GLUCONEOGENESIS AND AMMONIAGENESIS IN RAT KIDNEY: EFFECT OF 3-MERCAPTOPICOLINIC ACID

F. I. BENNETT and G. A. O. ALLEYNE*
Department of Medicine, University of the West Indies, Kingston 7, Jamaica, W.I.

Received 7 April 1976

1. Introduction

Metabolic acidosis in the rat results in increased renal gluconeogenesis and ammoniagenesis [1,2]. The site of stimulation for gluconeogenesis is phosphoenol pyruvate carboxykinase (PEPCK, EC 4.1.32) [3] but the rate determining step for ammoniagenesis is not clear. The relationship between the two processes is even less certain and there are studies which show an increase in gluconeogenesis without any change in ammoniagenesis [4,5] and conversely that gluconeogenesis can be blocked without affecting ammoniagenesis [6]. An earlier proposal that enhancement of PEPCK may be important in augmenting ammoniagenesis [3] has been re-emphasised recently by Krebs and Vinay [7]. 3-Mercaptopicolinic acid (3-MPA) is an inhibitor of PEPCK [8–11]. We have therefore used this substance to re-examine the relationship between ammonia and glucose production by the kidney in normal and acidotic rats.

2. Materials and methods

2.1. *Experiments in vivo*

Adult Sprague-Dawley rats were used. Acidosis was produced by tube feeding 3 doses of 400 mM NH_4Cl (2.5 ml/100 gm body weight) at 0800, 2000 and 0800 hours. Control animals received 400 mM NaCl and all rats were sacrificed 2 h after the last feed. Animals were anaesthetized and blood acid–base parameters measured as described previously [5]. In experiments with 3-MPA, rats were fed 100 mg/kg 3-MPA in 1% methyl cellulose or 0.9% NaCl in 1% methyl cellulose: they were anaesthetized 30 min later, the kidneys were rapidly removed, quick frozen and powdered in liquid nitrogen. The powder was extracted in 6% PCA for measurement of intermediates [12].

2.2. *Experiments in vitro*

For measurements of gluconeogenesis and ammoniagenesis in vitro, slices of kidney cortex were cut and incubated in Krebs-Ringer bicarbonate buffer as described previously [5] and glutamine, glutamate, glucose and ammonia were measured in neutralized samples of the medium [12]. At the end of some experiments slices were rapidly removed, quick frozen, powdered and extracted in 6% PCA for measurement of glutamate and aspartate.

3. Results

3.1. *Degree of acidosis*

NH_4Cl produced significant metabolic acidosis, as the blood pH and plasma bicarbonate fell from the control value of 7.37 ± 0.01 and 23.9 ± 0.5 mM (n = 13) to values of 7.09 ± 0.2 and 12.3 ± 1.7 (n = 7).

3.2. *Site of action of 3-MPA*

Intermediates measured 30 min after the 3-MPA was given show an increase in 2-oxoglutarate, malate and aspartate, with a decrease in phosphoenolpyruvate and glycerol-3-phosphate. Glutamine and glutamate were unchanged. There was significant hypoglycaemia at this time (table 1). Glucose production by

* To whom correspondence should be addressed.

Table 1
Effect of 3-MPA in vivo on blood glucose and kidney metabolite levels

	Plasma glucose (mg/100 ml)	Glutamine (μmoles/g protein)	Glutamate (μmoles/g protein)	2-Oxoglutalate (nmoles/g protein)	Aspartate (μmoles/g protein)	Malate (μmoles/g protein)	Phosphoenol pyruvate (nmoles/g protein)	Glycerol-3-phosphate (nmoles/g protein)
Control	129.4 ± 7.3 (3)	4.5 ± 0.4 (5)	10.8 ± 1.0 (5)	486 ± 6.87 (7)	2.9 ± 0.4 (5)	1.1 ± 0.1 (5)	176 ± 20.4 (8)	129.4 ± 7.3 (3)
Merapto-picolinate fed	67.6 ± 0.9[b] (3)	5.3 ± 0.5 (6)	9.5 ± 0.9 (6)	774 ± 87.6[a] (9)	12.1 ± 1.9[b] (6)	9.7 ± 1.2[c] (6)	56 ± 7.5 (8)	67.6 ± 0.9[b] (3)

Values are mean ± s.e.m., for the numbers of observations in parentheses.
[a,b,c] Indicate statistical significance $p = 0.05$, 0.01 and 0.001 respectively, compared with controls.

