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Treatment of rats with glucagon or mannoheptulose increases mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity and decreases succinyl-CoA content in liver

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1. The activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (EC 4.1.3.5) in extracts of rapidly frozen rat livers was doubled in animals treated in various ways to increase ketogenic flux. 2. Some 90% of the activity measured was mitochondrial, and changes in mitochondrial activity dominated changes in total enzyme activity. 3. The elevated HMG-CoA synthase activities persisted throughout the isolation of liver mitochondria. 4. Intramitochondrial succinyl-CoA content was lower in whole liver homogenates and in mitochondria isolated from animals treated with glucagon or mannoheptulose. 5. HMG-CoA synthase activity in mitochondria from both ox and rat liver was negatively correlated with intramitochondrial succinyl-CoA levels when these were manipulated artificially. Under these conditions, the differences between mitochondria from control and hormone-treated rats were abolished. 6. These findings show that glucagon can decrease intramitochondrial succinyl-CoA concentration, and that this in turn can regulate mitochondrial HMG-CoA synthase. They support the hypothesis that the formation of ketone bodies from acetyl-CoA may be regulated by the extent of succinylation of mitochondrial HMG-CoA synthase.

INTRODUCTION

Regulation of the ketogenic pathway is complex and incompletely understood (McGarry & Foster, 1971*a*,*b*; Zammit, 1984). However, it is clear that there is control, since ketogenesis may be stimulated *in vivo* by glucagon (McGarry *et al.*, 1975; Unger & Orci, 1975; Unger, 1978, 1985; McGarry & Foster, 1980; Foster, 1984).

There is considerable evidence that inhibition of carnitine palmitoyltransferase I by malonyl-CoA in liver is important in control of ketogenesis (McGarry *et al.*, 1977; Zammit, 1981, 1984; Sugden & Williamson, 1982; Foster, 1984). However, under certain conditions control shifts away from this enzyme to an unidentified intramitochondrial control site (Palmer *et al.*, 1983; Grantham & Zammit, 1986, 1988; Holness *et al.*, 1987; Schofield *et al.*, 1987*a*,*b*; Boon & Zammit, 1988; Decaux *et al.*, 1988). This allows a more rapid response to the changing metabolic state.

Regulation within the pathway from acetyl-CoA to ketone bodies (the HMG-CoA pathway) has been suggested by many groups to be a factor in the control of liver ketogenesis (Bush & Milligan, 1971; Huth *et al.*, 1973, 1978; Menahan *et al.*, 1981; Zammit, 1981, 1984; Sugden & Williamson, 1982; Lowe & Tubbs, 1985*b*; Holness *et al.*, 1987; Decaux *et al.*, 1988). However, there is no direct evidence for such regulation. HMG-CoA synthase, the second enzyme of the pathway, appears to have a high flux control coefficient over ketogenesis, although this has not been measured directly. The enzyme has been described as 'rate-limiting' in the conversion of acetyl-CoA into acetoacetate (Williamson *et al.*, 1968; Dashti & Ontko, 1979). It is not known whether it is allosterically controlled (Sugden & Williamson, 1982) nor whether its rate is affected *in vivo* other than by substrate and product concentrations.

Lowe & Tubbs (1985*b*) studied the succinylation and inactivation of purified ox liver mitochondrial HMG-CoA synthase by succinyl-CoA, and showed that this inactivation is due to the enzyme catalysing its own succinylation at the active site. Although these studies were performed *in vitro* with purified enzyme, Lowe & Tubbs (1985*b*) postulated a control mechanism in which the flux through the HMG-CoA pathway *in vivo* is regulated according to the extent of succinylation of HMG-CoA synthase by succinyl-CoA.

The mitochondrial concentration of succinyl-CoA in normal rat liver is thought to be about 0.2 mM (Barritt *et al.*, 1976), although under some contrived circumstances it may be much higher, with much of the CoA pool in the form of succinyl-CoA (Smith *et al.*, 1973). In addition, Siess *et al.* (1980) have reported that glucagon may decrease the succinyl-CoA content of liver, although Ochs (1984) was unable to reproduce this result. These concentrations are in the range that might inhibit HMG-CoA synthase *in vivo*.

In the present paper we examine the hypothesis that glucagon may control HMG-CoA synthase *in vivo* via its effects on mitochondrial succinyl-CoA content (Lowe & Tubbs, 1985*b*), and provide evidence that this is the case. Some of the results have been presented in preliminary form (Quant *et al.*, 1987*a*,*b*, 1988).

