FORMATION OF FREE ACETATE BY ISOLATED PERFUSED LIVERS FROM NORMAL, STARVED AND DIABETIC RATS

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Summary

Isolated rat livers perfused in an open system exhibited a continous net release of free acetate. Upon intraportal infusion of hexanoate the net release of total ketone bodies and of free acetate increased significantly in livers from fed and 48 hours starved rats. The ratio ketone body production/acetate production during infusion of hexanoate was similar with livers from fed and starved rats. Livers from diabetic rats, however, did not only exhibit a higher rate of ketone body and acetate production, but also a significant decrease of the ratio ketone body production/acetate production. Intraportal infusion of oleate led also to an enhanced release of free acetate. An examination of the activities of 5 enzymes involved in ketone body and acetate metabolism showed no correlation with the higher rate of acetate production by diabetic livers.

INTRODUCTION

Usually the metabolic disorder in diabetic ketosis is more severe than in starvation ketosis, even when the plasma levels of ketone bodies are in the same range under both conditions. Moreover, in balance studies total oxidation, ketone body production, and synthesis of triglycerides could not completely account for the total amount of non-esterified fatty acids (NEFA) taken up by the liver, and this discrepancy increased further during starvation (1).

Therefore, we have studied whether the liver is able to release free acetate, and if so, whether the rate of acetate release might be influenced by the supply of free fatty acids and by the metabolic state.

MATERIALS AND METHODS

For the study of acetate metabolism in isolated perfused rat liver the glasware and tubings of the perfusion apparatus had to be sterilized. The perfusion was performed in an open system in order to permit the measurement of porto-caval concentration differences across the liver. The medium consisted of Krebs-Henseleit bicarbonate buffer plus 5 $\mu g \cdot ml^{-1}$ ampicillin with or without bovine serum albumin. When albumin was used (as in experiments with long-chain fatty acids), it was extensively dialysed and concentrated in a hollow fiber dialysis apparatus and finally lyophilized. The medium was filtered through a Millipore filter (pore size 0.45 μ) immediately before use.

The analysis of acetate was performed according to Bergmeyer and Moellering (2). For this purpose 2 ml of the medium were brought to pH 3.0 with 0.5 M citrate buffer, and a microdistillation according to Bartley (3) was performed. A correction for the losses during the distillation procedure was made by the aid of (1-^{14}C) acetate. After transfer of the liver to the perfusion chamber, the organ was first perfused in a recirculating system for 15 minutes. Thereafter, the non-recirculating perfusion was started (6 $ml \cdot g^{-1} \cdot min^{-1}$, temperature 32^{o}C).

The activity of ß-ketothiolase (EC. 2.3.1.9), ß-hydroxy, ß-methyl-glutaryl-CoA synthase (EC. 4.1.3.5), ß-hydroxy, ß-methyl-glutaryl-CoA lyase (EC. 4.1.3.4) were determined according to Williamson et al. (4), acetate thiokinase (EC. 6.2.1.1) according to Barth et al. (5), and acetyl-CoA deacylase (EC. 3.1.2.1) according to Hepp et al. (6).

Alloxan diabetes was induced by intravenous injection of

50 mg $\cdot kg^{-1}$ of alloxan monohydrate into 24 hours starved male Wistar rats. The animals were treated with 4-8 units of insulin (Lente-Insulin, NOVO) per day for about 2 weeks. Thereafter the insulin treatment was stopped. The experiments were performed 60 hours after the last injection of insulin.

RESULTS AND DISCUSSION

The results are summarized in tables 1 to 3. Livers from chow-fed control rats produced very little ketone bodies during this period (table 1).

In the presence of hexanoate the production of ketone bodies increased but there was no significant difference between livers from normal and from starved rats (table 1). Livers from diabetic animals again produced significantly more ketone bodies than was found in the other groups. After cessation of the hexanoate infusion, ketogenesis by livers from fed or starved rats returned to the low basal values, but remained high in experiments with livers from diabetic rats (table 1).

A net release of acetate occured with livers from all 3 groups (table 1). However, this release was small with livers from fed and 48 hours starved rats, whereas livers from diabetic animals released 3-4 times more acetate (table 1). When hexanoate was infused a significant increase in the production of free acetate was measured in all 3 groups. Again, there was no significant difference between livers from fed and starved animals, while livers from diabetic animals released significantly more acetate. Moreover, the absolute increase of acetate release following the infusion of hexanoate, was higher with livers from diabetic animals. After cessation of the hexanoate infusion, the release of acetate decreased to the same extent

Table 1

Net production of total ketone bodies (acetoacetate+3-hydroxybutyrate) and of free acetate, and ketone body/acetate ratio in experiments with isolated perfused livers from fed (n=12), 48 hours starved (n=8), and from fed alloxandiabetic rats (n=25). From the 4^{th} to the 16^{th} minute 1 mmole $\cdot h^{-1}$ of hexanoate was infused intraportally. The hexanoate was free of acetate and ethanol. Mean values ± SEM.

