Adaptive Changes of H^+ and/or Bicarbonate Transport in Chronic Metabolic Acidosis: Its Quantitative Evaluation by Using Isolated Perfused Kidney

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SUZUKI, M., TERAQ, N., ASANO, Y. & HOSODA, S. *Adaptive Changes of H^+ and/or Bicarbonate Transport in Chronic Metabolic Acidosis: Its Quantitative Evaluation by Using Isolated Perfused Kidney*. Tohoku J. exp. Med., 1987, **152** (3), 283-290 — We have recently improved the technique of the isolated perfused rat kidney by addition of amino acids to the perfusion solution. Utilizing this improved preparation, we examined to what extent chronic acid-base status can alter H^+ and/or bicarbonate transport in the whole kidney and the effect of acetazolamide (10^{-4} M) on this process. Kidneys from rats that had been drinking tap water (control), 1.5% NH_4Cl (acidotic), or 1.5% $NaHCO_3$ (alkalotic) were perfused with a solution of identical pH and PCO_2 . During the control period, the fractional bicarbonate reabsorption was higher in acidotic rats than in control rats by 1.0% and urine pH was lower than those in control. On the other hand, the two parameters were not different between control and alkalotic rats. Acetazolamide increased urine pH and decreased the fractional bicarbonate reabsorption in all, but these parameters in acidotic and alkalotic rats were not different compared with those in control. We conclude that adaptive changes of H^+ and/or bicarbonate transport in chronic metabolic acidosis corresponds to about 1.0% as fractional bicarbonate reabsorption by the isolated perfused kidney and is largely carbonic anhydrase dependent. ——— adaptation; acid-base; isolated perfused kidney; acetazolamide

In response to chronic acid-base alterations, adaptive changes in H^+ and/or bicarbonate transport occur. However, most of these studies have been performed in the individual segments of the nephron (McKinney and Burg 1977; Cogan et al. 1979; Ulrich and Papavassiliou 1981; Lucci et al. 1982; Laski and Kurtzman 1983; Lombard et al. 1983; Tsai et al. 1984). On the other hand, although quantitative evaluation of adaptive changes in H^+ and/or bicarbonate transport has been attempted in the whole kidney in vivo, reduced serum bicarbonate levels in chronic metabolic acidosis make impossible to provide the comparable filtered

bicarbonate load with those under normal conditions, and acute correction of it by administering NaHCO_3 causes volume overload (Levine and Nash 1973). Therefore, it is very difficult to evaluate the quantitative changes of H^+ and/or bicarbonate transport in the whole kidney in vivo.

In this regard, the isolated perfused kidney could be the ideal preparation for this purpose. However, in this preparation, an impairment of renal function and urinary acidification was noted (Nizet 1975). Therefore, adaptive changes of H^+ and/or bicarbonate transport were only studied in the distal nephron with an extremely low bicarbonate concentration in perfusion solution which could exclude the large effect of more proximal portions of nephron (Terao and Tannen 1981; Kornandakiet al. 1983).

Recently we demonstrated the improvement of functional state of the isolated perfused rat kidney as the preparation for the study hydrogen transport in the whole kidney by adding an amino acids mixture to the perfusion solution (Terao et al. 1985); there was a marked increase and stability of the absolute amount and the rate of reabsorption of bicarbonate as well as phosphorus associated with constant urine pH. In the present study, we attempted to estimate quantitative alterations of H^+ and/or bicarbonate transport in response to chronic acid-base changes in the whole kidney by perfusing kidneys pretreated with acid and alkali with normal bicarbonate perfusion solutions.