EXPERIMENTAL

Materials

CoA was obtained from P-L Biochemicals. Acetyl-CoA was prepared by treating CoA with acetic anhydride.

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; PTA, phosphotransacetylase; PDS, 4,4'-dithiobispyridine.

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Acetoacetyl-CoA, succinyl-CoA, acetyl phosphate, alloxan monohydrate, D-mannoheptulose, glucagon, citrate synthase (EC 4.1.3.7; from pig heart) and succinyl-CoA synthetase (EC 6.2.1.4; from pig heart) were obtained from Sigma. Phosphotransacetylase (PTA; EC 2.3.1.8; from *Bacillus stearothermophilus*) was from Boehringer. Ox liver mitochondrial HMG-CoA synthase (EC 4.1.3.5) was purified as described by Lowe & Tubbs (1985*a*).

Animals

Female Wistar rats weighing approx. 200 g were anaesthetized with diethyl ether and then by intraperitoneal injection with Nembutal (1 μl/g body wt.). After 20 min, glucagon or mannoheptulose was administered (see below), or the livers were removed (other conditions). For 'glucagon-treated rats', glucagon (20 μg/200 g body wt., prepared as described by Yamazaki, 1975) was injected into the tail vein. The livers were removed 10 min later. Control rats were injected with the same solution but containing no glucagon. For 'mannoheptulose-treated rats', mannoheptulose (500 mg of mannoheptulose/ml of 0.9% NaCl solution, at a dose of 2 g mannoheptulose/kg body wt.) was administered by intraperitoneal injection as described by Tullson & Aprille (1986). The livers were removed 60 min later. Control rats were injected in the same way but with saline containing no mannoheptulose. 'Alloxan-diabetic rats' were lightly anaesthetized with diethyl ether 48 h before experiments, and then alloxan monohydrate (70 mg/kg body wt. in 0.3 ml of 0.9% NaCl) was injected into the tail vein (Williamson *et al.*, 1967). Control rats were injected with saline, but were otherwise treated identically.

All rats were allowed free access to Diet 41B (Oxoid) ('fed rats'), except for 'starved rats', which were starved for 48 h in cages with gridded bases, and 'fat-fed rats', which were given a high-fat diet (low in carbohydrate) consisting of (by wt.) 66% margarine, 32% soluble casein and 2% inorganic-salt mixture with vitamin supplement (specially prepared by W. Lillico and Son, Aylesford, Kent, U.K.) for 3 days before use.

Preparation of crude extracts

Whole livers were rapidly excised and submersed in liquid N_2 for 2 min. They were then pulverized in a percussion mortar (precooled in solid CO_2). Subsequent operations were carried out at 4 °C. The liver powder was homogenized with 3 ml of isolation buffer/g in a loosely fitting Dounce homogenizer, then centrifuged at 600 *g* for 5 min. The resulting supernatant is referred to as the 'crude extract'. Isolation buffer contained 0.3 M-sucrose, 0.5 mM-EDTA and 10 mM-Mops/KOH, pH 7.2.

Fractionation of the liver

Whole livers were removed, cut up finely and homogenized in a loosely fitting glass homogenizer with 3 ml of isolation buffer/g wet wt. All operations were conducted at 4 °C. The supernatant after centrifugation at 600 *g* for 5 min is referred to as the 'homogenate'. This was centrifuged at 15000 *g* for 20 min, and the supernatant, containing the cytoplasmic fraction, was assayed without further preparation. The pellet was washed once by gentle resuspension in buffer and resedimented as above. This mitochondrial pellet was resuspended in isolation buffer. This procedure gave < 5% cross-contamination between cytoplasm and mitochondria, based on the activities of lactate dehydrogenase and citrate synthase.

Isolation of mitochondria

Whole rat livers were placed in ice-cold mitochondrial isolation medium (250 mM-sucrose, 5 mM-Tris/HCl, 1 mM-EGTA, pH 7.4). Excised ox liver was transported from the abattoir packed in ice, and about 100 g was removed for subsequent use. All subsequent operations were conducted at 4 °C in mitochondrial isolation medium as described for fractionation of the liver, but the mitochondrial pellet was washed twice and resuspended in mitochondrial isolation medium.

