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DECREASE BY GLUCAGON IN HEPATIC SUCCINYL-CoA

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SUMMARY: Succinyl-CoA has been determined in freeze-clamped rat liver tissue to amount to 9.5 ± 0.7 nmoles/g fresh wt. (n=11). Intravenous injection of glucagon lowers this level within 10 min to 4.5 nmoles/g fresh wt. (n=11; $P < 0.0005$). Isolated hepatocytes also show a significant decrease in their succinyl-CoA content when incubated with glucagon or dibutyryl cyclic AMP. This effect appears to be fully expressed already 1 min after exposure of isolated hepatocytes to glucagon.

INTRODUCTION

In liver perfusion studies glucagon was found to markedly lower the levels of 2-oxoglutarate and glutamate (1,2). Ui et al. (2) observed that the hormone stimulated the release of $^{14}CO_2$ from [1-^{14}C]-glutamate. Moreover, it was shown that mitochondria isolated from glucagon-treated rats display an accelerated flux through the 2-oxoglutarate dehydrogenase complex but no increase in extractable enzyme activity (3). Studies on the subcellular distribution of the C_5-dicarboxylates have revealed that they were affected by glucagon nearly to the same extent both in the cytosolic and mitochondrial compartments (4). The possibility to explain these hormone effects by the increase in succinate dehydrogenase (EC 1.3.99.1) activity (3) has become unlikely as no change after glucagon in the hepatic amount or the subcellular location of succinate was discernible (5). We therefore considered the possibility of an effect of glucagon at the succinyl-CoA synthetase (EC 6.2.1.4) step. The data of the present study indicate a decrease by glucagon in the hepatic succinyl-CoA con-

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tent and are compatible with the view that the flux through the 2-oxoglutarate dehydrogenase complex is enhanced by relief of its feed-back control by succinyl-CoA (6).

MATERIALS AND METHODS

Glucagon was a gift from Hoechst (Frankfurt, Germany). Succinyl-CoA was obtained from Sigma (Taufkirchen, Germany). Charcoal Norit A, DL-carnitine and gelatin "non plus ultra" were purchased from Serva (Heidelberg, Germany). Na^+-L-lactate was bought from Roth (Karlsruhe, Germany). Bovine serum albumin was a product of Behring (Marburg, Germany). L-lysine and thiogenal® were obtained from Merck (Darmstadt, Germany). Succinyl-CoA-3-oxoacid CoA-transferase (EC 2.8.3.5) was prepared by the method of Sharp and Edwards (7). Other biochemicals and enzymes came from Boehringer (Mannheim, Germany).

Succinyl-CoA in liver tissue

Normal fed male Sprague-Dawley rats (200-300 g) were anaesthetized by intraperitoneal injection of 1 ml/kg thiogenal®. They were allowed to sleep for about 20 min prior to a tail vein injection of 20 µg glucagon in 0.1 ml solvent (8). Control animals received the solvent only. The right lobe of the liver was removed 10 min after the injection and immediately deep-frozen in a Wollenberger clamp (9) precooled in liquid nitrogen. The tissue was powdered with the aid of a Mikrodismembrator (Braun, Melsungen, Germany) and extracted at 4°C with 2 ml/g of 6 % (w/v) $HClO_4$ using a motor-driven glass-teflon homogenizer. After centrifugation for 15 min at 14000 x g at 4°C the precipitate was extracted as before. The clear extracts were combined and neutralized with solid $KHCO_3$.

Succinyl-CoA in isolated liver cells

Hepatocytes were isolated from 48 h-starved male Sprague-Dawley rats (200-300 g) in principle according to Berry and Friend (10) as described (11). The incubation medium was composed of 1 ml of Ca^{2+}-free Krebs-Henseleit bicarbonate buffer (12) pH 7.4, containing 1.5 % gelatin, 2mM-DL-carnitine and 2 mM-lysine, 0.1 ml of 10 % defatted (13) bovine serum albumin in 0.9 % NaCl, 0.01 ml of 110 mM-$CaCl_2$ and 0.2 ml of the liver cell suspension, corresponding to about 20 mg dry wt. After preincubation for 30 min at 37°C glucagon, dibutyryl cyclic AMP or solvent (1.3 % $NaHCO_3$) only was added to yield a medium concentration of 2 µM and 0.36 mM, respectively, and incubation was continued for the times indicated in Table 2. For determination of succinyl-CoA the liver cells of 1 ml of the incubation mixture were collected by centrifugation for 5 sec in an Eppendorf centrifuge (model 3200) and immediately extracted with 0.64 ml of ice-cold 12 % (w/v) $HClO_4$. After centrifugation the pellet was reextracted with 0.2 ml of this $HClO_4$. The supernatants were combined and neutralized with solid $KHCO_3$.

Succinyl-CoA assay

Succinyl-CoA was determined by the method of Smith et al. (14) using a Perkin-Elmer double beam spectrophotometer (model 356). The reaction cuvette contained 1.5 ml of 100 mM-potassium phosphate buffer pH 7.0, 1 ml of tissue extract, 0.05 ml of 200 mM-lithium acetoacetate, 0.1 ml of 2 mM-NADH and 0.05 ml of 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (corresponding to 0.05 mg). The reaction was initiated with 0.02 ml of succinyl-CoA-3-oxoacid CoA-transferase (corresponding to 0.026 mg or 0.37 U). To correct for unspecific NADH consumption the extract was analyzed in parallel after hydrolysis of succinyl-CoA, which was accomplished by incubation of 1 ml of extract with 0.1 ml of 1N-KOH for 30 min at 37°C prior to neutralization with 2N-HCl (0.05 ml).

RESULTS AND DISCUSSION

Assay and normal content of succinyl-CoA.

