KETOGENIC ACTION OF FRUCTOSE IN THE ISOLATED PERFUSED RAT AND GUINEA PIG LIVER

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1. Introduction

During studies with fructose as a precursor for gluconeogenesis in isolated perfused livers from rats and guinea pigs a significant rate of ketogenesis was observed which could not be detected with other gluconeogenic precursors (glycerol, pyruvate, lactate) or with glucose. The data presented in this paper show that fructose enhances the formation of acetyl-*S*-CoA from pyruvate by stimulating pyruvate oxidation. The stimulation of pyruvate oxidation results from an increased activity of pyruvate dehydrogenase due to the fructose induced fall in the concentrations of adenine nucleotides.

2. Methods

Isolated livers from 48 hr starved male Wistar rats (220–250 g) and male guinea pigs (320–350 g) were perfused with an artificial medium containing washed beef red cells as described earlier [1]. The experiments were started 45 min after connecting the liver to the artificial circulation. Fructose was added to the medium at the beginning of the experiment to give a final concentration of 20 mM. In the experiments with oleate the fatty acid was added as an albumin oleate complex [2] to give a final concentration of 2 mM.

Fructose, glucose, lactate, pyruvate, 3-hydroxybutyrate, aceto-acetate, ATP, ADP, and AMP were determined enzymic-optically [3]. Acetyl-*S*-CoA was measured according to Wieland and Weiss [4] with the modification described earlier [1]. Radioactive ketone bodies were isolated and counted according to Mayes and Felts [5] and $^{14}CO_2$ was collected and counted as described earlier [1]. U-^{14}C-fructose and 1-^{14}C-pyruvate were purchased from the Radiochemical Centre Amersham/England.

Table 1

Net formation of ketone bodies by isolated perfused livers from 48 hr starved *guinea pigs* under various conditions. Fructose, glycerol, pyruvate and lactate were added to the medium at the beginning of the experiments. The duration of perfusion was 60 min.

Substrate (mM)	Net formation of total ketone bodies (nmoles/g liver/min)	*n*
No external substrate	190 ± 70	18
Fructose (20)	370 ± 10	5
Glycerol (10)	90 ± 50	5
Pyruvate (20)	70 ± 30	6
L-Lactate (20)	210 ± 40	10

3. Results and discussion

Ketogenesis rose significantly in the presence of fructose as well in rat as in guinea pig liver (tables 1 and 2). In experiments with guinea pig livers the rate of ketogenesis was also determined in the presence of lactate, pyruvate and glycerol. Only in the presence of fructose was the rate of ketogenesis higher compared with endogenous ketogenesis (table 1).

In the presence of 2 mM oleate on the other hand, fructose inhibited ketogenesis (table 2).

MICA (5-methoxy-2-indolecarbonic acid) which is known to inhibit pyruvate oxidation and pyruvate carboxylation [6], abolished ketogenesis in the pres-

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Table 2
Net formation of ketone bodies by isolated perfused livers from 48 hr starved *rats*. In the experiments with oleate, the fatty acid–albumin complex. The duration of the perfusion was 60 min.

Substrate (mM)	Net formation of total ketone bodies (nmoles/g liver/min)	*n*
No external substrate	73 ± 21	10
Fructose (20)	231 ± 56	8
Oleate (2)	1950 ± 140	3
Fructose (20) + oleate (2)	1050 ± 40	3

ence of fructose but not in the presence of oleate (fig.1). This made it likely that the carbon of the ketone bodies produced in presence of fructose was derived from fructose itself and not from endogenous fatty acids. To prove this, U-^{14}C-fructose was added to the medium together with cold fructose, and the incorporation of radioactivity into the ketone bodies formed in presence of fructose was measured. The ratio of the specific activities of fructose and total ketone bodies was close to unity (table 3). This means that the ketone bodies were derived from fructose. The higher rate of ketogenesis in

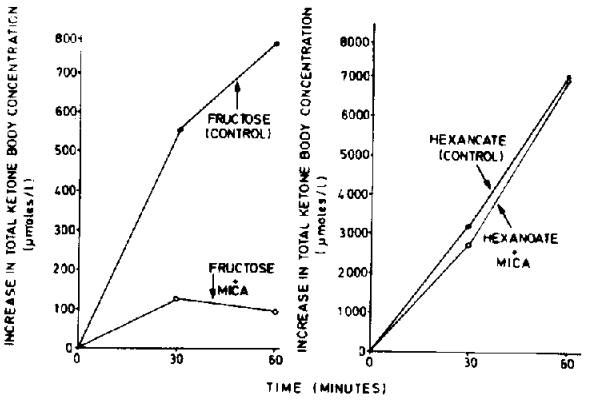

Fig. 1. Effects of 5-methoxy-2-indole carbonic acid (MICA) on the net formation of total ketone bodies from fructose or from hexanoate by isolated perfused livers from 48 hr starved rats. Fructose was added to the perfusion medium at zero time to give a final concentration of 20 mM. Hexanoate was infused intraportally at a rate of 0.72 mmole/hr. The concentration of MICA was 0.1 mM in all experiments. The points are mean values from 3 experiments under each condition.