Assay of HMG-CoA synthase activity

Samples (100 μl) of crude extracts, homogenates or mitochondria were treated with 7.5 μl of 20% (w/v) Triton X-100 to expose HMG-CoA synthase, and 5 μl samples (25 μg of protein) were assayed immediately.

Lowe & Tubbs (1985*a*) assayed purified HMG-CoA synthase by measuring the disappearance of the enol form of acetoacetyl-CoA by monitoring A_{303}. In crude liver extracts the activities of contaminating acetoacetyl-CoA thiolase and acetyl-CoA hydrolase contribute to the disappearance of acetoacetyl-CoA. Acetyl-CoA hydrolase activity produces CoA. This provides the second substrate for acetoacetyl-CoA thiolase, and so allows the breakdown of acetoacetyl-CoA to acetyl-CoA. We eliminated this contaminating thiolase activity by preventing accumulation of CoA. This was done by addition of acetyl phosphate and PTA to regenerate acetyl-CoA from any CoA produced. We assessed the efficiency of the acetyl-CoA regenerating system by challenging the assay system by addition of 5 μM-CoA (Fig. 1*a*). In the absence of acetyl phosphate and PTA the disappearance of acetoacetyl-CoA was fast, owing to thiolase activity. In the presence of the acetyl-CoA-regenerating system the rates were less. Addition of CoA had no effect, indicating that the system satisfactorily eliminates the activities of the interfering enzymes.

The standard 1 ml assay system contained 50 mM-Tris/HCl, pH 8.0, 10 mM-$MgCl_2$ and 0.2 mM-dithiothreitol at 30 °C; 5 mM-acetyl phosphate, 10 μM-acetoacetyl-CoA and 5 μl samples of extracts were added, followed by simultaneous addition of acetyl-CoA (100 μM) and 10 units of PTA. HMG-CoA synthase activity was measured as the difference in the rate before and after acetyl-CoA addition (Fig. 1*b*). The absorption coefficient of acetoacetyl-CoA under the conditions used (pH 8.0, 10 mM-$MgCl_2$), is 12.2×10^3 $M^{-1} \cdot cm^{-1}$ (Lowe & Tubbs, 1985*a*). By definition, 1 unit of enzyme activity causes 1 μmol of acetoacetyl-CoA to be transformed/min. The reaction was linear with the amount of protein added over the range studied.

Other assays

Citrate synthase, lactate dehydrogenase and protein were assayed as described by Lowe & Tubbs (1985*a*), Bergmeyer & Bernt (1974) and Gornall *et al.* (1949) respectively.

Preparation of samples for assay of succinyl-CoA and acetyl-CoA content

(*a*) Whole liver extracts. The liver powder (see above) was homogenized at 4 °C with 2 ml of 6% (w/v) $HClO_4$/g

