INHIBITION OF LINOLEIC ACID DEGRADATION BY HYPOGLYCIN A

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1. Introduction

Unripe fruits and seeds of ackee (*Blighia sapida*) grown in Jamaica contain the plant toxin hypoglycin A [1]. It has been shown that ingestion of this substance causes the Jamaican vomiting sickness [2,3]. The most pronounced clinical symptom of this disease is severe hypoglycemia, followed by coma and death [4]. Extensive biochemical studies revealed methylenecyclopropylacetyl CoA, a metabolite of hypoglycin A, to be the actual toxic compound [5]. It has been reported to inhibit dehydrogenases involved in amino acid degradation [6,7] as well as those which are part of the β-oxidation system [8,9].

Massive urinary excretion of dicarboxylic acids with 5–10 carbon atoms in hypoglycin-treated rats has been observed [10]. Most of these compounds have also been detected in the urine of cases of vomiting sickness [4]. Some of these dicarboxylic acids have been proposed to accumulate due to the inhibition of fatty acid degradation [10]. However, significant radioactivity was not found in any of the urinary metabolites after simultaneous administration of hypoglycin A and [U-^{14}C]palmitic acid or [U-^{14}C]oleic acid [10].

In the present paper we describe results of experiments which show that 4*cis*-decene-1,10-dioic acid, the main urinary metabolite, as well as 4*cis*-octene-1,8-dioic acid arise from linoleic acid.

2. Materials and methods

Male albino Wistar rats (200–250 g) which had been deprived of food for 12 h were given intramuscular injection of hypoglycin A in a single dose of 10 mg/100 g body wt. Some rats were subsequently injected intravenously with [U-^{14}C]stearic acid, [U-^{14}C]oleic acid or [U-^{14}C]linoleic acid (5 μCi/mg) in form of a complex with 5% bovine serum albumin in saline. Control rats were given a similar volume of saline. The rats were deprived of food for the duration of the experiment. Urine specimens were collected after 24 h individually from rats in metabolic cages. Bacterial growth was prevented by adding toluene.

Extraction of organic acids from aliquots of a 24 h specimen and subsequent methylation was carried out as in [10]. The resulting methyl esters were further purified by thin-layer chromatography (TLC) on silica gel H using hexane : acetone (8:2) as developing system. The fraction containing the methyl esters of dicarboxylic acids was analyzed by radiogas chromatography. Details of radiogas chromatography are in [11]. The structures of the individual esters were identified by combined gas chromatography–mass spectrometry (GC–MS).

Purified hypoglycin A was a gift from Dr C. von Holt, Department of Biochemistry, University of Cape Town. [U-^{14}C]Stearic acid, [U-^{14}C]oleic acid as well as [U-^{14}C]linoleic acid were obtained from NEN Chemicals GmbH (Dreieich).

3. Results and discussion

The similarities of the structure of the 3 unsaturated urinary metabolites with parts of those of linoleic acid and linolenic acid (fig.1) prompted us to consider

