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Tissue levels of *S*-adenosylhomocysteine in the rat kidney: effects of ischemia and homocysteine

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Abstract

Most *S*-adenosylmethionine (AdoMet)-dependent methyltransferases are regulated *in vivo* by the AdoMet/*S*-adenosylhomocysteine (AdoHcy) ratio, also termed as ''methylation potential.'' Since adenosine inhibits *in vitro* AdoHcy hydrolysis and since adenosine tissue levels increase during hypoxia, it can be predicted that AdoHcy levels may increase in the rat kidney in parallel of those of adenosine. Therefore, the present investigation was performed to assess changes of renal AdoHcy and AdoMet tissue contents during ischemia and after administration of adenosine and homocysteine or both in the ischemic rat kidney. In anesthetized rats ischemia of the kidney was induced by renal artery occlusion for various time intervals. Adenosine and homocysteine were infused into the renal artery of the ischemic kidney. To induce a hyperhomocysteinemia homocysteine was continuously infused. The kidneys were removed and immediately snap-frozen. Tissue contents of AdoHcy, AdoMet, adenosine and adenine nucleotides were analyzed by means of HPLC. Under normoxic condition the tissue contents of AdoHcy, AdoMet and adenosine were 0.7 ± 0.05, 44.1 ± 1.0 and 3.8 ± 0.1 nmol/g wet weight, respectively. Renal ischemia for 30 min resulted in an increase of AdoHcy levels from 0.7 ± 0.05 to 9.1 ± 0.6 nmol/g wet weight and in a dramatic decrease of the AdoMet/AdoHcy ratio and energy charge from 65.1 ± 5.6 to 2.8 ± 0.2 and from 0.87 ± 0.01 to 0.25 ± 0.01, respectively. Application of exogenous adenosine into the ischemic kidney did not result in further AdoHcy accumulation. However, when homocysteine was infused into the ischemic kidney, AdoHcy increased five-fold above control levels, during 5 min ischemia. Systemic infusion of homocysteine leads to a reduction of the methylation potential also in the normoxic kidney. We conclude that (i) the methylation potential in the kidney is markedly reduced during ischemia, mainly due to accumulation of AdoHcy; (ii) elevation of AdoHcy tissue content during ischemia is the result of the inhibition of AdoHcy hydrolysis; (iii) homocysteine is rate limiting for AdoHcy synthesis in the ischemic kidney; (iv) under normoxic conditions hyperhomocysteinemia can affect the methylation potential in the renal tissue.

Keywords: *S*-Adenosylhomocysteine; *S*-Adenosylmethionine; Adenosine; Homocysteine; Methylation potential; Energy charge

1. Introduction

The tissue level of AdoHcy, generated from AdoMet, is controlled *in vivo* by the activity of *S*-adenosyl-L-homocysteine hydrolase (AdoHcyase, EC 3.3.1.1). Inhibition of this cellular enzyme results in intracellular accumulation of AdoHcy which is a potent inhibitor of AdoMet-dependent methyltransferases [1–3]. The ratio of AdoMet/AdoHcy is therefore considered to be a reliable indicator of the flow of methyl groups transferred from AdoMet to methyl acceptors within the cells [4]. Since hydrolysis of AdoHcy results in adenosine and homocysteine formation and since AdoHcyase catalyzes the reversible reaction of adenosine and homocysteine to AdoHcy [5,6], the metabolisms of adenosine, AdoHcy and AdoMet are functionally linked and alterations in one may affect the other.

Adenosine formation in the kidney is enhanced when ATP hydrolysis prevails over ATP synthesis [7]. The major site of adenosine production in the kidney is assumed to be most likely located in the cytosol [8,9]. Adenosine can be deaminated by adenosine deaminase or enters the purine nucleotide pool by adenosine kinase (Fig. 1). Homocysteine, once formed, can enter the transsulfuration pathway and is metabolized to cystathionine, or can be re-methylated to methionine (Fig. 1). Plasma homocysteine is mainly eliminated by the kidneys, either by excretion into

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Abbreviations: AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; AdoHcyase, *S*-adenosylhoocysteine hydrolase; CRF, chronic renal failure.