METHOD

Preparations and experimental procedures

Three groups of male Sprague-Dawley rats were prepared by allowing free access to tap water (control group, $n=5$), 1.5% NH_4Cl (NH_4Cl group, $n=5$), or 1.5% NaHCO_3 (NaHCO_3 group, $n=5$) for 4 to 11 days. The rats weighing 340 to 400 g were anesthetized with pentobarbital (50 mg/kg, i.p.) and they received 1 g of mannitol i.v. The ureter was

TABLE 1. *Amino acid composition of perfusion solution*

Amino acids from concentrated	mg/100 ml solutions	Amino acids from individual	mg/100 ml reagents
Leucine	5.2	Alanine	17.8
Phenylalanine	3.4	Glycine	17.3
Methionine	1.6	Cysteine	8.8
Lysine	7.4	Aspartate	2.7
Isoleucine	5.2	Glutamate	7.4
Valine	4.8	Asparagine	3.0
Histidine	2.2	Glutamine	2.9
Threonine	4.8	Serine	1.1
Tryptophan	0.8	Proline	3.6
Arginine	4.2		
Tyrosine	3.6		

cannulated with PE-10 catheter, the renal artery was cannulated with 18 gauge metal needle without ischemia, and the kidney was removed within 15 min after a laparotomy and perfused in a pulsatile fashion at 37°C using a recirculating system with a reservoir as described previously (Terao and Tannen 1981).

Before each experiment, perfusion medium was prepared as follows: to 100 ml of 6.7% bovine albumin solution, were added, with 5 mM glucose, 2 ml of concentrated essential amino acid solution (BME amino acid solution, M.A. Bioproducts, Walkersville, MA, USA) and other amino acids individually, of which composition is described in Table 1 as we used previously (Terao et al. 1985). Equilibrated with 95% O₂ and 5% CO₂, pH of perfusion solution was adjusted to about 7.40.

The perfusion was carried out for 55 min with the last 30 min divided into two clearance periods. Urine and perfusate samples were obtained at 15 min interval during clearance studies. Urine samples were collected under mineral oil.

During the first clearance period, the quantitative evaluation of adaptive changes to chronic acid or alkali ingestion was made with respect to urine pH and fractional reabsorption of bicarbonate. At the end of the first clearance period (control time) acetazolamide 10⁻⁴ M was added.

Analysis

Pco₂ and pH of perfusion solution, perfusate and urine were measured by a blood gas system (BMS3-MK2, Radiometer, Copenhagen, Denmark). Na and K were measured with a flame photometer. ¹⁴C-inulin was used for the determination of GFR. Urinary ammonia was determined by the colorimetric method (McCullough 1967) and urinary phosphorus by the method of Chen, Toribara and Warner (Chen et al. 1957). Phosphorus levels in perfusion solution and perfusate were measured by the Coulter phosphour reagent kit supplied by Coulter Electronics, INC. (Hialeah, FL, USA) according to the modification of Fiske-Subbarow method (Fiske and Subbarow 1925). Phosphorus in perfusion solution was entirely ultrafiltrable in this preparation (Steele and Underwood 1978), so that filtered phosphorus was calculated as the product of GFR and phosphorus concentrations in perfusion solution and perfusate. The perfusion solution and perfusate bicarbonate concentrations were calculated from the Henderson-Hasselbalch equation. A pK' of 6.10 and solubility coefficient of 0.0301 were used for perfusion solution and perfusate. In the urine, a solubility coefficient of 0.0309 was used. The pK' calculated from urinary ionic strength was $6.33 - 0.5 \sqrt{(\text{Na}^+) + (\text{K}^+)}$, with the concentration of Na and K being expressed in equivalents per liter (Hasting and Sendroy 1925). Paired and non paired Student's *t*-test were used for the statistical analysis.

RESULT

During the first clearance time (control time) of perfusing kidneys from normal rats or those drinking either acid or alkali, with the perfusion solution of the same pH and Pco₂ (Table 3), urine pH was significantly lower in the NH₄Cl group (6.65 ± 0.03) than in the control group (7.00 ± 0.06 , $p < 0.01$) (Fig. 1). In addition, as shown in Fig. 2, the fractional bicarbonate reabsorption was also increased from the control group ($98.3 \pm 0.3\%$) to the NH₄Cl group ($99.3 \pm 0.1\%$, $p < 0.01$) by 1.0%. However, during the control time, in the NaHCO₃ group, there were only tendency of the higher urine pH (7.22 ± 0.15 , N.S.) and of the lower fractional bicarbonate reabsorption ($97.6 \pm 0.8\%$, N.S.) comparing with those in the control group.

