THE EFFECT OF METABOLIC ACIDOSIS ON γ-GLUTAMYLTRANSPEPTIDASE ACTIVITY IN THE RAT KIDNEY

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Received 5 May 1977

1. Introduction

As part of the renal response to metabolic acidosis in the rat, there are increases in ammonia production and uptake of glutamine [1] and enhanced activity of the mitochondrial phosphate-dependent glutaminase [2]. There are no data on the effect of acidosis on γ-glutamyl transpeptidase which has been shown to be identical with the maleate stimulated glutaminase [3,4]. The enzyme which is thought to mediate aminoacid transport [5] is located on the external surface of the kidney cell with its catalytic activity probably restricted to the brush border membrane of the proximal straight tubule [6]. This paper reports studies on the effect of metabolic acidosis on the activity of γ-glutamyl transpeptidase in crude homogenates and in purified brush border membranes of the rat kidney.

2. Materials and methods

Adult Sprague Dawley rats were used. Chronic acidosis was induced by placing rats on 280 mM NH_4Cl as the sole drinking fluid for 7–10 days. Controls received 280 mM NaCl. In short term studies rats were given 400 mM NH_4Cl (2.5 ml/100 g body wt) by intragastric tube at 12 h intervals for 24 h, 36 h and 48 h and were sacrificed 2 h after the last feeding. Controls received 400 mM NaCl. All rats were starved overnight prior to sacrifice.

Homogenates (5%) of kidney cortex were prepared in 250 mM sucrose. Suspensions of brush border membranes were prepared by the method of Aronson and Sacktor [7]. Enzyme activity was measured as described by Tate and Meister [5]. Protein in homogenates was estimated by the Biuret method; in brush border preparations by the Lowry method [8].

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3. Results

3.1. *Enzyme properties*

Enzyme activity in homogenates from control and chronically acidotic animals is linear over a 1 h incubation, and with respect to homogenate concentration. The variation of activity with glutamine concentration is shown in fig.1. Homogenates from kidneys of rats made acidotic for 8 days show an increase in enzyme activity.

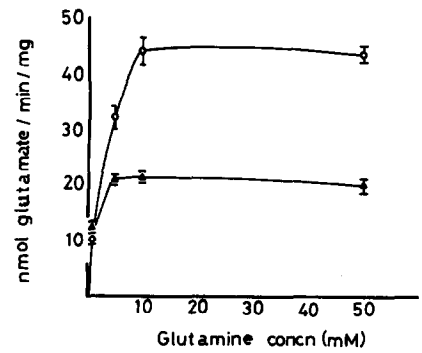

Fig.1. Variation of enzyme activity with glutamine concentration. Control rats (△——△). Chronically acidotic rats (8 days) (○——○). Values plotted are the mean ± SEM.

Table 1
Effect of maleate and serine-borate on enzyme activity in homogenates from renal cortex of normal rats and rats made acidotic for 8 days

Condition	Rate of product formation (nmol glutamate/min/mg)	
	Control	Acidotic
+ 50 mM maleate	27.2 ± 0.7 (4)	39.9 ± 2.5 (4)
− maleate	1.29 ± 0.4 (4)	1.91 ± 0.5 (4)
+ 2 mM serine-borate	8.9 ± 1.7 (4)	35.6 ± 2.3 (4)

Figure in parentheses represents number of observations

In the absence of the activator, maleate, activity is almost abolished in homogenates from both control and acidotic rats. However, the enzymes show a difference in reactivity to 2 mM serine-borate. Whereas the control enzyme is inhibited 67.3% the acidotic enzyme is inhibited only 10% (table 1).

3.2. *Acidosis*

Acidosis for 24 h does not result in increased levels of enzyme activity but at 36 h there is a significant increase in activity which is still present at 48 h and 8 days (table 2).

3.3. *Brush border preparations*

Enzyme activity was measured at various stages during a 5-fold purification of brush border plasma membrane in control and 8 day acidotic rats. The

Table 2
Effect of acidosis on level of γ-glutamyl transpeptidase in crude homogenates

Duration of acidosis	Control (nmol glutamate/min/mg)	Acidotic (nmol glutamate/min/mg)
24 h	21.7 ± 0.98 (10)	21.2 ± 1.3 (5)
36 h	26.4 ± 1.5 (14)	40.2 ± 2.2 (14)[b]
48 h	23.1 ± 1.8 (12)	31.1 ± 0.58 (12)[a]
7–10 days	24.3 ± 1.7 (10)	36.6 ± 1.7 (10)[b]

[a,b]Indicate statistical significance $p<0.005$ and $p<0.001$, respectively, between control and acidotic

Figure in parentheses represents number of observations

Table 3
γ-Glutamyl transpeptidase activity during brush border membrane purification

Stage	Control (nmol glutamate/min/mg)	Acidotic (nmol glutamate/min/mg)	% Increase
1	48.4 ± 5.7 (5)	66.9 ± 6.7 (5)	38
2	71.7 ± 12.1 (5)	97.8 ± 12.5 (5)	36
3	216.6 ± 19.2 (5)	305.4 ± 21.3 (5)	41

Stage 1 Combined supernatants after homogenisation
Stage 2 Brush border layer from density gradient centrifugation
Stage 3 Final suspension

Figure in parentheses represents number of observations

enzyme specific activity increases with purification of the brush border membranes and the increase due to acidosis persists table 3.

The enzymes at various stages of purification behave similarily in respect to maleate activation, activity is almost abolished in its absence. With the enzyme from control rats, presence of the inhibitor serine-borate results in 75% inhibition at stage 1 and almost total loss of activity in the final suspension. With the enzyme from acidotic rats at stage 1 there is approximately 20% inhibition while in the final suspension there is 87% inhibition. Enzyme activity was measured in a brush border preparation from 24 h acidotic rats since the crude homogenate might have masked increases in activity. No increased activity was detectable at any stage of the purification.

4. Discussion

These experiments show a significant increase in γ-glutamyl transpeptidase activity in response to metabolic acidosis. Glutamine is normally a poor substrate for the enzyme but in the presence of maleate there is inhibition of transpeptidation and stimulation of its glutaminase activity. Curthoys and Kuhlenschmidt [4] suggest that there may be a physiological counterpart to maleate such as the factor reported by Alleyne and Roobol [9] which appears in the plasma of acidotic rats. The increased ammoniagenesis observed in acidosis could be partially due to γ-glutamyl transpeptidase activity since the increased activity of the enzyme could result in enhanced

glutamine transport. Potassium deficiency, another condition in which ammonia genesis is enhanced, also results in increased enzyme activity (unpublished observations). It has been suggested that the early increase in ammonia production in acidosis is due to increased phosphate dependent glutaminase synthesis in the proximal convoluted tubule [10]. Since increased γ-glutamyl transpeptidase activity is not measurable until acidosis has persisted for 36 h, it is envisaged that adaptation of this enzyme is a secondary response to acidosis.

The behaviour of the enzyme during purification and the reactivity to maleate would indicate that the enzymes from control and acidotic rats are identical. The reason for the difference in reactivity with serine-borate is not clear but the mechanism whereby this mixture inhibits enzyme activity has not been elucidated [3]. A natural extension of these experiments will be measurement of the enzyme activity in species such as the dog which show an adaptive increase in ammoniagenesis in response to acidosis but no increase in activity of the phosphate-dependent glutaminase.

Acknowledgements

Dr McFarlane Anderson was supported by a grant from the Wellcome Trust.

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