INHIBITION OF UREA SYNTHESIS BY 4-PENTENOIC ACID

Allen M. Glasgow and H. Peter Chase

University of Colorado Medical Center
Department of Pediatrics
R F. Stolinsky Laboratories
Denver, Colorado

Received November 19,1974

SUMMARY: 4-Pentenoic acid (1 mM) inhibited ureagenesis approximately 70% in rat liver slices with ammonia as substrate. Inhibition of ureagenesis was much less with aspartate and citrulline as substrates. The block in ureagenesis was not reversed by the addition of glucose, glutamate or N-acetyl glutamate to the medium. There was no direct inhibition of either carbamyl phosphate synthetase or ornithine carbamyl transferase by 4-pentenoic acid. The inhibition of ureagenesis probably accounts for the elevated plasma ammonia in rats injected with 4-pentenoic acid.

4-Pentenoic acid is an analog of hypoglycin the compound thought to cause Jamaican vomiting sickness (1,2). 4-Pentenoic acid is a potent inhibitor of fatty acid oxidation (3). It also causes hypoglycemia in several species primarily because of impaired gluconeogenesis which in turn is probably secondary to impaired fatty acid oxidation (4,5).

We have recently shown (6) that 4-pentenoic acid administered to rats produces many of the essential features of Reye's syndrome, an increasingly recognized, often fatal encephalopathy of children (7). As part of these studies it was found that giving 200 mg/kg of 4-pentenoic acid intraperitoneally to rats results in a 4 fold elevation in plasma ammonia in 20-25 minutes (6) We report here the effects of 4-pentenoic acid on ureagenesis in vitro.

METHODS

Adult male Sprague-Dawley rats that had been allowed free access to Purina Rat Chow were decapitated, the livers rapidly removed and sliced with the aid of a Stadie Riggs microtome. To measure urea production, liver slices weighing 100-150 mg were incubated in 5 ml Krebs ringer phosphate buffer, pH = 7.2, gassed with 100% oxygen and shaken at 120 cycles/min at 37 °C. Substrates and other compounds were added as indicated in the Table. Two samples were

taken for baseline urea concentration, and another two samples, to which 10 µMoles urea was added, were used for standards. The reaction was terminated by the addition of 0.5 ml of 3 M perchloric acid. The tissue was homogenized in the medium and the protein removed by centrifugation. An aliquot of supernatant was neutralized with an appropriate volume of 3 M KOH, and the potassium perchlorate removed by centrifugation. In experiments in which ammonia was not a substrate (See Table) the resultant supernatant was used for the assay of urea.

When ammonia was a substrate, 2 ml neutralized supernatant was passed through a 0.5 x 5 cm column (Pasteur pipette) of BIO REX 70 resin, Na^+ form, followed by water until 5 ml of effluent was collected. Blanks and standards were similarly treated. An aliquot (50 µl if not passed through a column; 100 µl if passed through a column) was added to 0.2 ml urease (Type III from Sigma; 0.5 mg/ml in 0.01 M $NaPO_4$ buffer, pH 7.0, checked daily to make sure hydrolysis was complete) and incubated 30 min at 37 °C. Then 1 ml phenol reagent (8) and 1 ml hypochlorite reagent (8) were added and after a further 30 min at 37 °C the absorbancy at 640 mµ was measured. Correction was made for the absorbancy with water in the place of enzyme and the absorbancy due to the enzyme without substrate. The recovery of the standard averaged 92% (range 87-94%).

Urea cycle enzymes were assayed in duplicate using liver from one rat homogenized in 0.1% cetyltrimethylbromide. For carbamyl phosphate synthetase (ammonia) (EC 2·7·2·5) 1.0 ml assay medium contained 100 µMoles tris buffer pH 8.0, 20 µMoles ATP, 20 µMoles $MgCl_2$, 16 µMoles reduced glutathione, 80 µMoles $KHCO_3$, 20 µMoles NH_4Cl, 20 µMoles L-ornithine, 20 µMoles N-acetyl glutamate, 0.2 µCi D-L ornithine ^{14}C, 400 units (µMoles/hr) ornithine carbamyl transferase (EC 2·1·3·3) and 100 µl of 1:10 liver homogenate. Incubation was at 37 °C for 20 minutes. N-acetyl glutamate was omitted from the blank. For ornithine carbamyl transferase 1.0 ml assay medium containing 50 µMoles glycylglycine pH 8.3, 8.3 µMoles carbamyl phosphate, 10 µMoles L-ornithine, 1 µCi D-L ornithine ^{14}C and 100 µl of a 1:100 liver homogenate was incubated 5 minutes at 37 °C. A blank contained homogenate boiled for 3 minutes. Both reactions were stopped with

0.1 ml 90% formic acid. An aliquot was spotted on Whatman #3 paper and run overnight with butanol, acetic acid, water (12:3:5). Spots corresponding to ornithine and citrulline, located by staining parallel runs of standard, were cut out and counted by liquid scintillation.

RESULTS AND DISCUSSION

4-Pentenoic acid at a final concentration of 1 mM consistently inhibited ureagenesis (Table).

