin present in perfusates do not significantly alter preischemic dialysate purine metabolite levels, whereas 100 µmol/l EHNA significantly decreases inosine and hypoxanthine levels. In the present study, we do not have an explanation as to why iodotubercidin did not have an effect on inosine and hypoxanthine levels under resting conditions. Previous dose-response studies demonstrated that 10 µmol/l iodotubercidin effectively inhibits adenosine kinase in the brain (34). Furthermore, we have observed that a higher dose of iodotubercidin (100 µmol/l) did not alter renal interstitial concentrations of adenosine under resting conditions. Although these data suggest that the lack of response of iodotubercidin to renal interstitial adenosine levels may not have been solely dose related, it remains unclear whether an inhibition of adenosine kinase altered renal interstitial levels of purine metabolites. Other adenosine metabolic pathways cannot be ruled out and need to be examined further. During ischemia, calculated net increases of adenosine, inosine, and hypoxanthine were 0.60, 1.82, and 21.5 µmol/l, respectively, which were not affected by treatment with iodotubercidin alone. After treatment with iodotubercidin plus EHNA, net increases of adenosine, inosine, and hypoxanthine during ischemia were 15.4, 0.70, and 9.7 µmol/l, respectively. These were similar to the increases with EHNA alone. Thus the net increase in adenosine was significantly augmented by the treatment with EHNA, whereas the net increases in inosine and hypoxanthine were inversely diminished. These data support the hypothesis that the accumulation of a large interstitial concentration of adenosine is prevented by adenosine deaminase-mediated further degradation to inosine and hypoxanthine.

The primary actions of iodotubercidin and EHNA involve the inhibition of adenosine deaminase and adenosine kinase, respectively (19, 23, 29, 32, 34, 35, 37, 38); however, the exact mechanism by which these drugs affect interstitial adenosine levels remains to be identified. Both adenosine kinase and adenosine deaminase may be primarily intracellular enzymes (37, 38), although recent studies have shown activity of extracellular adenosine deaminase in renal proximal tubules (7). In the present experimental settings, it is difficult to determine whether iodotubercidin and EHNA at the concentrations used in the perfusate can inhibit extracellular and/or intracellular enzyme activities. It has been reported that adenosine can also be transported into renal cells by an energy-dependent system (15), which may be an important point of control of renal interstitial adenosine levels. Studies performed in erythrocytes (10) and in brain slices (9) suggest the possibility that iodotubercidin may have an influence on cellular uptake of adenosine. Thus future studies will be needed to determine the effects of iodotubercidin and EHNA on intracellular and extracellular enzyme activities as well as cellular uptake of adenosine.

Studies using an in vitro juxtamedullary nephron technique in the rat (13, 14) have shown that extracellular administration of adenosine, superfused over the renal microvessels, can exert a significant vasoconstriction in afferent arterioles at very high concentrations (10 µmol/l range). The results from previous (6, 25–27, 36, 40) and present microdialysis experiments have shown that resting renal interstitial adenosine concentrations normally remain below 1 µmol/l. Therefore, it is possible that basal adenosine concentrations are lower than appear necessary for significant interstitial adenosine-mediated renal vasoconstriction. Indeed, we have previously reported that intrarenal administration of a selective adenosine A1 receptor antagonist, KW-3902, which prevents the vasoconstrictor influence of exogenous adenosine, does not cause significant changes in basal RBF or GFR (3). The present experiments have shown, however, that renal interstitial adenosine levels are significantly increased during ischemia. In addition, several studies have indicated that angiotensin II (2, 22, 39) and norepinephrine (2) can amplify adenosine A1 receptor-mediated renal vasoconstriction. Collectively, it is possible that ischemia-induced accumulation of renal interstitial adenosine can elicit renal vasoconstriction, which may be involved, at least in part, in renal hemodynamic changes in the initiation phase of posts ischemic acute renal failure. Because the results of the present study
have shown that renal interstitial adenosine levels returned to the preischemic levels within 60 min after recirculation, adenosine may not have been the primary mediator of renal vasoconstriction in the maintenance phase of posts ischemic acute renal failure.

In summary, the present study provides evidence for the metabolism of renal interstitial adenosine. Under resting conditions, neither the adenosine kinase inhibitor iodotubercidin nor the adenosine deaminase inhibitor EHNA changed adenosine concentrations. On the other hand, treatment with iodotubercidin plus EHNA significantly increased the adenosine concentration. During ischemia, renal interstitial adenosine was significantly increased. The treatment with iodotubercidin did not alter the increase in adenosine during ischemia; however, EHNA markedly enhanced adenosine accumulation, the value of which was not augmented by an addition of iodotubercidin. These results support the concept that both adenosine kinase and adenosine deaminase contribute to the metabolism of renal interstitial adenosine under resting conditions, whereas accumulated interstitial adenosine during ischemia is mainly degraded by adenosine deaminase.

We are grateful to Hidehiko Sakurai and Motoki Kyo (Toyobo, Otsu, Japan) for supplying the dialysis membrane and steel needles and to Yumi Ihara and Yukari Moriyasu for secretarial service. Jennifer A. Giffin reviewed the manuscript.

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science, and Culture of Japan.

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