The data in the literature on hepatic succinyl-CoA measurements are scarce and divergent. Values of about 600 nmoles/g wet wt. (15,16) and 100 nmoles/g wet wt. (17) have been published, yet no details on the assay(s) employed were reported. In the present study (Table 1) considerably lower values have been obtained by an assay following the NADH consumption during conversion of succinyl-CoA to 3-hydroxybutyryl-CoA by highly purified succinyl-CoA-3-oxoacid CoA-transferase and 3-hydroxyacyl-CoA dehydrogenase. As to the reliability of this assay it appears noteworthy that succinyl-CoA added to the liver tissue powder prior to the extraction in amounts of 0.1-0.4 nmoles/g was recovered by about 80 %. The recovery of succinyl-CoA from the acid extracts both from liver tissue and isolated hepatocytes was in the range of 80-90 %. It is further worth mentioning that on the basis of succinyl-CoA measurements in isolated rat liver mitochondria incubated with 2-oxoglutarate under state 3 conditions a value of 36 nmoles/g wet wt. can be calculated (14). Moreover, succinyl-CoA, in freeze-clamped mouse liver has been found (by a different approach) to amount to 24 nmoles/g wet wt. (18).

Table 1

Effect of i.v. glucagon injection into intact rats on hepatic succinyl-CoA content.

	Succinyl-CoA (nmoles/g wet wt.)		
Animals	Control		Glucagon
Fed	9.5 ± 0.7	(11)	4.5 ± 0.6
		P < 0.0005	
48 h-Starved	13.0 ± 1.6	(3)	3.3 ± 0.7
		P < 0.005	

Mean values ± SEM for the numbers of animals in parentheses are given. Statistical significance P was calculated according to Student's t-test.

Effect of glucagon on succinyl-CoA in liver tissue.

Our previous measurements of citric acid cycle intermediates after glucagon treatment of intact rats have revealed an increase in malate and citrate by 121 % and 44 % respectively, in fed animals (3). The data summarized in Table 1 now indicate the fact that a short-term treatment with glucagon also causes a marked change in the hepatic succinyl-CoA concentration. In normal fed and 48 h fasted rats the level of succinyl-CoA was found to be diminished by 53 % and 75 %, respectively (Table 1). This opens the possibility that the lowering by glucagon in C_5-dicarboxylates results from a relief of the feed-back inhibition of 2-oxoglutarate dehydrogenase by succinyl-CoA, first described by Garland (6).

Effect of glucagon on succinyl-CoA in isolated hepatocytes.

The possibility that the glucagon effect observed in the intact animal was mediated indirectly by extrahepatic action(s) of the hormone has been ruled out by experiments with isolated hepatocytes. The results recorded in Table 2 demonstrate that glucagon or dibutyryl cyclic AMP added to the incubation medium effectively lowered the cellular amounts of succinyl-CoA. The magnitude of the hormone effect *in vitro* is well comparable with that

Table 2
Effect of glucagon and dibutyryl cyclic AMP (db-cAMP) on the amount of succinyl-CoA in isolated hepatocytes.

Time of exposure	Succinyl-CoA (nmoles/g dry wt.) Control	Glucagon	db-cAMP
1 min	57.1 ± 11.6 (3)	27.4 ± 11.4[a] (3)	
10 min	50.9 ± 8.6 (4)	32.2 ± 9.8[b] (4)	
	44.7 ± 6.1 (8)		23.6 ± 3.8[c] (8)

Isolated liver cells were incubated as described in the Methods section. Mean values ± SEM for the numbers of different cell preparations in parentheses are given. Statistical significance P was calculated according to Student's t-test for paired data; [a] $P < 0.0125$; [b] $P < 0.05$; [c] $P < 0.0005$.

observed in vivo. The finding (Table 2) that the glucagon effect is maximal 1 min after exposure of the cells to the hormone is of special interest. In a study (19) on the kinetics of the changes in 2-oxoglutarate and glutamate in isolated hepatocytes, these metabolites, although being significantly lowered 1 min after glucagon, still continued to fall thereafter, reaching a minimum after about 15 min. Thus it seems possible that the decrease by glucagon in succinyl-CoA precedes that in the C_5-dicarboxylates. This might indicate that the hormone stimulates the flux at the succinyl-CoA synthetase step. This interpretation is in accordance with the observation that the mitochondrial GTP/GDP ratio tends to rise after glucagon (5) and that the flux from 2-oxoglutarate to malate in mitochondria isolated from glucagon-treated rats is increased (3). The fact that the level of succinate remains unchanged after glucagon (5) is not in conflict with this view, as the succinate dehydrogenase activity is increased after glucagon (3). The possibility that the fall in succinyl-CoA is merely the consequence of 2-oxoglutarate

consumption is unlikely because of the temporal relationship mentioned above and because of the fact that the 2-oxoglutarate concentration in the mitochondrial compartment of hepatocytes still is 0.8 mM even 10 min after glucagon (4).

The lowering effect of glucagon on hepatic succinyl-CoA might be of potential interest with respect to the regulation of the pyruvate carboxylase (EC 6.4.1.1) activity. According to (20) pyruvate carboxylase from chicken liver is inhibited by succinyl-CoA, displaying a K_i of 0.25 mM. If the rat liver enzyme behaves alike, it is noteworthy that the mitochondrial succinyl-CoA concentration of control livers amounts to 0.2 mM, as according to our preliminary experience on succinyl-CoA compartmentation this metabolite is confined to the mitochondrial matrix space. Consequently, the fall in succinyl-CoA after glucagon, in addition to that of glutamate (4), another metabolic inhibitor of pyruvate carboxylase (21), might lead to de-inhibition of this gluconeogenic key enzyme.

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