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TECHNICAL NOTE

Enhanced reabsorption of bicarbonate and phosphorus by the addition of amino acids in the isolated perfused rat kidney

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In the isolated perfused kidney, a defect in urinary acidification has been noted [1]. Nevertheless, this preparation which used rat kidney with albumin saline solution as perfusate including glucose as substrate has also been used for the study of distal acidification, because of urinary acidification reached in the extreme metabolic acidosis [2, 3].

Recently, in a model of the isolated perfused rat kidney, it was demonstrated clearly that adding amino acids results in the improvement and stability of renal function which are associated with the amelioration of histological damage of the thick ascending limb of Henle [4]. This observation prompted us to re-evaluate acid-base parameters of this preparation with respect to the effect of amino acids. Our results show that the addition of amino acids mixture leads to significant increase and stability of the reabsorption of HCO_3^- and phosphorus as well as the constant urine pH.

Methods. Kidneys from male Sprague-Dawley rats weighing 340 to 400 g were perfused in vitro as previously described [2, 5] except for using 18-gauge metal needles instead of glass cannulas. Calculated mean artery pressure was maintained at 90 mm Hg during the experiments. Perfusion was carried out for 85 min with the last 60 min divided into four clearance periods with 6.7% bovine albumin Krebs-Henseleit saline solution, equilibrated with 97% O_2/5% CO_2. The perfusion medium contained 5 mM glucose with ($N = 5$), or without amino acids ($N = 5$), the composition of which was similar to that of a previous study [4]. Prior to each experiment 2 ml of concentrated essential amino acid solution (M. A. Bioproducts, Walkersville, Maryland, USA) were added to 100 ml of a prepared perfusate. The amino acid solution contained 12 amino acids including cystine (2.4 mg) and nine other amino acids individually. This procedure provides the similar composition of amino acids as previously described [4] except for the presence of cystine. Perfusate pH was adjusted to about 7.40. Urine and perfusate samples were obtained at 15-min intervals; urine samples were collected under mineral oil.

Analysis. Pco_2 and pH of perfusate and urine were measured by a blood gas system (BMS3-MK2 , Radiometer, Copenhagen, Denmark). Sodium and potassium were measured with a flame photometer, and ^{14}C-inulin was used for the determination of glomerular filtration rate (GFR). Urinary ammonia was determined by the calorimetric method [6] and urinary phosphorus by the method of Chen, Toribara, and Warner [7]. Perfusate phosphorus was measured by a phosphorus reagent kit supplied by Coulter Electronics, Inc. (Hialeah, Florida, USA) according to the modification of the Fiske-Subbarow method [8]. Perfusate phosphorus was entirely ultrafiltrable in this preparation [9], so that filtered phosphorus was calculated as the product of GFR and its perfusate concentrations. The perfusate HCO_3^- concentration was calculated from the Handerson-Haaselbalch equation. A pK′ of 6.10 was used for carbonic acid, and a solubility factor of 0.0301 was used for carbon dioxide. A solubility coefficient of 0.0309 was used for urine samples. The pK′ calculated from urinary ionic strength was $6.33-0.5\sqrt{(Na^+)+(K^+)}$; the concentration of sodium and potassium were expressed in equivalents per liter [10]. Paired and nonpaired Student t tests were used for the statistical analysis.

Results. Table 1 indicates, as reported in the previous study [4], improvement of renal functions in the amino acid-added group and compares with those of the nonamino acid-added group; both GFR and fractional sodium reabsorption (FR Na) were higher and fractional potassium excretion (E/F K) less.

Keeping in mind that a similar perfusate pH and Pco_2 were in the two groups (Table 2), Figure 1 shows that in the group with amino acids, as reflected in an increase in GFR, the filtered load of HCO_3^- was much greater than in the one without amino acids. Despite this larger filtered HCO_3^- load, fractional reabsorption of HCO_3^- in the amino acid-added group which was stable and ranged from 98.2 ± 0.8% to 97.2 ± 0.8% exceeded that in the nonamino acid-added group with a range from 94.4 ± 0.8% to 92.5 ± 1.3%. Moreover, urine pH was constant from 7.00 ± 0.08 to 7.08 ± 0.06 in the amino acid-added group, in contrast to the significant decrement in urine pH from 7.36 ± 0.06 to 7.02 ± 0.07 ($P < 0.05$) in the nonamino acid-added group. In addition to this remarkable increase in HCO_3^- reabsorption, as shown in Table 2 and Figure 2, the effect of adding amino acids on the transport of phosphorus was also striking; with amino acids, the significantly lower urinary excretion was accompanied by the substantially higher and stable reabsorption rate with the range from 98.2 ± 0.3% to 97.0 ± 0.5 (NS) in contrast to the rapid decrease in the reabsorption rate from 90.8 ± 2.0% to 70.3 ± 6.8% ($P < 0.05$) without amino acids. As far as urinary ammonium excretion, it was higher in the amino acid-added group with the tendency to increase with time (Table 2). Nevertheless, in the group with amino acids, the