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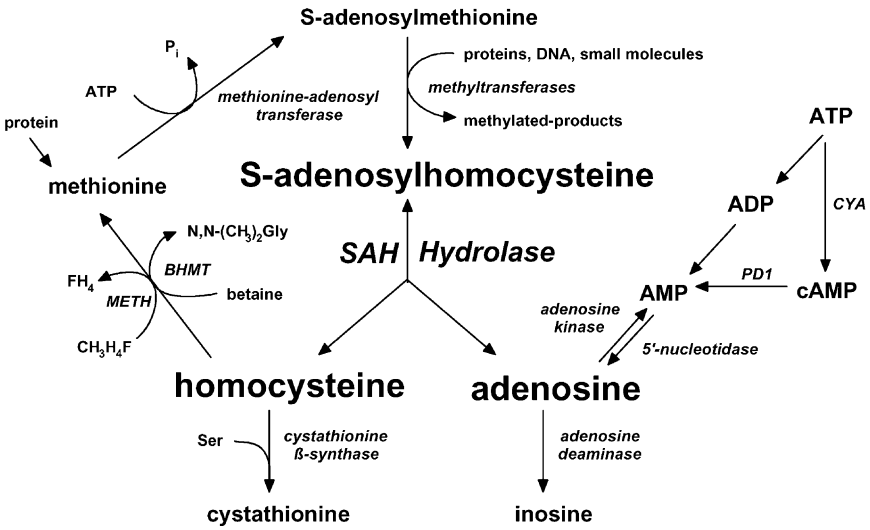

Fig. 1. Pathway of intracellular AdoHcy, AdoMet, homocysteine and adenosine metabolism. AdoHcy: *S*-adenosylhomocysteine; AdoMet: *S*-adenosylmethionine; METH: 5-methyltetrahydrofolate-homocysteine *S*-methyltransferase; BHMT: betaine-homocysteine *S*-methyltransferase; CYA: adenylate cyclase; PD1: phosphodiesterase.

the urine, even if this mechanism represents a minor elimination pathway [10] or by metabolism. Chronic renal failure (CRF) has been reported to be associated with elevated plasma concentrations of homocysteine, not because of impaired urinary excretion but because of impaired metabolism of homocysteine by the kidney [11,12]. Moderate hyperhomocysteinemia is commonly found in patients with cardiovascular disease [13,14] whereas the plasma homocysteine concentrations are considerably higher in patients with CRF [15]. These patients are known to have a higher incidence of vascular complications [15].

Since homocysteine is taken up by cells, the intracellular concentration of AdoHcy is also dependent on the concentration of homocysteine in the extracellular medium [16].

Although extensive studies of the extracellular actions of adenosine on adenosine A_1-, $A_{2A,2B}$- and A_3-receptors were performed [17–19], limited information is available about the intracellular action of adenosine. Therefore, the objectives of this study were to determine (i) the tissue content of AdoHcy, AdoMet, adenosine and the adenine nucleotides in the rat kidney under normoxic conditions; (ii) the effect of renal ischemia on the tissue levels of these intermediate metabolites in order to assess their mutual dependency; (iii) the concentration of AdoHcy and AdoMet in the ischemic kidney after application of exogenous adenosine or homocysteine, or both, adenosine and homocysteine and after induction of an acute hyperhomocysteinemia.

2. Materials and methods

The following materials were purchased from the sources indicated: homocysteine, adenosine, AdoHcy, Sigma; all other chemicals were of analytical grade and obtained from Merck Darmstadt, Germany.

3. Animal preparation

Male Sprague–Dawley rats (280–350 g) were obtained from Charles River, UK and fed with standard laboratory rat chow and tap water *ad libitum*.

Rats were anesthetized with thiopental 90 mg/kg i.p. (Trapanal® Byk Gulden, Konstanz, Germany) and placed on a heated table, which was thermo-controlled to keep the rectal temperature constant at 37.4° (RT® Effenberger, München, Germany). Tracheostomy was performed to keep the airways open. A polyethylene catheter (o.d. 0.4 mm approximately) was placed into the right jugular vein for intravenous infusion. The left kidney was exposed by flank incision and the kidney was placed on a Lucite holder. The occlusion of the renal artery was performed after careful preparation of the kidney hilus so that only the renal artery was occluded with an artery clamp.