During the control period, with regard to the parameters of renal function and

Fig. 1. Effect on urine pH of chronic acid and alkali ingestion, and of acetazolamide (10^{-4} M).

● NH₄Cl; ○ control; ▲ NaHCO₃. ** $p < 0.01$.

Fig. 2. Effect of chronic acid and alkali ingestion on fractional reabsorption of bicarbonate during the control time.

● NH₄Cl; ○ control; ▲ NaHCO₃. ** $p < 0.01$.

of acid-base (Tables 2, 3), the values of either the NH₄Cl group or the NaHCO₃ group compared with those of the control group were not statistically significant except for lower urinary HCO₃⁻ concentration in the NH₄Cl group.

The addition of acetazolamide (10^{-4} M) at the end of the control period, was followed by marked diuresis associated with natriuresis and kaliuresis (Table 2). Fig. 1 indicates that the additions of acetazolamide uniformly resulted in significant elevation of urine pH. However, during the acetazolamide period,

TABLE 2. *Renal function*

	Group	Control period	Acetazolamide period
GFR (ml/min)	NH ₄ Cl	1.00 ± 0.20	0.71 ± 0.09
	Control	1.23 ± 0.07	0.73 ± 0.04*
	NaHCO ₃	1.27 ± 0.22	0.77 ± 0.02
FR Na (%)	NH ₄ Cl	97.6 ± 0.2	89.5 ± 0.9**
	Control	97.2 ± 0.6	90.4 ± 1.2*
	NaHCO ₃	98.3 ± 0.4	93.2 ± 0.6**
E/F K	NH ₄ Cl	0.48 ± 0.09	1.17 ± 0.13**
	Control	0.29 ± 0.04	1.51 ± 0.14**
	NaHCO ₃	0.23 ± 0.02	0.98 ± 0.07**
Urine volume (ml/min)	NH ₄ Cl	0.044 ± 0.009	0.122 ± 0.015**
	Control	0.052 ± 0.013	0.126 ± 0.010**
	NaHCO ₃	0.041 ± 0.005	0.117 ± 0.010**
Kidney dry weight (g)	NH ₄ Cl	0.31 ± 0.03	
	Control	0.28 ± 0.03	
	NaHCO ₃	0.29 ± 0.04	

Values are means ± s.e. * $p < 0.05$; ** $p < 0.01$ against the values in the control period.

urine pH and fractional bicarbonate reabsorption in the control group (7.57 ± 0.04 , 69.5%, respectively) were not statistically different either from those in the NH₄Cl group (7.48 ± 0.02 , $74.0 \pm 21\%$) and/or from those in the NaHCO₃ group (7.60 ± 0.03 , $73.7 \pm 1.8\%$).

DISCUSSION

The results of the present study show that the enhancement of bicarbonate reabsorption rate was approximately 1.0% associated with a decrease in urine pH in response to chronic NH₄Cl drinking. In the present experiments, serum HCO₃⁻ levels were not measured after the dietary manipulation. However, the dietary maneuver in the present experiments similar to those of previous studies (Kornandakietti et al. 1983), in which the diet caused significant chronic metabolic acidosis or alkalosis. Therefore, it is reasonable to consider that, in the present experiments, the rats sacrificed for perfusion had chronic metabolic acidosis or alkalosis compared with the control rats.