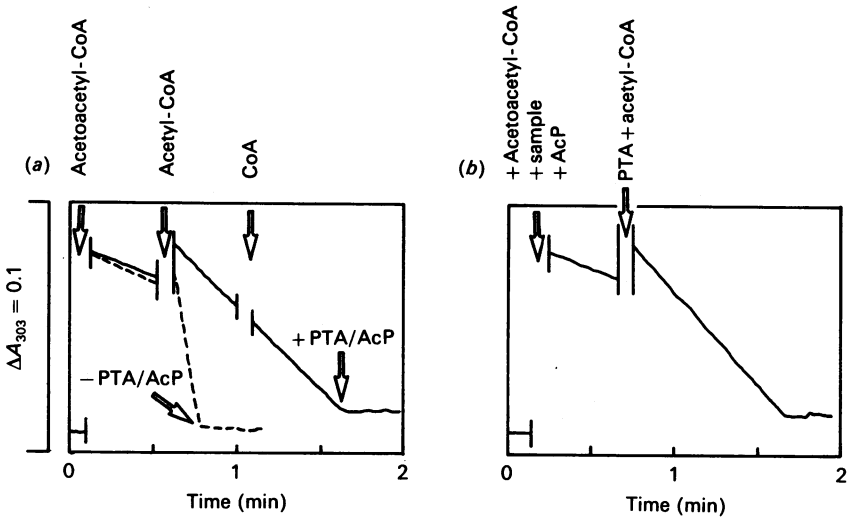

Fig. 1. Assay of HMG-CoA synthase

(*a*) In the presence of the acetyl-CoA-regenerating system [5 mM-acetyl phosphate (AcP) plus 10 units of PTA], acetoacetyl-CoA thiolase activity is eliminated and the addition of 5 μM-CoA does not increase the rate of disappearance of acetoacetyl-CoA. In its absence, the disappearance of acetoacetyl-CoA is faster, owing to thiolase activity. (*b*) Standard HMG-CoA synthase assay. The 1 ml system at 30 °C contained 50 mM-Tris/HCl (pH 8.0), 10 mM-$MgCl_2$ and 0.2 mM-dithiothreitol; 10 μM-acetoacetyl-CoA, 5 mM-acetyl phosphate and 5 μl of sample were added, followed by 10 units of PTA and 100 μM-acetyl-CoA. The rate of disappearance of the enol form of acetoacetyl-CoA was monitored at 303 nm. For details see the Experimental section.

and centrifuged for 15 min at 14000 *g*. The precipitate was re-extracted and the clear supernatants were combined and neutralized (to about pH 6.0) with solid $KHCO_3$ as described by Siess *et al.* (1980). (*b*) Samples (1 ml) of mitochondrial incubations or liver homogenates (about 5 mg of protein) were treated with 100 μl of ice-cold 3.4 M-$HClO_4$ and centrifuged for 2 min in a bench Minifuge. A 600 μl portion of supernatant was neutralized with 150 μl of 0.5 M-KOH/2 M-potassium acetate to pH 5–6 and immediately re-centrifuged. Then 500 μl of supernatant was assayed. Recovery of added succinyl-CoA was 85(±5)%.

Succinyl-CoA and acetyl-CoA assays

CoA was released from succinyl-CoA by using succinyl-CoA synthetase and allowed to react with 4,4′-dithiobispyridine (PDS). The stoichiometric production of 4-pyridone was monitored spectrophotometrically at 324 nm.

The standard 1 ml assay system contained 10 mM-$MgCl_2$, 2 mM-GDP, 50 μM-PDS, 5 mM-potassium phosphate and 100 mM-Hepes, pH 7.2. After addition of 500 μl of extract, approx. 3 min was allowed for any thiol groups (e.g. in free CoA or glutathione) to react with PDS. When a stable value of A_{324} was reached, succinyl-CoA synthetase (0.7 unit) was added. The small absorbance due to the enzyme was corrected for. There was a linear relationship between known succinyl-CoA concentrations and A_{324}. The apparent adsorption coefficient for 4-thiopyridone was 19.8×10^3 $M^{-1} \cdot cm^{-1}$, in agreement with Grassetti & Murray (1967).

Acetyl-CoA was assayed by the same method, in the same cuvettes immediately after the succinyl-CoA assays, by addition of 0.1 mM-oxaloacetate and 0.9 unit of citrate synthase.

RESULTS

The main aim of the experiments reported here was to test the hypothesis that glucagon and other ketogenic stimuli *in vivo* activate HMG-CoA synthase by lowering the hepatic succinyl-CoA content (Lowe & Tubbs, 1985*b*).

Glucagon injection into fed rats may result in high glucagon and high insulin concentrations in blood, whereas mannoheptulose injection results in high glucagon and low insulin levels (Tullson & Aprille, 1986; Aprille *et al.*, 1987). Mannoheptulose may therefore provide a more appropriate model for the study of the effects of glucagon *in vivo* than injection of glucagon itself.

Table 1 (column 1) shows the total activities of HMG-CoA synthase present in crude extracts of livers of control rats and rats treated in different ways. All the treatments resulted in significant increases (about 2-fold) in the activity of HMG-CoA synthase, with the high-fat diet being particularly effective.

As HMG-CoA synthases are soluble enzymes present in both cytoplasmic and mitochondrial compartments of rat liver cells (Clinkenbeard *et al.*, 1975*a*), it was necessary to establish the subcellular origin of the activity changes. Table 1 (columns 2 and 3) shows that both mitochondrial and cytoplasmic activities were increased, with changes in the mitochondrial enzyme dominating the effects. The change in cytoplasmic activity was not due to contaminating mitochondrial enzyme, as this was corrected for by using citrate synthase, another soluble matrix enzyme. Approx. 90% of the total HMG-CoA synthase activity of rat liver was mitochondrial, in reasonable agreement with previous reports on the cytoplasmic HMG-CoA synthase activity in liver from ox, chicken and rat (Baird *et al.*, 1970; Clinkenbeard *et al.*, 1975*a*,*b*;

Table 1. HMG-CoA synthase activity and succinyl-CoA content of liver fractions from rats treated in different ways

See the Experimental section for details. Values are ± S.E.M. with the number of animals in parentheses. Significantly different from controls: [a] $P < 0.02$; [b] $P < 0.01$; [c] $P < 0.002$; [d] $P < 0.001$.