	Total ketone bodies			Acetate			Ketone body production / Acetate production		
Time	Controls	Starved	Diabetic	Controls	Starved	Diabetic	Controls	Starved	Diabetic
(min)	(nmoles $\cdot$ g liver^{-1} $\cdot$ min^{-1})			(nmoles $\cdot$ g liver^{-1} $\cdot$ min^{-1})			(ratio)		
0	71± 16	351± 79	1132±195	45±18	19±11	218±31	1.6	18.4	5.1
1	85± 23	225± 56	661± 87	48± 9	49±28	178±51	1.8	4.6	3.7
2	87± 26	218± 62	561± 63	22± 1	88±37	162±30	4.0	2.5	3.4
4	103± 26	198± 54	629± 85	39±22	71±37	165±31	2.6	2.8	3.8
9	851±153	973±197	1164±112	131±28	190±78	306±39	6.5	5.1	3.8
11	832±125	1059±180	1231±120	122±18	157±53	301±39	6.9	5.7	4.1
13	744±148	1049±216	1285±110	141±23	114±19	327±42	5.3	9.2	3.9
16	961±125	923±138	1296±110	169±28	80±25	332±46	5.7	11.5	3.9
21	140± 24	88± 37	824±110	47± 8	127±72	195±40	3.0	0.7	4.2
23	112± 24	73± 15	789±115	67±25	85±30	199±45	1.7	0.9	3.9
25	116± 35	71± 14	801±125	64±12	42±13	170±35	1.8	1.7	4.7

with livers from fed and from starved rats. Acetate production by livers from diabetic animals decreased, but remained elevated as it was before the infusion of hexanoate. Looking at the ratio $\frac{\text{ketone body release}}{\text{acetate release}}$ one can see (table 1) that this ratio is similar with livers from fed and from starved rats during the infusion of hexanoate, but much lower with livers from diabetic rats. In cases of very severe diabetic ketosis ratios below 2 were observed (figure 1).

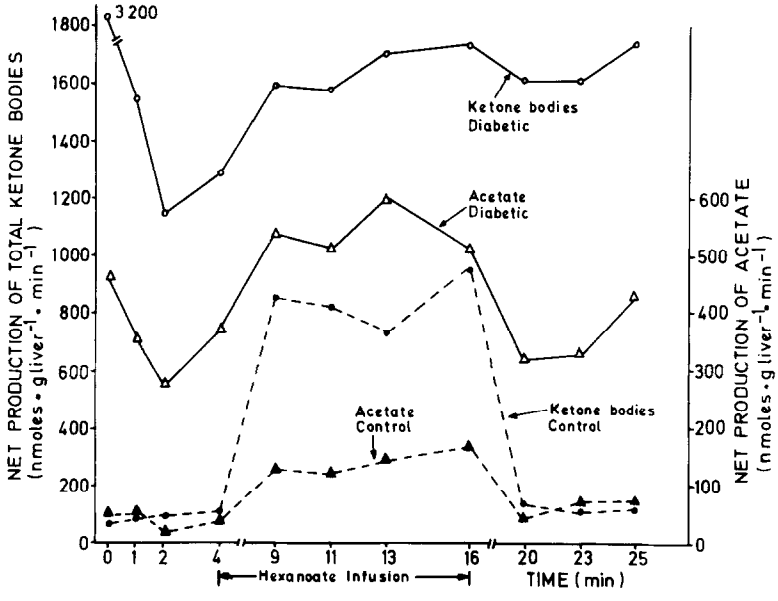

Figure 1

Net production of total ketone bodies and acetate by an isolated perfused liver from a severely alloxan diabetic rat. The dotted lines refer to the release of total ketone bodies and acetate by isolated perfused livers from non-diabetic fed rats. In all experiments 1 mmole $\cdot h^{-1}$ of hexanoate was infused intraportally from the 4^{th} to the 16^{th} minute.

We are reluctant to bear too much on the ratios in the absence of hexanoate infusion, since at least in the non-diabetic animals the rates of production of ketone bodies and acetate

Table 2

Net formation of total ketone bodies (acetoacetate+3-hydroxybutyrate) and of free acetate by the isolated perfused liver from an alloxandiabetic rat. The perfusion was performed in an open system with Krebs-Ringer bicarbonate medium containing 2% bovine albumine. From the 6^{th} to the 14^{th} minute oleate (2 mmoles $\cdot h^{-1}$) was infused intraportally. The albumin and the oleate were free of acetate.
For further details see Materials and Methods.