For studies of inulin clearance to assess glomerular filtration rate (GFR) during homocysteine infusion, additional to the preparation already described, another catheter was placed in the left carotid artery serving for blood sampling and for continuously monitoring of arterial blood pressure (pressure transducer TBM 4® WPI, Heidelberg, Germany connected to a recorder WK 280® WKK, Kaltbrunn, Switzerland). After a suprapubic incision, a catheter was placed into the bladder for collection of urine.

4. Experimental protocol

For measurement of *tissue contents*, the animals were allowed to stabilize from surgical procedure for 30 min. Isotonic saline (0.87% NaCl) was infused at a rate of 3 mL/hr.

In a first set of experiments, the normoxic kidneys were removed and immediately snap-frozen with clamps pre-

cooled to the temperature of liquid nitrogen within a time lag of 1–3 s. To induce ischemia, the renal artery was occluded for various time intervals 0.5, 1, 5, 10 and 30 and 45 min. Four kidneys were also snap-frozen at 15 min after release of the artery clamp of 30 min occlusion.

In a second set of experiments, (i) 1 μmol adenosine and/or homocysteine, respectively, and (ii) 10, 30, 100 and 300 nmol homocysteine in isotonic saline was infused into the renal artery immediately after occlusion of the renal artery which lasted for 5 min. Thereafter kidneys were snap-frozen.

For studies of *inulin clearance* the animals were allowed to stabilize from surgical procedure for 60–90 min, until urine flow was stable. ^{3}H-Inulin (NEN, Dreieich, Germany) in isotonic saline (0.87%) was infused at a rate of 3.0 mL/hr for assessment of GFR throughout the entire experiment. In addition, this infusion line contained either isotonic saline or homocysteine. Homocysteine administration was started with a prime dose of 690 μg per 100 g body weight and a subsequent infusion of 216 μg/hr per 100 g body weight in the continuous infusion dissolved in isotonic saline. This dose regimen was shown to induce a sustained hyperhomocysteinemia (36.49 μM) according to House *et al*. [20], 20 min after start of homocysteine administration urine collection was started for three consecutive 20 min clearance periods with blood sampling at the mid point of the clearance periods. Thereafter kidneys were removed and snap-frozen as already described.

5. Tissue preparation and analysis

The frozen kidneys were pulverized under liquid nitrogen and the tissue protein was precipitated with 0.6 N ice-cold perchloric acid. After centrifugation at 11,000 *g* for 30 min at 4° the supernatants were recovered and 2 mL of the supernatants were adjusted to a pH between 5.5 and 6.5 by adding potassium carbonate 2 M to measure AdoHcy, AdoMet and adenosine. For adenine nucleotide measurement, 500 μL supernatant was neutralized with potassium carbonate 2 M and potassium dihydrogen phosphate 1 M, pH 9.5.

AdoHcy, AdoMet, adenosine [21] and adenine nucleotides [22] were assayed as previously described. In brief, AdoHcy, AdoMet, adenosine were analyzed with HPLC using a Nucleosil 100 C_{18} (3 μm, 125 × 4 mm i.d.) column. Eluent A consisted of 10 mM ammonium dihydogenphosphate and 0.6 mM heptanesulfonic acid sodium salt as the ion-pair forming agent in 3% methanol. Solvent B contained, in addition, 10% (v/v) acetonitrile. The adenine nucleotides were also analyzed with HPLC using a Grom-Sil 120 ODS-3 CP (5 μm, 125 × 4 mm i.d.) column. Eluent A consisted of 65 mM potassium phosphate pH 4.6 and 5 mM tetrabutylammonium sulfate as the ion-pair forming agent. Solvent B was identical to solvent A but, in addition, contained 40% acetonitrile. Sodium in urine was measured by flame photometry. Inulin clearance was used for GFR determination.