	HMG-CoA synthase activity				
	Total activity in crude extracts (munits/mg of protein)	Activity in fractions (% of total control)		Activity in homogenates (munits/mg of protein)	Activity in mitochondria (munits/mg of protein)
Condition		Mitochondrial	Cytoplasmic		
Control (fed)	2.09 ± 0.22 (24)	91.0 (20)	9.0 (20)	1.78 ± 0.39 (5)	16.17 ± 0.82 (3)
48 h-starved	3.79 ± 0.50 (12)[b]	148.4 ± 22.3 (4)[d]	32.6 ± 6.5 (4)[d]	–	–
Alloxan-diabetic	4.12 ± 0.62 (4)[c]	169.4 ± 22.6 (4)[d]	27.6 ± 8.5 (4)[d]	–	–
Glucagon-treated	4.00 ± 0.56 (4)[c]	166.2 ± 20.4 (4)[d]	24.8 ± 4.5 (4)[d]	2.91 ± 0.40 (5)[d]	23.77 ± 3.62 (3)[a]
Glucagon-treated, starved	4.08 ± 0.26 (4)[c]	–	–	–	–
Mannoheptulose-treated	3.63 ± 0.19 (4)[c]	–	–	3.36 ± 0.13 (3)[d]	21.98 ± 3.40 (4)[b]
Fat-fed	6.04 ± 0.88 (4)[d]	265.9 ± 24.2 (4)[d]	23.1 ± 5.5 (4)[d]	–	–

	Succinyl-CoA content (% of control)		
Condition	Crude extracts	Homogenates	Mitochondria
Control (fed)	100 (4) (8.6 nmol/g wet wt.)	100 (3) (7.9 nmol/g wet wt.)	100 (4) (0.18 nmol/mg of protein)
Glucagon-treated	60.00 (3)[a]	66.67 (3)[a]	71.43 (3)[a]
Mannoheptulose-treated	63.00 (3)[b]	69.00 (3)[a]	70.05 (4)[a]

Aragon & Lowenstein, 1983; Lowe & Tubbs, 1985*a*). Even in starvation, where the proportion of the total activity due to the cytoplasmic enzyme increased significantly, the assumption that total enzyme activity primarily reflects the mitochondrial enzyme activity is valid.

Table 1 (columns 1, 4 and 5) also reports the persistence of the increased HMG-CoA synthase activity after treatment of fed rats with glucagon or mannoheptulose. The elevated activity in crude extracts prepared from rapidly frozen tissue persisted in homogenates of unfrozen livers and in isolated mitochondria even after 1 h of preparation time. The difference between control and treated animals was still present in mitochondria 5 h after preparation (results not shown).

Siess *et al.* (1980) reported a decrease in succinyl-CoA content of extracts prepared from rapidly frozen livers of glucagon-treated rats. We have confirmed this result (Table 1, column 6). The change persists in homogenates and in mitochondria isolated from glucagon-treated rats (Table 1, columns 7 and 8). Mannoheptulose treatment of fed rats resulted in similar persistent decreases in succinyl-CoA content.

To investigate whether the changes in HMG-CoA synthase activity were related to the changes in succinyl-CoA content, we generated a range of succinyl-CoA concentrations in isolated rat and ox liver mitochondria by adding 2-oxoglutarate to uncoupled mitochondria in the presence of malonate (Fig. 2*a*). Intramitochondrial succinyl-CoA increased from 0.05 to 0.7 nmol/mg of mitochondrial protein with increasing 2-oxoglutarate concentrations. Assuming 1 μl of matrix water per mg of mitochondrial protein, this corresponds to a range of 0.05–0.7 mM-succinyl-CoA. Acetyl-CoA remained low and approximately constant at about 0.07 nmol/mg of mitochondrial protein.