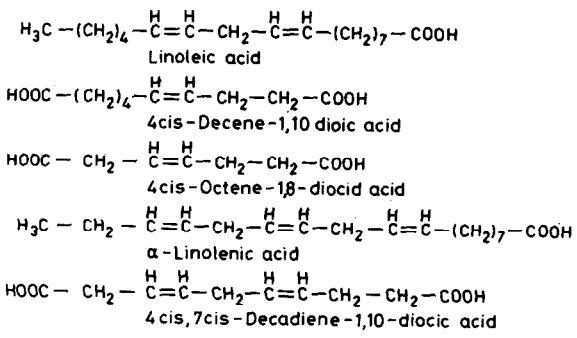
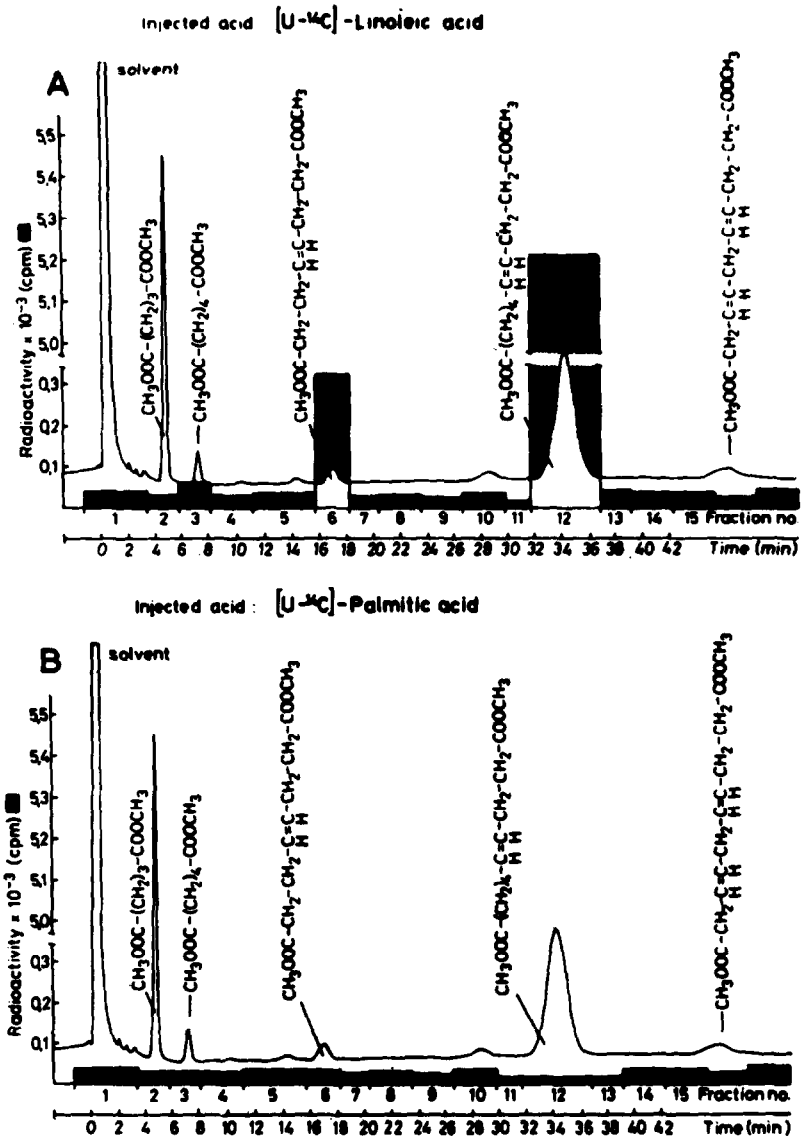


Fig.1. Comparison of the structure of three unusual urinary metabolites with those of linoleic and α-linolenic acid.

the possibility of a biogenic relationship. The radiogas chromatograms of fig.2 strengthen this assumption. Injection of [U-^{14}C]linoleic acid into hypoglycin A-treated rats led to 2 radioactive urinary metabolites, 4*cis*-decene-1,10-dioic acid and 4*cis*-octene-1,8-dioic acid. The almost identical specific radioactivities of