6. Calculation and statistics

The energy charge of the adenylate system was calculated according to Atkinson and Walton [23], defined as

$$\frac{\mathrm{ATP} + (1/2)\mathrm{ADP}}{\mathrm{AMP} + \mathrm{ADP} + \mathrm{ATP}}$$

Results are expressed as means ± SEM. Data were analyzed by ANOVA and Student–Neuman–Keul's test for multiple comparison. A difference between group data was considered to be significant when P-value was <0.05.

7. Results

7.1. Tissue levels of AdoHcy, AdoMet and adenosine during ischemia

Under control conditions, i.e. the shortest time interval between removal of kidney and snap-freezing, the tissue content of AdoHcy, AdoMet and adenosine was 0.7 ± 0.05, 44.1 ± 1.0 and 3.8 ± 0.1 nmol/g wet weight, respectively. Measurements of low AdoHcy tissue content requires immediate inactivation of the metabolism by snap-freezing. As previously reported, the tissue content of AdoHcy in kidney is between 2 and 34 nmol/g wet weight [24–26]. The major point why the values are so high might be due to the methods used for tissue removal and preparation.

Within 60 s of renal artery occlusion tissue levels of AdoHcy and adenosine were increased four-fold from 0.7 to 2.7 and from 3.8 to 16.2 nmol/g wet weight, respectively. Whereas the highest adenosine tissue level was achieved after 5 min ischemia, the AdoHcy tissue level increased continuously and achieved the highest value after 30 min ischemia (Table 1). Renal recirculation of 15 min after release of a 30 min renal artery occlusion resulted in a fall of adenosine levels close to control values whereas the AdoHcy levels remained elevated eight-fold compared to control values. The increase of AdoMet tissue content in the first 60 s of ischemia was lower as that observed for adenosine and AdoHcy. After 30 min renal artery occlusion, the AdoMet tissue level decreased to a value of 25.6 ± 1.6 nmol/g wet weight and reached the control value at 15 min renal recirculation after 30 min renal artery occlusion (Table 1).

The changes of AdoHcy and AdoMet tissue levels during ischemia lead to a decrease of the AdoMet/AdoHcy ratio (methylation potential). As expected, also the energy charge (phosphorylation potential) decreased during ischemia within 10 min from 0.87 ± 0.01 to 0.26 ± 0.01 (Table 1). Whereas the phosphorylation potential reached

Table 1
Tissue content of AdoHcy, AdoMet and adenosine in the rat kidney (nmol/g) wet weight after ischemia and post-ischemic recirculation

Experimental condition	*n*	AdoHcy	AdoMet	Adenosine	AdoMet/AdoHcy	EC
Control	6	0.7 ± 0.05	44.1 ± 1.0	3.8 ± 0.1	65.1 ± 5.6	0.87 ± 0.01
Ischemia						
0.5 min	6	1.8 ± 0.3*	50.8 ± 2.3*	11.1 ± 2.0*	30.5 ± 4.1	0.53 ± 0.02
1.0 min	5	2.7 ± 0.2*	60.0 ± 2.0*	16.2 ± 2.3*	23.1 ± 3.5	0.38 ± 0.01
5.0 min	4	5.8 ± 0.4*	48.5 ± 2.8	22.5 ± 1.3*	8.5 ± 0.7	0.28 ± 0.01
10.0 min	8	7.8 ± 0.9*	44.2 ± 5.1	19.0 ± 1.7*	6.1 ± 1.4	0.26 ± 0.01
30.0 min	5	9.1 ± 0.6*	25.6 ± 1.6*	14.7 ± 1.0*	2.8 ± 0.2	0.25 ± 0.01
45.0 min	4	7.7 ± 0.4*	21.1 ± 1.1*	14.2 ± 1.0*	2.7 ± 0.1	0.34 ± 0.02
30.0 min[a]	4	5.6 ± 0.7*	46.4 ± 6.5	5.20 ± 0.1	10.1 ± 2.0	0.82 ± 0.01

Ischemia was performed as described in Section 4. Values are means ± SEM for *n* animals. AdoMet/AdoHcy ratio and energy charge (EC).
* $P < 0.01$ vs. control.
[a] With 15 min recirculation.