Fig. 2(*b*) shows that HMG-CoA synthase activity in intact mitochondria from ox or rat liver decreased with increased 2-oxoglutarate in the medium. Fig. 2(*c*) shows the data for succinyl-CoA content from Fig. 2(*a*) plotted against the data for HMG-CoA synthase activity from the same incubations (Fig. 2*b*). Increased succinyl-CoA levels correlated with decreased HMG-CoA synthase activities.

Short-term treatment of fed rats with glucagon or mannoheptulose increased HMG-CoA synthase activity and decreased succinyl-CoA levels in subsequently isolated mitochondria (Table 1). However, incubation with 2-oxoglutarate abolished these differences. Fig. 3 shows that there is the same negative correlation between succinyl-CoA content and HMG-CoA synthase activity in mitochondria isolated from glucagon-treated, mannoheptulose-treated or control rats. Under these artificial conditions in which differences in endogenous succinyl-CoA levels were abolished, the enzyme in mitochondria from rats treated in different ways responds to generated succinyl-CoA in the same way, supporting the suggestion that the hormone effects are mediated entirely through the succinyl-CoA content of the mitochondria.

DISCUSSION

In this paper we show that mitochondrial HMG-CoA synthase activity of rat liver increases substantially with a number of treatments designed to increase ketogenic

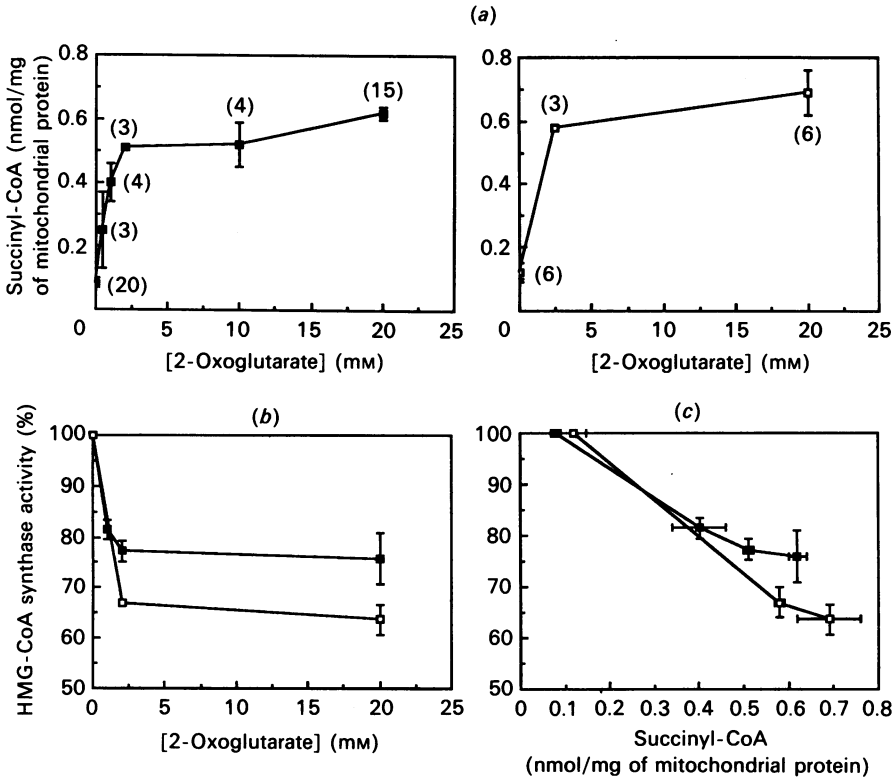

Fig. 2. Effect on intramitochondrial succinyl-CoA concentration and HMG-CoA synthase activity of varying the concentration of 2-oxoglutarate in the incubation system

Rat (■) or ox (□) liver mitochondria (5 mg of mitochondrial protein/ml) were incubated aerobically at 30 °C for 4 min in 1 ml of medium containing 130 mM-KCl, 20 mM-Tris/HCl, 5 mM-$MgCl_2$, 0.2 mM-dithiothreitol, pH 7.2, 1 mM-malonate, 0.5 μM-carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, and 2-oxoglutarate as indicated. Values shown are means ± S.E.M. for the numbers of experiments shown in parentheses. (*a*) Effect on intramitochondrial succinyl-CoA. (*b*) Effect on HMG-CoA synthase activity. This was measured in duplicate assays and expressed as the percentage of activity in the controls assayed at the same time, but containing no 2-oxoglutarate. (*c*) Correlation between rat and ox liver HMG-CoA synthase activity and succinyl-CoA content in isolated liver mitochondria. Data are from (*a*) and (*b*).

