THE MECHANISM OF THE INCREASE IN RENAL AMMONIAGENESIS IN THE RAT WITH ACUTE METABOLIC ACIDOSIS

Jose A. LUPIANEZ, Paloma HORTELANO, Fermin SANCHEZ-MEDINA, Antonio SANCHEZ-POGO, Norma McFARLANE-ANDERSON+, Jennifer BARNSWELL+ and George A. O. ALLEYNE+

Department of Biochemistry, University of Granada, Spain and *+Department of Medicine, University of the West Indies, Kingston 7, Jamaica*

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1. Introduction

Chronic metabolic acidosis causes several biochemical changes in the rat kidney: perhaps the most prominent of these is the increase in ammonia production from glutamine and the increase in activity of phosphate-dependent glutaminase [1]. The changes with acute acidosis are less clear and although there is an early increase in total renal production of ammonia [2] no enzymatic change has been described to explain this. Since changes in pH and bicarbonate alone could not be the stimulus for this renal metabolic adaptation, we looked for and demonstrated the presence of a factor in the plasma of acutely acidotic rats which stimulated glutamine uptake and ammoniagenesis by renal slices from normal rats [3]. We now describe a possible mechanism for this ammoniagenic response to acute acidosis in the rat. Our studies have focussed on the kidney since although the liver, brain and intestine metabolise glutamine, none of these organs show any enzymatic adaptation in response to metabolic acidosis.

2. Materials and methods

Male Sprague-Dawley rats (150–200 g) were used. After an overnight fast they were tube-fed 4 ml/100 g body wt of 400 mM NaCl (controls) or an equivalent volume of 400 mM NH_4Cl (acidotic). Animals were sacrificed 0.5 h later after being anaesthetized with sodium pentobarbital 40 mg/kg body wt. Blood was drawn from the abdominal aorta into heparinized syringes and plasma separated immediately. The blood pH of acidotic rats had dropped from a control value of 7.39 ± 0.01 (mean ± SEM) to 7.13 ± 0.01 ($n = 4$). The measurement of ammonia production from glutamine 2.0 mM by renal cortical slices has been described [4]. To study the effect of plasma on ammoniagenesis, slices were incubated for 0.5 h in a 1:5 dilution of plasma in Krebs-Ringer bicarbonate buffer (pH 7.4) with glutamine 2.0 mM as substrate. PDG was assayed in homogenates of kidney cortex with glutamine at 1.0 and 20.0 mM [5]. To measure the effect of plasma on PDG, cortical slices were first preincubated in a 1:5 dilution of plasma in Krebs-Ringer bicarbonate buffer without substrate for 0.5 h. Ammonia was measured by the alkaline phenate method [6] and protein as in [7].

3. Results

3.1. *Ammonia production*

Table 1 shows that slices from acutely acidotic rats produced significantly more ammonia ($p < 0.001$)

Table 1
Ammonia production by renal cortical slices from control and acutely acidotic rats and the effect of plasma from control and acidotic rats on ammonia production by normal rats

Cortical slices from		Effect on normal slices of plasma from	
Control	Acidotic rats	Control	Acidotic rats
(μmol . h^{-1} . g dry wt^{-1})		(μmol . 30 min^{-1} . g dry wt^{-1})	
369 ± 14 (20)[a]	439 ± 13 (20)	182 ± 8 (12)	223 ± 3 (12)

[a] Number of slices

Table 2
The effect of acute acidosis on renal glutaminase measured at 1.0 and 20.0 mM glutamine

Glutamine	Glutaminase (nmol . min^{-1} . mg protein^{-1}) Control	Acidotic
1.0 mM	5.2 ± 0.7 (9)[a]	21.1 ± 1.6 (6)
20.0 mM	56.8 ± 4.1 (9)	52.3 ± 1.5 (6)
($V_{1.0\ mM}/V_{max}$) × 100	9 + 1 (9)	40 ± 3 (6)

[a] Number of experiments

and plasma taken from acidotic rats stimulated ammonia production significantly in slices taken from normal rats ($p < 0.001$).