Table 2
Effect of intra-arterial infusion of adenosine and/or homocysteine in the ischemic kidney

	n	AdoHcy	AdoMet	Adenosine	AMP	ADP	ATP	AdoMet/AdoHcy	EC
Control (NaCl 0.87%)	5	6.7 ± 0.3	47.5 ± 1.9	22.1 ± 1.6	1098.1 ± 19	361.1 ± 10	293.7 ± 20	7.1 ± 0.2	0.26 ± 0.01
Adenosine	5	7.7 ± 0.3	46.7 ± 1.0	57.1 ± 4.5*	1220.2 ± 28*	358.9 ± 21	489.1 ± 11*	6.0 ± 0.2	0.32 ± 0.01*
Homocysteine	7	35.3 ± 3.2*	48.2 ± 1.3	20.3 ± 1.0	1175.6 ± 64	304.0 ± 19	296.6 ± 24	1.4 ± 0.1	0.25 ± 0.01
Adenosine + homocysteine	4	60.6 ± 2.6*	39.4 ± 3.0*	29.7 ± 1.5	1122.8 ± 14	264.0 ± 20	562.9 ± 15*	0.65 ± 0.04	0.35 ± 0.01*

The time of ischemia was 5 min. Data are expressed in nmol/g wet weight. Adenosine and/or homocysteine were infused in a concentration of 1 μmol as described in Section 4. Values are means ± SEM for *n* animals. AdoMet/AdoHcy ratio and EC.
* $P < 0.01$ vs. control.

Table 3
Effect of homocysteine on AdoHcy, AdoMet, adenosine and adenine nucleotides tissue content in the ischemic kidney

Homocysteine (nmol)	*n*	AdoHcy	AdoMet	Adenosine	AMP	ADP	ATP	AdoMet/AdoHcy	EC
Control (NaCl 0.87%)	5	6.7 ± 0.3	47.5 ± 1.9	22.1 ± 1.6	1098.1 ± 19	361.1 ± 10	293.7 ± 20	7.1 ± 0.2	0.26 ± 0.01
10	4	5.5 ± 0.5	48.5 ± 2.2	22.4 ± 0.6	1324.2 ± 60*	280.4 ± 9	403.5 ± 10*	9.6 ± 0.6	0.28 ± 0.01
30	4	5.9 ± 0.2	47.7 ± 1.5	16.3 ± 1.0*	1025.0 ± 20	288.9 ± 34	681.7 ± 26*	8.2 ± 0.3	0.38 ± 0.01*
100	4	9.1 ± 0.8*	46.6 ± 1.0	21.4 ± 1.7	1216.4 ± 58	269.7 ± 10	431.6 ± 23*	5.3 ± 0.6*	0.29 ± 0.01
300	4	21.2 ± 1.5*	44.4 ± 0.8*	25.0 ± 1.4	1239.2 ± 60	249.1 ± 6	290.3 ± 23	2.1 ± 0.1*	0.24 ± 0.01
1000	7	35.3 ± 3.2*	48.2 ± 1.3	20.3 ± 1.0	1175.6 ± 64	304.0 ± 19	296.6 ± 24	1.4 ± 0.1	0.25 ± 0.01

The time of ischemia was 5 min. Data are expressed in nmol/g wet weight. Homocysteine was infused in different doses as described in Section 4. Values are means ± SEM for *n* animals. AdoMet/AdoHcy ratio and EC.
* $P < 0.01$ vs. control.

the control value at 15 min renal recirculation, the AdoMet/AdoHcy ratio remained reduced to 15% of the control.

7.2. Tissue content of AdoHcy after intrarenal infusion of homocysteine and/or adenosine into the ischemic kidney

Intrarenal infusion of 1 μmol adenosine into the ischemic kidney increased the adenosine tissue content up to 57.1 ± 5.4 nmol/g wet weight and increased slightly the energy charge whereas AdoHcy tissue content remained unchanged (Table 2). However, when homocysteine (1 μmol) was administered into the ischemic kidney AdoHcy tissue levels increased up to 35.3 ± 3.2 nmol/g wet weight, whereas adenosine, AdoMet and AMP remained unaltered. Highest AdoHcy tissue levels were measured after infusion of both, adenosine and homocysteine (1 μmol each) into the ischemic kidney.