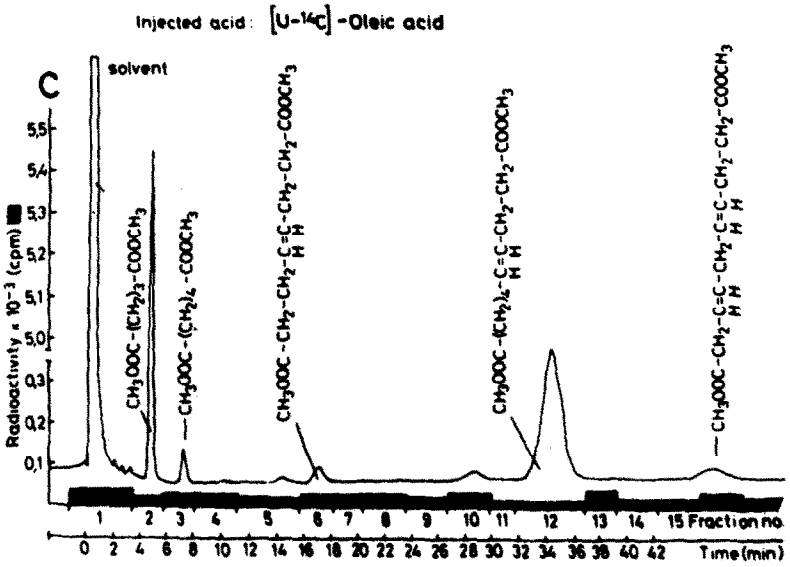


Fig.2. Radiogas chromatograms of aliquots of the organic dicarboxylic acids extracted from urine of rats after simultaneous administration of hypoglycin A and a [U-^{14}C]fatty acid. (A) [U-^{14}C]Linoleic acid. (B) [U-^{14}C]Stearic acid. (C) [U-^{14}C]Oleic acid.

the two compounds (5190 cpm/756 mm^2 peak area and 350 cpm/47 mm^2 peak area) suggest a precursor–product relationship. In the case of [U-^{14}C]stearic and [U-^{14}C]oleic acid injected together with hypoglycin A into rats the urine contained the same unusual metabolites, but none of them possessed significant amounts of radioactivity. Whereas the control rats (no hypoglycin A) excreted none of these compounds in detectable amounts.

These observations are not consistent with an initial proposal for the mechanism of inhibition of fatty acid oxidation by hypoglycin (reviewed [12]). This assumption centered around the concept of hypoglycin A preventing the translocation of long chain fatty acids from cytosol into mitochondria due to decreased concentrations of free carnitine and coenzyme A. However, our results confirm the findings of Tanaka [10] who failed to verify his hypothesis that the unusual urinary compounds possessing one double bond are metabolites of oleic acid. The data presented in this paper strongly suggest the following conclusions:

1. The toxic metabolite of hypoglycin A inhibits an enzyme which is specific for the degradation of linoleic acid. This enzyme is not required for the oxidation of stearic and oleic acid.
2. According to the structure of the unusual metabolites the inhibition must occur at the level of a chain length of 10 carbon atoms.
3. The metabolism of the first double bond of linoleic acid (originally at position 9) via isomerisation of 3*cis*, 6*cis*-dodecadienoyl-CoA to 2*trans*, 6*cis*-dodecadienoyl-CoA with subsequent β-oxidation to 4*cis*-decenoyl-CoA is not inhibited.

These conclusions are in agreement with recent results from our in vitro studies concerning β-oxidation of unsaturated fatty acids in bovine liver mitochondria [11,13]. It has been demonstrated that two enzymes, acyl-CoA dehydrogenase II (EC 1.3.99) [11,13] and 4-enoyl-CoA reductase (EC 1.3.1) [11], are specifically required for the degradation of 4*cis*-decenoyl-CoA. Inhibition of one of the enzymes followed by ω-oxidation of 4*cis*-decenoyl-CoA should directly lead to one of the urinary metabolites 4*cis*-decene-1,10-dioic acid (fig.3). Activation of the new carboxyl group and one subsequent β-oxidation cycle would give rise to the second detected unusual metabolite of linoleic acid, 4*cis*-octene-1,8-dioic acid. The double bond of this compound is symmetrically located in the middle of the molecule, separated from both activated carboxyl groups by two carbon atoms (position 4). Thus, further β-oxidation at both end