3.2. *Phosphate-dependent glutaminase (PDG) activity*

PDG activity in acutely acidotic rats was not demonstrably higher with saturating concentrations of glutamine, but at 1 mM there was a 4-fold increase in activity (table 2). $V_{1\ mM}$ as a % of V_{max} was also increased 4-fold in the kidneys of acidotic rats. Table 3 shows that when kidney cortical slices were pre-incubated for 0.5 h in plasma and then the PDG assayed, the plasma from acidotic rats caused a 3-fold increase in activity at 1.0 mM glutamine but only a 34% at 20.0 mM glutamine. Again $V_{1.0\ mM}$ as a % of V_{max} was twice as high in the slices pre-incubated in plasma from acidotic rats compared with plasma from control rats.

4. Discussion

These data show that changes in PDG activity in acute acidosis appear when the enzyme is measured at a concentration of substrate approximating that found in vivo. One interpretation of our data is that acute acidosis induces a conformational change in the PDG and this effect of acidosis is mediated through a factor in plasma. This conformational change in PDG clearly precedes the increased enzyme synthesis which occurs with chronic acidosis and may represent the mechanism whereby there is increased renal ammoniagenesis in response to acute acidosis. Other studies of ours on the plasma factor have now established that it is a non-protein substance which is dialysable, is not of adrenal origin and exerts its effect on the kidney by an α-adrenergic like mechanism. The method of inducing metabolic acidosis is not critical. We have shown that administration of HCl rather than NH_4Cl also leads to the appearance of the ammoniagenic factor in plasma (G. A. O. A. et al., submitted). Rats have also been made to swim for 15 min thereby inducing acute lactic acidosis and glutamine metabolism is affected in a manner identical to that seen here. Ammoniagenesis increases, a conformational change in renal PDG is seen and plasma from the rats after swimming stimulates ammoniagenesis from glutamine in cortical slices and produces the same enzymic changes as are seen in the experiments described here (P. H. et al., submitted). Parenthetically, we have found that administration of 400 mM sodium bicarbonate, 4 ml/100 g body wt, does not lead to any changes in the glutaminase.

Table 3
The effect on glutaminase of preincubation of renal cortex in plasma from control and acutely acidotic rats

Glutamine	Glutaminase (nmol . min^{-1} . mg protein^{-1}) after preincubation in plasma from Control rats	Acidotic rats
1.0 mM	18.4 ± 1.4 (6)	54.8 ± 3.4 (10)
20.0 mM	78.8 ± 5.1 (6)	106.0 ± 4.7 (10)
($V_{1.0\ mM}/V_{max}$) × 100	23 ± 2 (6)	52 ± 3 (10)

The activity of PDG in slices preincubated in plasma is higher than in the untreated kidneys: this may indicate the presence of an activator in plasma which increases in acidosis. There is no immunological difference between the glutaminases in kidneys from normal or chronically acidotic rats [8] indicating that in acidosis there is an increased quantity of enzyme. It has been shown however that glutaminase may exist in different forms [9]. During purification a Tris form of the enzyme has been identified with a lower molecular mass and properties different from those of the enzyme equilibrated with a borate phosphate buffer. The Tris form is stimulated 50–100-fold with 150 mM phosphate whereas the borate form is only stimulated 3–30-fold. It is not clear what form of enzyme exists in vivo. One suggestion is that the enzyme is mainly in the form of a monomer and that in vivo there is equilibrium between monomeric and dimeric forms of the enzyme [10]. It has also been proposed that in chronically acidotic Wistar and not Sprague-Dawley rats the enzyme is in a dif-

ferent molecular form or in a different conformational state [10]. On the basis of the marked differences in the properties of the Tris and borate forms of the enzymes a conformational change has been speculated to contribute to the regulation of glutaminase in vivo [9]. There are of course other enzymes for which this mechanism of control has been established [11]. These enzyme changes are usually hormonally-induced through the action of specific messengers such as the cyclic nucleotides. It has not been possible to establish as yet whether the plasma factor we have described is a known hormone and whether its effect is expressed via a known messenger.

Phosphate-dependent glutaminase is tightly bound to the inner mitochondrial membrane and it has been shown that in chronic acidosis increased mitochondrial permeability may contribute to the increase in renal ammoniagenic capacity [10]. We do not think that permeability changes can account for the phenomena we have described since a change in permeability should affect both K_m and V_{max} and we would not have observed increase in $V_{1.0\ mM}/V_{max}$ which was seen in kidneys from acutely acidotic rats or kidney cortices treated with plasma from acutely acidotic rats.

We think that these data provide a new insight into the mechanisms of the ammoniagenic response to acute acidosis in the rat.

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