When homocysteine was administered dose-dependently into the 5 min ischemic kidney, AdoHcy tissue content accumulated only at homocysteine concentrations higher than 100 nmol. AdoMet and adenosine tissue

Table 4
Renal hemodynamic parameters of rats with hyperhomocysteinemia resulting from infusion of homocysteine

Parameter	Control ($n = 4$)	Homocysteine ($n = 4$)
Body weight (g)	330 ± 12.6	324 ± 12
GFR (mL/min 100 g)	0.80 ± 0.05	0.86 ± 0.05
UV (μL/min 100 g)	2.5 ± 0.3	2.5 ± 0.2
$U_{Na}V$ (μmol/min)	0.9 ± 0.09	1.6 ± 0.2
MAP (mmHg)	111 ± 3.0	106 ± 3.5
Hct (%)	46.5 ± 0.5	45.2 ± 0.4

Glomerular filtration rate (GFR), urine flow (UV), urine sodium excretion ($U_{Na}V$), mean arterial blood pressure (MAP) and hematocrit (Hct). Values are means ± SEM for *n* animals.

Table 5
Renal AdoHcy metabolism in rats with acute hyperhomocysteinemia

	n	AdoHcy (nmol/g wet weight)	AdoMet	Adenosine	AMP	ADP	ATP	AdoMet/ AdoHcy	EC
Control (NaCl 0.87%)	4	0.7 ± 0.04	49.8 ± 0.3	4.45 ± 0.3	152.6 ± 3.6	360.5 ± 10	1978.5 ± 23	65.7 ± 3.6	0.86 ± 0.01
Homocysteine	4	1.5 ± 0.1*	51.3 ± 0.5	7.23 ± 0.9*	400.0 ± 31*	528.4 ± 41*	1531.5 ± 35*	32.3 ± 3.4*	0.73 ± 0.01*

AdoMet/AdoHcy ratio and EC. Values are means ± SEM for *n* animals. The homocysteine concentration infused was 690 μg per 100 g body weight in a prime dose and 216 μg/hr per 100 g body weight in the continous infusion as described in Section 4.

* $P < 0.01$ vs. control.

levels did not alter during the homocysteine infusion (Table 3).

7.3. Tissue content of AdoHcy in hyperhomocysteinemia

Renal hemodynamic parameters are present in Table 4. There were no significant effects of homocysteine infusion on urine flow, GFR, MAP and $U_{Na}V$. The acute hyperhomocysteinemia produced by an i.v. infusion of homocysteine yielded significantly higher AdoHcy and adenosine tissue levels. The AdoMet tissue level was elevated but not significantly (Table 5). The calculated AdoMet/AdoHcy ratio and the energy charge was significantly reduced in the group with i.v. infusion of homocysteine.

8. Discussion

AdoHcy, the product of AdoMet-dependent transmethylation reactions is a potent endogenous inhibitor of transmethylations [1–3]. It is the ratio of AdoMet/AdoHcy concentrations, also termed as methylation potential, that determines the level of transmethylation [1,2,27]. Recent studies [28,29] have shown that the action of aldosterone to increase sodium transport in A6 cells requires a high methylation potential including an increased hydrolysis of AdoHcy by the AdoHcyase. In order to investigate further factors that can affect the methylation potential, we examined the influence of ischemia, adenosine and homocysteine on the renal AdoHcy and AdoMet tissue content.

The AdoHcy tissue content of 0.7 nmol/g wet weight in the normoxic kidney increases 11-fold during 10 min of ischemia induced by renal artery occlusion. In the same time period, the AdoMet tissue content is nearly unchanged. Accordingly, the reduction of the methylation potential from 65.1 to 6.1 is due to the increase of AdoHcy tissue content.