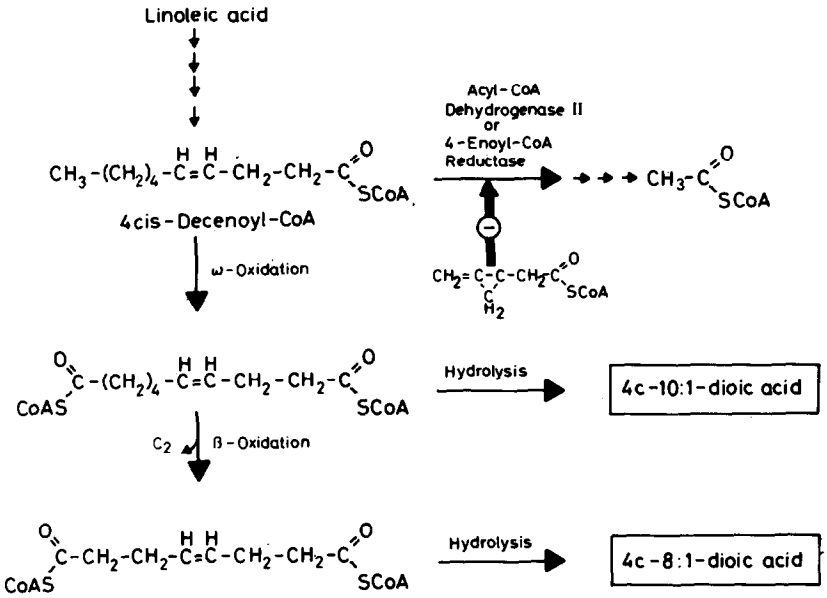


Fig.3. Proposed pathway for the formation of 4*cis*-decene-1,10-dioic (4*c*-10:1-dioic acid) and 4*cis*-octene-1,8-dioic acid (4*c*-8:1-dioic acid) from linoleic acid.

would require the specific enzymes mentioned above.

Results which complement and support the present findings are in [9]. From inhibition studies employing a crude mixture of acyl-CoA dehydrogenases (EC 1.3.99.3) from rabbit liver it was concluded that methylenecyclopropylacetyl-CoA severely inhibits not only acyl-CoA dehydrogenase I (butyryl-CoA dehydrogenase, EC 1.3.99.2) but in addition acyl-CoA dehydrogenase II (octanyl-CoA dehydrogenase, EC 1.3.99).

According to the proposed pathway for the formation of the two unusual metabolites of linoleic acid (fig.3), the data reported here present the first, although indirect, evidence for 4*cis*-decenoyl-CoA being an intermediate of linoleic acid degradation in vivo. This has been inferred so far only from in vitro studies [14].

In addition, our interpretation of the presented data suggest further that 4*cis*, 7*cis*-decadiene-1,10-dioic acid should be a metabolite of linolenic acid.

The results reported here support the view that the influence of hypoglycin A on the metabolism of unsaturated fatty acids contributes to its toxic effect and will help to gain deeper insight into the enzymology of β-oxidation.

Acknowledgements

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References

[1] Hassall, C. H., Reyle, K. and Feng, P. (1954) Nature 173, 356–357.
[2] Chen, K. K., Anderson, R. C., McCowen, M. C. and Harris, P. N. (1957) J. Pharmacol. Exp. Ther. 121, 272–285.
[3] Feng, P. C. and Patrick, S. J. (1958) Brit. J. Pharmacol. 13, 125–130.
[4] Tanaka, K., Kean, E. A. and Johnson, B. (1976) New. Engl. J. Med. 295, 461–467.
[5] Von Holt, C., Von Holt, M. and Böhm, H. (1966) Biochim. Biophys. Acta 125, 11–21.

[6] Tanaka, K., Miller, E. M. and Isselbacher, K. J. (1971) Proc. Natl. Acad. Sci. 68, 20–24.
[7] Tanaka, K. (1975) in: Hypoglycin (Kean, E. A. ed) pp. 67–92, Academic Press, New York.
[8] Osmundsen, H. and Sherratt, H. S. A. (1975) FEBS Lett. 55, 38–41.
[9] Kean, E. A. (1976) Biochim. Biophys. Acta (1976) 422, 8–14.
[10] Tanaka, K. (1972) J. Biol. Chem. 247, 7465–7478.
[11] Kunau, W. H. and Dommes, P. (1978) Eur. J. Biochem. in press.
[12] Manchester, K. L. (1974) FEBS Lett. 40, 133–139.
[13] Dommes, V. and Kunau, W. H. (1978) J. Biol. Chem. submitted.
[14] Stoffel, W. (1966) Naturwissensch. 53, 621–630.