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Table 1. Amino acid study: renal function[a]

	Amino acids	Perfusion time, *min*			
		25 to 40	40 to 55	55 to 70	70 to 85
GFR, *ml/min*	(+)	1.31 ± 0.10	1.10 ± 0.07	0.91 ± 0.13	0.81 ± 0.13
	(−)	0.87 ± 0.02	0.58 ± 0.04	0.42 ± 0.04	0.33 ± 0.05
P		< 0.01	< 0.01	< 0.01	< 0.01
FR Na, %	(+)	97.8 ± 0.9	97.0 ± 0.9	96.9 ± 1.0	96.6 ± 1.4
	(−)	97.1 ± 1.1	95.4 ± 1.4	94.5 ± 2.0	94.4 ± 3.0
P		NS	NS	NS	NS
E/F K	(+)	0.33 ± 0.09	0.48 ± 0.08	0.51 ± 0.08	0.47 ± 0.07
	(−)	0.58 ± 0.10	1.12 ± 0.09	1.40 ± 0.13	1.46 ± 0.15
P		NS	< 0.01	< 0.01	< 0.01
Urine volume, *ml/min*	(+)	0.045 ± 0.010	0.066 ± 0.008	0.064 ± 0.007	0.061 ± 0.009
	(−)	0.057 ± 0.011	0.076 ± 0.011	0.070 ± 0.014	0.059 ± 0.015
P		NS	NS	NS	NS

Abbreviations: GFR, glomerular filtration rate; FR Na, fractional sodium reabsorption; E/F K, fractional potassium excretion.
[a] Values are means ± SE.

Table 2. Amino acids study: acid-base parameters[a]

	Amino acids	Perfusion time, *min*			
		25 to 40	40 to 55	55 to 70	70 to 85
Perfusate pH	(+)	7.43 ± 0.01	7.44 ± 0.01	7.44 ± 0.01	7.44 ± 0.01
	(−)	7.39 ± 0.01	7.40 ± 0.01	7.40 ± 0.01	7.40 ± 0.01
P_{CO_2}, *mm Hg*	(+)	36.4 ± 1.8	36.0 ± 1.7	36.3 ± 1.5	36.2 ± 1.4
	(−)	38.6 ± 1.3	37.6 ± 1.2	38.4 ± 1.4	38.4 ± 1.4
HCO_3^- reabsorption, *μmoles/min*	(+)	29.9 ± 3.1	25.2 ± 2.2	21.2 ± 2.9	18.9 ± 3.4
	(−)	18.7 ± 1.0	12.1 ± 0.7	9.0 ± 0.8	6.9 ± 0.8
P		< 0.01	<0.001	< 0.01	< 0.01
Urine NH_4^+, *μmoles/min*	(+)	0.13 ± 0.01	0.24 ± 0.03	0.40 ± 0.07	0.31 ± 0.06
	(−)	0.03 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.02[b]
P		< 0.01	<0.01	< 0.01	< 0.05
Urine phosphorus, *μmoles/min*	(+)	0.02 ± 0.00[b]	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	(−)	0.05 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.02
P		NS	< 0.01	< 0.01	< 0.02

[a] Values are means ± SE.
[b] $N = 4$.

amount of ammonium excretion was trivial compared with that of HCO_3^- reabsorption, which was much higher than the one without amino acids.

Discussion. We could show that in the isolated perfused rat kidney, the addition of amino acids not only improves renal function but also enhances the reabsorption of both HCO_3^- and phosphorus.

The exact location responsible for the stimulation of the reabsorption of the two anions is not known. The possibility of the thick ascending limb of Henle might be raised in view of the association of improvement of renal function and histological change [4]. The recent report of the presence of significant HCO_3^- reabsorption in this part of the nephron of the rat by the isolated perfused tubule technique [11] is also consistent with this possibility. However, the contribution of other parts of the nephron could be possible as well. The available evidence indicates the lack of phosphorus transport in the thick ascending limb of Henle [12]; therefore, at least concerning phosphorus, the substantial augmentation of its reabsorption is likely due to the alteration of the transport in the nephron segment other than in the thick ascending limb of Henle.