One explanation of the AdoHcy accumulation in the ischemic kidney could be the inhibition of AdoHcy hydrolysis by adenosine. As reported previously in *in vitro* studies [30], adenosine inhibits AdoHcy hydrolysis with a K_i of 3 μM and leads in concentrations of 3–10 μM to a gradually inactivation of AdoHcyase based on the oxidation at C3′ of the ribose residue of adenosine with concomitant reducing the tightly bound NAD^+ to NADH [31]. The adenosine accumulation in the ischemic kidney reaches concentrations that are in the range of 5–20 μM. Thus, increased AdoHcy levels during ischemia could be the result of AdoHcyase inhibition also under *in vivo* conditions. This inhibition of AdoHcyase may be maximal at 20 nmol/g wet weight adenosine, since additional administration of exogenous adenosine into the ischemic kidney leads not to a further increase of AdoHcy tissue levels, when adenosine tissue content could be elevated from 22.1 to 57.1 nmol/g wet weight (Table 2).

Another explanation for the accumulation of AdoHcy tissue content in the ischemic kidney could be an enhanced rate of AdoHcy synthesis from adenosine and homocysteine. To test this possibility we infused homocysteine into the ischemic kidney. Addition of homocysteine to the enzymatic inactive AdoHcyase in the presence of adenosine lead in *in vitro* studies to reconversion of NADH to NAD^+ thus the enzymatic reaction in the direction of AdoHcy synthesis is favored [32]. Since the K_M for homocysteine in this reaction is 100 μM [33] we injected homocysteine with concentrations of 10–1000 nmol into the renal artery of the ischemic kidney to achieve a similar homocysteine concentration also under *in vivo* conditions. Since AdoHcy tissue levels increased in this set of experiments in a concentration dependent manner up to 35.3 nmol/g wet weight (Table 3), we conclude that homocysteine is rate limiting for AdoHcy synthesis under conditions of ischemia when adenosine tissue levels are elevated.

A support for this conclusion is provided by an even further increase in AdoHcy tissue levels up to 60.6 nmol/g wet weight when both, adenosine and homocysteine, were administered into the renal artery of the ischemic kidney (Table 2). This *in vivo* finding is in accordance with the results from *in vitro* studies, that the thermodynamic equilibrium of AdoHcyase reaction is on the side of AdoHcy synthesis [5,30].

Both, ischemia and elevated homocysteine concentrations lead to a substantial decrease of the methylation potential in the cell. Thus, it is conceivable that under these conditions, AdoMet-dependent methyltransferases such as thiopurine methyltransferase, cathechol-*O*-methyltransferase or histamine *N*-methyltransferase may be almost completely inhibited [34–36].

In CRF the high plasma concentrations of homocysteine correlates with an increased AdoHcy concentration in red

blood cells and a reduced methylation potential, resulting in a reduced membrane protein methyl esterification [16,37]. Whether CRF and high homocysteine blood levels affect the methylation potential also in the kidney remains to be determined.

To analyze the renal response to hyperhomocysteinemia under normoxic conditions we infused homocysteine i.v. according to House *et al*. [20]. We found that AdoHcy tissue content increases under these experimental conditions two-fold, without changes in AdoMet tissue by slight reduction in the energy charge. The overall kidney function was unchanged in accordance with the data of House *et al*. [20]. Thus, it appears that the availability of free adenosine is rate limiting for AdoHcy synthesis in the kidney under normoxic conditions. Whereas under ischemic conditions when adenosine accumulates the availability of homocysteine becomes rate limiting for AdoHcy synthesis.

We are aware that the result of the experiment of hyperhomocysteinemia may not be quantitatively extrapolated to human renal physiology, since the major difference between rat and human with respect to homocysteine is that in rats homocysteine is mainly present in the unbound form [20], while in human it is mainly protein-bound [10]. However, the enzymes metabolizing homocysteine are the same in rats and human.

We conclude that (i) during ischemia of kidney, the methylation potential is markedly reduced, mainly due to accumulation of AdoHcy; (ii) the increased AdoHcy levels during ischemia are the result of the inhibition of AdoHcy hydrolysis; (iii) homocysteine is rate limiting for AdoHcy synthesis in the ischemic kidney; (iv) under normoxic conditions hyperhomocysteinemia can affect the methylation potential in the renal tissue. Our data strongly support the interaction between the energy metabolism and transmethylation reactions.

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