Table 2
Effect of 3-MPA on gluconeogenesis

Substrate	0 mercaptopicolinate (μmoles glucose/ h g dry wt.)	0.1 mM Mercaptopicolinate (μmoles Glucose/h g dry wt.)
Fructose 2 mM	112.3 ± 4.9 (17)	96.6 ± 8.9 (9)
Dihydroxy-acetone 2 mM	127.3 ± 10.6 (8)	117.5 ± 7.3 (10)
Malate 2 mM	83.8 ± 3.6 (18)	53.7 ± 3.4[c] (9)
Oxogluterate 2 mM	46.7 ± 2.8 (6)	22.3 ± 2.2[c] (6)
Fumarate 2 mM	107.1 ± 7.4 (12)	44.5 ± 2.3[c] (12)

Values are mean ± s.e.m., for the numbers of observations in parentheses.
[c] Indicates statistical significance $p < 0.001$ compared with controls.

cortical slices from fructose and dihydroxyacetone was not inhibited by 3-MPA while that from oxoglutarate, fumarate and malate was significantly inhibited (table 2). These two sets of data show that 3-MPA acts at the PEPCK step to inhibit renal gluconeogenesis.

3.3. *Effect of 3-MPA on gluconeogenesis and ammoniagenesis*

With glutamine as substrate, 3-MPA inhibited ammoniagenesis (23%) and gluconeogenesis (82%). Kidney cortex slices from acidotic rats showed a significant increase in ammonia and glucose production, both of which were inhibited by 3-MPA. However, even with 3-MPA, slices from acidotic rats still produced more ammonia and glucose than slices from controls (table 3). Glutamate output was unaffected by 3-MPA.

3.4. *Glutamine uptake and source of ammonia*

The contribution of the amide and amino groups of glutamine to net ammonia production was calculated [13]. Kidney slices from acidotic rats showed

Table 3
Effect of 0.5 mM 3-MPA on ammonia, glucose and glutamate production from 2 mM glutamine

	Control		Acidotic	
	0 MPA	0.5 mM MPA	0 MPA	0.5 mM MPA
Total (μmoles NH_3/h g dry wt.)	373.0 ± 12.6 (34)	287.1 ± 8.2 (32)	535.4 ± 20.1 (32)	380.8 ± 13.5 (32)
Total (μmoles glucose/h g dry wt.)	38.0 ± 1.7 (35)	6.7 ± 1.2 (15)	78.6 ± 4.0 (36)	13.6 ± 1.4 (34)
Total (μmoles glutamate/h g dry wt.)	66.4 ± 4.2 (31)	76.4 ± 3.1 (32)	71.9 ± 5.9 (33)	75.3 ± 3.3 (34)

Values are mean ± s.e.m. for the numbers of observations in parentheses.

Table 4
Effect of 3-MPA on the contribution of the amide and amino groups of glutamine (2 mM) to ammoniagenesis

Slices	Net ammonia production (μmoles/h g dry wt.)	Glutamine uptake (amide NH_3) (μmoles/h g dry wt.)	Amino NH_3 (μmoles/h g dry wt.)
Control	341.2 ± 13.9 (34)	219.2 ± 10.3 (34)	122.0 ± 12.2 (34)
Control in 0.5 mM MPA	257.9 ± 8.2 (32)	197.8 ± 7.9 (32)	60.1 ± 7.1 (32)
Acidotic	503.6 ± 20.4 (31)	301.1 ± 9.1 (31)	202.5 ± 18.6 (31)
Acidotic in 0.5 mM MPA	354.6 ± 13.7 (32)	240.2 ± 9.0 (32)	114.4 ± 13.5 (32)

Values are mean ± s.e.m., for the numbers of observations in parentheses.

enhanced uptake of glutamine (table 4). 3-MPA did not affect glutamine uptake by slices from control rats whereas this was significantly inhibited in slices from acidotic rats. In control slices, depression of ammonia formation was due entirely to a reduction in amino ammonia while in acidotic slices there was reduction in deamidation and deamination with the latter being more severely depressed.