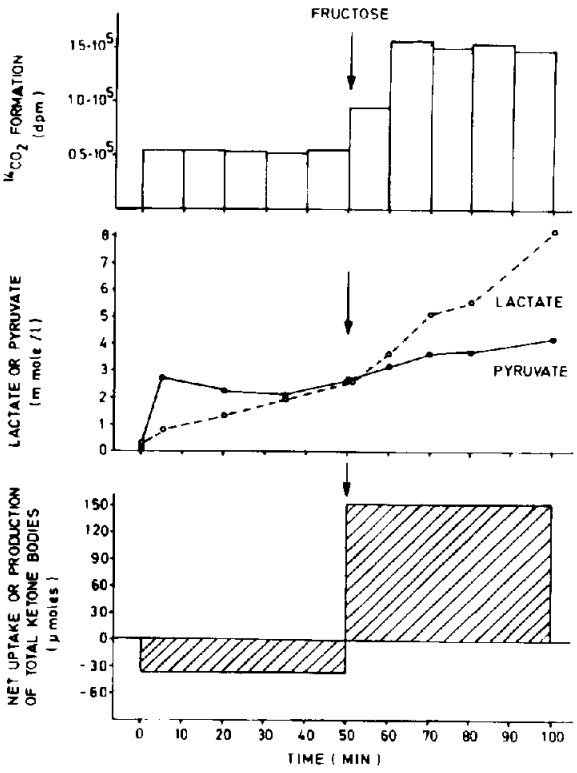

Fig. 2. Formation of $^{14}CO_2$ from 1-^{14}C-pyruvate, concentrations of lactate and pyruvate in the perfusion medium, and net balance of ketone bodies (cumulative) in experiments with isolated perfused rat livers. A priming dose of 1-^{14}C-pyruvate was given at zero time to give an initial concentration of 5 mM. This was followed by a constant intraportal infusion of 1 mmole/hr of 1-^{14}C-pyruvate of the same specific activity throughout the experiment. After 50 min (arrow) cold fructose (20 mM final concentration) was added. The ketone body balance refers to the total amount of ketone bodies taken up within the first 50 min or being produced during the second 50 min.

the presence of fructose was paralleled by an increase in the concentration of acetyl-*S*-CoA (table 4) and a fall in the concentration of adenine nucleotides (table 4). The concentration of ATP fell by more than 50% while the ATP/ADP ratio showed only a moderate decrease.

At the time when these experiments were done, we learned about the finding of Raivio et al. [7] who observed an increased breakdown of adenine nucleotides in the presence of fructose in rat liver. Their results fit very well with ours.

Table 3

Incorporation of radioactivity from U-^{14}C-labelled fructose (20 mM) into total ketone bodies by isolated perfused livers drom 48 hr starved rats. For calculation of the specific radioactivity of carbon from ketone bodies the measured values were corrected from the amount of cold ketone bodies present in the perfusion medium before the addition of fructose. The values given are mean values from 2 experiments.

Time	Radioactivity in total ketone bodies	Specific radioactivity in total ketone bodies	Specific radioactivity per μg atom carbon from fructose / Specific radioactivity per μg atom carbon from ketone bodies
(min)	(dpm)	(dpm/μg atom carbon)	(ratio)
5	199072	2621	1.21
30	372270	3478	0.89
60	692220	3243	0.96

Table 4

Concentrations of ATP, ADP, AMP, and acetyl-*S*-CoA in livers from 48 hr starved rats. The livers had been perfused in the presence of either 20 mM glucose or 20 mM fructose for 60 min. Liver tissue was obtained by freeze clamping.

	ATP	ADP	AMP	ATP/ADP	ATP+ADP+AMP	Acetyl-*S*-CoA	*n*
	(μmole/g liver)			(ratio)	(μmole/g liver)	(nmole/g liver)	
Glucose	2.340 ± 0.110	0.870 ± 0.120	0.106 ± 0.012	2.75 ± 0.23	3.503 ± 0.264	45.56 ± 6.09	5
Fructose	1.090 ± 0.080	0.550 ± 0.021	0.113 ± 0.010	1.90 ± 0.18	1.760 ± 0.094	76.71 ± 6.45	6

Linn et al. [8] (see also Wienland and v. Jagow-Westermann [9]) have described an inactivation of pyruvate dehydrogenase by phosphorylation with ATP as substrate and a specific kinase. The inactivated enzyme could be reactivated in presence of high concentrations of Mg^{2+} by a specific phosphatase [9]. We therefore checked the effect of fructose on the oxidation of 1-^{14}C-pyruvate by the isolated perfused rat liver. As can be seen from fig. 1 the rate of $^{14}CO_2$ for mation rose to about 300% when fructose was added to the medium although the radioactive lactate–pyruvate pool became considerably diluted with cold lactate and pyruvate generated from fructose. We conclude from these results that fructose enhances the formation of acetyl-*S*-CoA and ketone bodies by an activation of pyruvate dehydrogenase due to a decrease in the concentrations of ATP and ADP.

Further work is in progress to measure the active and inactive form of pyruvate dehydrogenase directly in presence and absence of fructose.

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References

[1] H.D. Söling, B. Willms, D. Friedrichs and J. Kleineke, European J. Biochem. 4 (1968) 364.
[2] B.D. Ross, R. Hems, R.A. Freedland and H.A. Krebs, Biochem. J. 105 (1967) 869.
[3] Methoden der enzymatischen Analyse, ed. H.U. Bergmeyer (Verlag Chemie, Weinheim, 1962).
[4] O. Wieland and L. Weiss, Biochem. Biophys. Res. Commun. 10 (1963) 333.
[5] P.K. Mayes and J.M. Felts, Nature 215 (1967) 716.
[6] R.L. Hanson, P.D. Ray, P. Walter and H.A. Lardy, Biochemistry 6 (1969) 4351.

[7] P.H. Mäenpää, K.O. Raivio and M.P. Kekomäki, Science 161 (1968) 1253;
[8] K.O. Raivio, M.P. Kekomäki and P.H. Mäenpää, Biochem. Pharmacol. 18 (1969) 2615.
[8] T.C. Linn, F.H. Pettit and L.J. Reed, Proc. Natl. Acad. Sci. U.S. 62 (1969) 235.
[9] O. Wieland and B. v. Jagow-Westermann, FEBS Letters 3 (1969) 271.