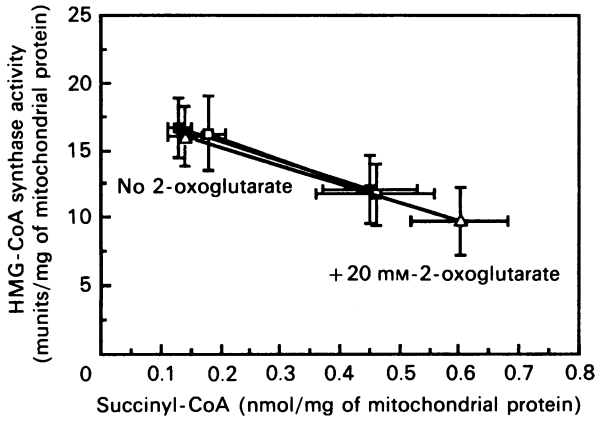

Fig. 3. Correlation between HMG-CoA synthase activity and succinyl-CoA content in rat liver mitochondria

Mitochondria isolated from rats treated as shown [■, glucagon; □, control (sham-injected); △, mannoheptulose] were incubated for 60 min in a medium in which succinyl-CoA levels could be manipulated (high with 20 mM-2-oxoglutarate present, low without), and HMG-CoA synthase activity and succinyl-CoA content were measured. Values are means ± S.E.M. of results from experiments with four rats.

capacity by direct action of glucagon on the liver. These effects are not caused by insulin. Insulin levels rise after glucagon injection, but fall after mannoheptulose injection (Tullson & Aprille, 1986), yet both treatments caused similar increases in HMG-CoA synthase activity. The effects of diet and other treatments on HMG-CoA synthase persist in isolated mitochondria.

Williamson *et al.* (1968) found that HMG-CoA synthase activity increased after alloxan-induced diabetes and fat feeding, but not after 48 h starvation. The effects that we report are substantially greater. One major difference is that we performed all experiments on anaesthetized rats. We have found that HMG-CoA synthase activity in unanaesthetized rats is relatively high and rather variable, perhaps because of differences in endogenous adrenaline levels at the time of killing. The experiments of Williamson *et al.* (1968) were conducted on animals that had not been anaesthetized, which may have caused the smaller differences that they reported and may have masked the effects of starvation on HMG-CoA synthase activity.

Our findings indicate that glucagon causes increased HMG-CoA synthase activity by modification of existing enzyme rather than by synthesis of new enzyme. Firstly, the effect is rapid: activity of HMG-CoA synthase is increased within 10 min of glucagon injection (Table 1).

Secondly, HMG-CoA synthase is present in very large amounts in liver mitochondria (approx. 1.7% of mitochondrial protein; Lowe & Tubbs, 1985*a*), so doubling the amount of enzyme would require a large amount of net protein synthesis to occur. Thirdly, the differences in activity are abolished by manipulation of the mitochondrial succinyl-CoA content.

We also show that the succinyl-CoA content of crude liver extracts, homogenates and isolated mitochondria is lowered by treatments that raise HMG-CoA synthase activity. By manipulation of succinyl-CoA concentrations *in situ* we show that there is a negative correlation between HMG-CoA synthase activity and succinyl-CoA levels in isolated control mitochondria. Incubation of mitochondria with 2-oxoglutarate to change succinyl-CoA content abolishes the differences in HMG-CoA synthase activity between mitochondria isolated from control rats and rats treated with glucagon or mannoheptulose. These observations strongly suggest that the elevated enzyme activity in treated rats may be explained by a decrease in the amount of enzyme which is succinylated. This conclusion is supported by measurement of the succinylation state (Quant *et al.*, 1989).

It therefore seems feasible that glucagon may stimulate HMG-CoA synthase by rapidly lowering the hepatic intramitochondrial succinyl-CoA concentration and hence causing de-inhibition of the enzyme by desuccinylation. The data presented in this paper strongly support the hypothesis proposed by Lowe & Tubbs (1985*b*), that the flux through the HMG-CoA cycle is regulated by the extent of succinylation of HMG-CoA synthase and that this may be a factor in the control of ketogenesis *in vivo*.

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