3.5. *Glutamate and aspartate in slices*

Glutamate and aspartate levels in slices incubated without 3-MPA were 12.2 ± 0.6 (13) and 3.0 ± 0.4 (8) μmol/mg Protein. In the presence of 3-MPA the glutamate levels did not change, 12.4 ± 0.7 (13) but aspartate rose to 10.5 ± 0.9 (10).

4. Discussion

Our studies in vivo and in vitro confirm that 3-MPA produces hypoglycemia and inhibits renal PEPCK. The metabolic profile we have found in the kidney of rats given 3-MPA is similar to that described for the liver [9,10]. We were unable to measure oxaloacetate but the increased levels of aspartate found probably reflect an increased level of oxaloacetate.

3-MPA reduced the amount of ammonia formed from the amino group of glutamine, and this was linked with a reduction in gluconeogenesis. Glutamate derived from glutamine probably has oxidation or gluconeogenesis as its major fate. In slices from control animals glutamate formation from glutamine remained unchanged with 3-MPA (table 3) but the amount of glutamate which was deaminated fell (table 4). In slices with or without 3-MPA the glutamate deaminated was 60 versus 120 μmol/h g dry wt. Glucose production in similar conditions was 6.7 and 38.0 μmol/h g dry wt. Therefore, with or without 3-MPA the amount of glutamate used in non-gluconeogenic pathways was the same (47 and 46 μmol/h g). This indicates that it is the reduction in utilization of glutamate for gluconeogenesis which is responsible for the reduction in ammoniagenesis.

Krebs and Vinay [7] have proposed that the increased ammonia production in acidosis is related to the stimulation of gluconeogenesis at the PECPK step. A decrease in 2-oxoglutarate leads to a displacement of glutamate dehydrogenase (EC 1.4.1.3) from equilibrium with resultant increased glutamate oxidation. Our data are in agreement with this, since in the reverse situation when there was inhibition of PEPCK we found elevated 2-oxoglutarate levels and decreased formation of ammonia from the glutamate derived from glutamine.

In slices from acidotic animals 3-MPA actually depressed glutamine uptake and the NH_3 production from both amide and amino groups was reduced. It has been shown in the acidotic rat that there is increased glutaminase activity as well as increased

transmitochondrial transport of glutamine. Our studies so far do not indicate which if either of these steps is affected by 3-MPA.

Acknowledgements

We thank Dr H. C. Saunders of Smith, Kline and French, Philadelphia, USA for a generous gift of 3-mercaptopicolinic acid. This work was supported by a grant from the Medical Research Council of Great Britain. We also thank Dr Derek Williamson for reviewing the manuscript critically.

References

[1] Goodman, A. D., Fuisz, R. E. and Cahill, G. F. Jr. (1966) J. Clin. Invest. 45, 612–619.
[2] Goorno, W. E., Rector, F. C. jr. and Seldin, D. W. (1967) Am. J. Physiol. 213, 969–974.
[3] Alleyne, G. A. O. and Scullard, G. H. (1969) J. Clin. Invest. 48, 364–370.
[4] Churchill, P. C. and Malvin, R. L. (1970) Am. J. Physiol. 218, 241–245.
[5] Bennett, F. I., Alexander, J. E., Roobol, A. and Alleyne, G. A. O. (1975) Kidney International 7, 380–384.
[6] Preuss, H. G. (1969) Nephron 6, 235–246.
[7] Krebs, H. A. and Vinay, P. (1975) in: Medical Clinics of North America 59, 595–603.
[8] Di Tullio, N. W., Berkoff, C. E., Blank, B., Kostos, V., Stack, E. J. and Saunders, H. L. (1974) Biochem. J. 138, 387–394.
[9] Blacksheer, P. J., Holloway, P. A. H. and Alberti, K. G. M. M. (1975) Biochem. J. 18, 353–362.
[10] Goodman, M. N. (1975) Biochem. J. 150, 137–139.
[11] Kostos, V., D. Tullio, N. W., Rush, J., Cieslinski, I., Saunders, H. L. (1975) Arch. Biochem. Biophys. 171, 459–465.
[12] Bergmeyer, H. U. (ed.) (1974) Methods of Enzymatic Analysis Vols 3 + 4 Academic Press, New York.
[13] Preuss, H. G. (1971) Am. J. Physiol 220(1) 54–58.