The adaptation to the chronic acid-base change was noted in the proximal convoluted tubules of the rat and the rabbit (Cogan et al. 1979; Tsai et al. 1984), the rat distal convoluted tubules (Lucci et al. 1982), the rabbit cortical and medullary collecting ducts (McKinney and Burg 1977; Laski and Kurtzman 1983; Lombard et al. 1983) and the rat papillary collecting ducts (Ulrich and Papavassiliou 1981), in addition to the rat distal nephron (Terao and Tannen 1981; Kornandakietti et al. 1983). The present results do not provide any insight into the localizations of the adaptation in the nephron and the relative contribu-

TABLE 3. *Acid-base parameters*

		Group	Control period	Acetazolamide period
Perfusate	pH	NH ₄ Cl	7.42±0.01	7.43±0.01
		Control	7.42±0.01	7.43±0.01
		NaHCO ₃	7.41±0.01	7.42±0.01
	Pco ₂ (mmHg)	NH ₄ Cl	38.7 ±2.7	37.8 ±2.9
		Control	38.4 ±0.8	37.7 ±0.9
		NaHCO ₃	36.4 ±3.9	36.2 ±4.8
	HCO ₃ ⁻ (mM)	NH ₄ Cl	24.3 ±1.1	24.3 ±1.4
		Control	25.1 ±1.9	24.0 ±1.4
		NaHCO ₃	22.7 ±3.4	21.9 ±3.8
	K (mM)	NH ₄ Cl	5.0 ±0.2	4.5 ±0.2
		Control	4.8 ±0.2	4.6 ±0.2
		NaHCO ₃	4.9 ±0.1	4.7 ±0.1*
Urine	Pco ₂ (mmHg)	NH ₄ Cl	36.7 ±0.5	53.7 ±3.1**
		Control	38.9 ±1.7	52.1 ±2.7*
		NaHCO ₃	36.3 ±2.2	47.7 ±2.7*
	HCO ₃ ⁻ (mM)	NH ₄ Cl	3.6 ±0.6††	36.2 ±2.6**
		Control	8.6 ±2.7	42.7 ±3.3**
		NaHCO ₃	15.2 ±5.0	39.5 ±3.0**
	NH ₄ ⁺ (μmol/min)	NH ₄ Cl	0.52±0.24	0.43±0.12
		Control	0.25±0.06	0.20±0.03
		NaHCO ₃	0.07±0.02	0.15±0.05
	Phosphorus (μmol/min)	NH ₄ Cl	0.02±0.00	0.04±0.01
		Control	0.03±0.00	0.03±0.01
		NaHCO ₃	0.01±0.03	0.04±0.01

Values are means±s.e. * $p < 0.05$; ** $p < 0.01$ against the values in the control period.

†† $p < 0.01$ against the value in the control group.

tions of those segments to it. Nevertheless, this is the first observations with regard to the quantitative changes of adaptation to chronic acid-base alterations in the whole kidney. Because of a considerable variation of the data, urine pH and fractional bicarbonate reabsorption in the NaHCO₃ group were not different from those in the control group. The reason for this variability is unknown. After acetazolamide was added, there was no statistically significant difference of urine pH and fractional bicarbonate reabsorption between the control group and the other two groups. This observation seems to imply that the adaptation of H⁺ and/or HCO₃⁻ transport is largely carbonic anhydrase dependent.

It is well known that, in chronic metabolic acidosis, urinary ammonia excretion increases due both to enhanced renal ammonia production and to decreased urine pH as a result of low blood HCO₃⁻ concentrations (Alpern et al. 1985). However, in the present experiments, urinary ammonia excretions was not significantly increased (Table 2). This finding could be at least partially explained by the fact that, kidneys were perfused with a perfusion solution with

normal HCO_3^- concentrations in the present experiments. Since urine pH was not so lowered as seen in ongoing metabolic acidosis, less amount of urinary ammonia was excreted under this particular experimental condition.

In summary, we concluded that the adaptive change of H^+ and/or HCO_3^- transport in the whole kidney due to chronic metabolic acidosis is about 1.0% as expressed by fractional bicarbonate reabsorption by using our isolated perfused rat kidney model, and that this adaptive response is largely carbonic anhydrase dependent.

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