Wherever the localization for enhanced reabsorption of HCO_3^- and phosphorus, both increase and stabilization in the reabsorption of these two buffers associated with the constant urine pH make this model more useful for the study of H^+ transport in the whole kidney.

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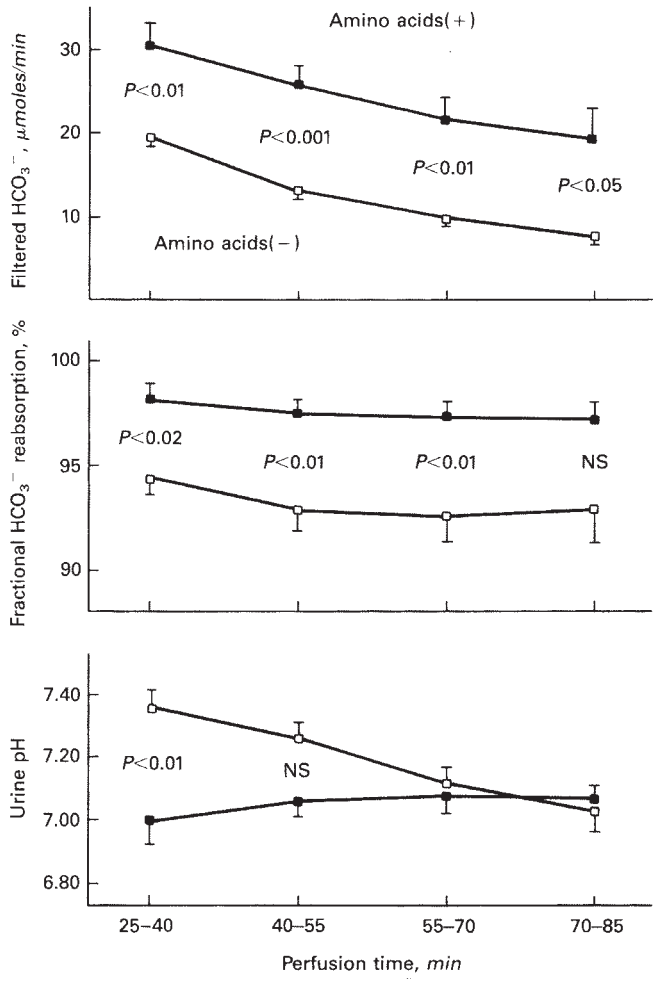

Fig. 1. *Effect of the addition of amino acids to perfusate on filtered HCO_3^-, fractional HCO_3^- reabsorption, and urine pH.* Despite the greater filtered load of HCO_3^-, fractional reabsorption of HCO_3^- in the amino acid group exceeded that in the nonamino acid group and was stable over time as urine pH.

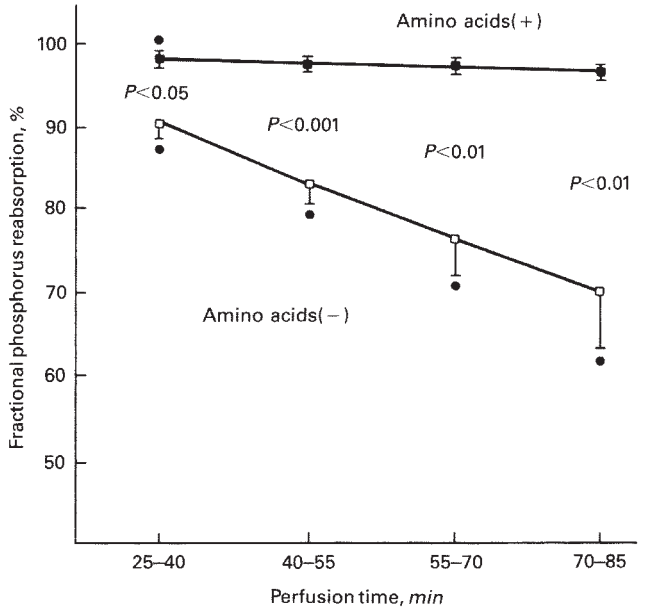

Fig. 2. *Effect of the addition of amino acids on fractional phosphorus reabsorption.* Fractional phosphorus reabsorption in the amino acid group was greater and constant compared to that in the nonamino acid group. Symbol: ●, $N = 4$.

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