Time	Total ketone bodies	Acetate	Ketone body production / Acetate production
	(nmoles $\cdot$ g liver^{-1} $\cdot$ min^{-1})		(ratio)
0	765	945	0.81
1	447	174	2.57
3	905	273	3.32
5	569	226	2.52
6	685	209	3.28
8	1080	226	4.78
10	1172	556	2.11
11	1056	586	1.80
12	915	590	1.55
14	810	493	1.64
15	465	475	0.98
16	545	302	1.80
18	500	255	1.96
19	800	145	5.52
20	185	138	1.34

were very low, and therefore the ratios showed a large variation. In order to avoid the argument that the high rates of acetate release by diabetic livers were the result of an insufficient oxygen supply in the hemoglobin-free system, we performed 3 ex-

periments with livers from severely diabetic rats in which the medium contained washed aged human red cells. These livers (not shown here) exhibited the same high rate of production of total ketone bodies and free acetate as seen without red cells.

In 3 experiments with alloxan-diabetic rats oleate instead of hexanoate was infused intraportally. In all 3 experiments the infusion of oleate led to a significant release of free acetate addition to ketone body production. A typical example is given in table 2.

Except under conditions where ethanol is oxidized free acetate arises mainly from the deacylation of acetyl-CoA. Therefore, the net formation of free acetate will be determined by the equilibrium between deacylation of acetyl-CoA and the reactivation in the acetate thiokinase reaction. For this reason, the activities of acetate thiokinase and acetyl-CoA deacylase together with the activities of ß-hydroxy, ß-methyl-glutaryl-CoA synthase, ß-hydroxy, ß-methyl-glutaryl-CoA lyase, and ß-ketothiolase were examined. The results are summarized in table 3.

The activities of ß-hydroxy, ß-methyl-glutaryl-CoA synthase and ß-hydroxy, ß-methyl-glutaryl-CoA lyase showed a small increase in both starvation and diabetes (table 3). This is in accordance with similar findings of Williamson et al. (4) during starvation. The decrease of the activity of acetate thiokinase during starvation is in accordance with the data of Barth et al. (5) and Murthy and Steiner (7).

The ratio acetyl-CoA deacylase/acetate thiokinase increased form 0.89 in fed rats to 2.65 in starved and to 1.79 in diabetic rats.

Therefore, the higher absolute and relative production of free acetate by livers from diabetic animals cannot be ex-

Table 3

Activities of enzymes involved in the formation of ketone bodies and in acetate metabolism in livers from fed (n=8), starved (n=4), and fed alloxandiabetic (n=12) male Wistar rats. ß-Hydroxy, ß-methylglutaryl CoA synthase (HMG-CoA synthase), ß-hydroxy, ß-methylglutaryl CoA lyase (HMG-CoA-lyase), and ß-ketothiolase were determined according to Williamson et al. (4). Acetate thiokinase activity was measured according to Sladek (9) and Barth et al. (5), Acetyl-CoA deacylase activity according to Hepp et al. (6). Mean values ± SEM.

	HMG-CoA synthase	HMG-CoA lyase	ß-Ketothiolase	Acetate Thiokinase (total)	Acetyl-CoA deacylase (soluble)
	(μmoles · g liver^{-1} · min^{-1})				
Fed rats	2.09±0.31	8.19±0.65	28.02±4.10	1.10±0.27	0.98±0.17
48 hrs. starved rats	2.69±0.55	11.17±2.29	24.36±5.60	0.51±0.13	1.35±0.35
Diabetic rats	3.24±0.6	10.07±0.97	31.52±5.06	0.73±0.20	1.31±0.16

plained by a higher degree of acetyl-CoA deacylation or a lower degree of acetate reactivation. An impaired oxidation of acetyl-CoA during severe diabetes is the most likely explanation. Studies performed in clinical patients in various metabolic states (Seufert,Mewes and Söling (8)) will demonstrate that the metabolism of free acetate has to be considered also in man.

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Refernces:

1) Cahill, G.F.Jr., Owen, O.E. and Morgan, A.P. (1968) Advanc.Enzyme Reg., 6, 143
2) Bergmeyer, H.U. and Moellering, H., (1966) Biochem. Z., 344, 157
3) Bartley, W., (1953) Biochem. J., 53, 305
4) Williamson, D.H., Bates, M.W. and Krebs, H.A., (1968) Biochem. J., 108, 353
5) Barth, C., Sladek, M. and Decker, K., (1972) Biochem.Biophys.Acta, 260, 1
6) Hepp, D., Prüsse, E., Weiss, H. and Wieland, O., (1966) Advanc.Enzyme Reg., 4, 89
7) Murthy, V.K. and Steiner, G., (1973) Metabolism, 21, 124
8) Seufert, C.D., Mewes, W. and Söling, H.D., unpublished results
9) Sladek, M. (1971) M.D. Thesis, University of Freiburg/Germany