4-Pentenoic acid inhibited ureagenesis much less with aspartate and citrulline as substrates than with ammonia as substrate (Table). This suggests that

TABLE : UREA PRODUCTION*

Substrate	Rat Number	No 4-Pentenoic	1 mM 4-Pentenoic	Mean % inhibition
10 mM NH_4^+	1	25.2	11.3	65
	2	20.2	6.0	
	3	35.5	10.8	
10 mM aspartate and 10 mM citrulline	1	24.2	17.2	20
	2	31.2	28.1	
	3	26.4	20.5	
10 mM NH_4^+ and 2 mM ornithine	1	37.1	16.4	67
	2	40.3	11.2	
	3	43.2	11.7	
10 mM NH_4^+	4	41.5	10.3	74
	5	33.8	9.3	
10 mM NH_4^+ and 10 mM glucose	4	35.5	10.3	69
	5	37.9	12.9	
10 mM NH_4^+	6	39.3	9.6	76
	7	38.6	9.0	
10 mM NH_4^+ and 10 mM N-acetyl glutamate	6	41.5	14.5	71
	7	53.8	12.0	
10 mM NH_4^+	8	39.3	10.9	69
	9	26.8	9.1	
10 mM NH_4^+ and 10 mM glutamate	8	32.1	11.6	64
	9	28.7	10.3	

* Liver slices with and without 4-pentenoic acid were incubated as described in Methods. Results are expressed as net production of urea in μMoles/g wet weight liver during a 2 hour incubation.

the major block precedes the argininosuccinate synthetase step.

The activity of ornithine carbamyl transferase and carbamyl phosphate synthetase were 9165 and 564 μMoles/g liver/hr respectively in the absence of 4-pentenoic acid and 8640 and 586 μMoles/g liver/hr in the presence of 1 mM 4-pentenoic acid. Thus the block in ureagenesis is probably due to substrate depletion, however, a block in transport (e.g., citrulline across the mitochondrial membrane) or a direct enzyme inhibition by a metabolite of 4-pentenoic acid have not been excluded.

That the defect is not due to a deficiency of ornithine is strongly suggested by the fact that added ornithine does not restore ureagenesis in the presence of 4-pentenoic acid (Table). Thus, the inhibition of ureagenesis by 4-pentenoic acid is probably due to depletion of one of the substrates for the carbamyl phosphate synthetase step. ATP of N-acetyl glutamate would seem most likely. Furthermore, the possibility that depletion of either ATP or N-acetyl glutamate accounts for the block is appealing because depletion of either might be accounted for by the known effects of 4-pentenoic acid. Thus one might postulate that 4-pentenoic acid inhibits fatty acid oxidation resulting in reduced tissue ATP or that the effect of 4-pentenoic acid in depleting acetyl CoA (4) in turn results in depleted N-acetyl glutamate.

However, 1 mM 4-pentenoic acid only reduced ATP levels about 20% in perfused rat livers(4). Furthermore, if the inhibition of fatty acid oxidation resulted in reduced ATP which in turn resulted in reduced ureagenesis one might expect that addition of glucose to the medium might reverse this effect. This is not the case (Table).

4-Pentenoic acid has previously been shown to cause a marked reduction of acetyl CoA levels in perfused rat liver, reducing levels from about 34 to about 4 mμMoles/g wet weight (4). Neither N-acetyl glutamate nor glutamate reverse the inhibition or ureagenesis caused by 4-pentenoic acid (Table). However, N-acetyl glutamate may not cross membranes and glutamate might not increase the synthesis of N-acetyl glutamate. Thus the possibility that 4-pen-

tenoic acid inhibits ureagenesis by depleting N-acetyl glutamate has not been excluded. If this is the case then the turnover of N-acetyl glutamate must be more rapid than the previously estimated half life of a few hours (9).

The inhibition of ureagenesis by 4-pentenoic acid probably accounts for the elevated plasma ammonia caused by 4-pentenoic acid in vivo. Since 4-pentenoic acid impairs fatty acid oxidation it may increase the metabolism of amino acids as an energy source. However, it is unlikely that the increased flux of ammonia that might result would cause an elevated plasma ammonia in an animal with an intact urea cycle.

ACKNOWLEDGMENT

This work was supported by NIH Training Grant HD-65.

REFERENCES

1. Sherratt, H.S.A. (1969) Br. Med. Bull. 25, 250-255.
2. Bressler, R., Corredor, C., Brendel, K. (1969) Pharmacol. Rev. 21, 105-130.
3. Holland, P.C., Sherratt, H.S.A. (1973) Biochem. J. 136, 157-171.
4. Toews, C.J., Lowy,C., Ruderman, N.B. (1970) J. Biol. Chem. 245, 818-824.
5. Senior, A.E., Sherratt, H.S.A. (1968) Biochem. J. 110, 521-527.
6. Glasgow, A.M., Chase, H.P. (1974) Pediatr. Res. 8, 432.
7. Reye, R.D.K., Morgan, G., Baral, J. (1963) Lancet 2, 749-752.
8. Glasgow, A.M., Dhiensiri, K. (1974) Clin. Chem. 20, 642-644.
9. Shigesada, K., Tatibana, M. (1971) J. Biol. Chem. 246, 5588-5595.