Distinct physiological roles of animal succinate thiokinases

Association of guanine nucleotide-linked succinate thiokinase with ketone body utilization

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Two distinct succinate thiokinases have recently been shown to exist in animal tissues, one specific for guanine nucleotide and the other for adenine nucleotide. Their physiological roles have here been investigated by comparing the levels of the two enzymes in liver and brain of normal and diabetic rats. A marked rise in the level of brain guanine nucleotide-linked succinate thiokinase in the diabetic condition is consistent with an enhanced utilization of ketone bodies and hence with the associated elevated demand for succinyl-CoA for the activation of acetoacetate. Taken together with the reported mitochondrial values of the ATP/ADP and GTP/GDP ratios, the results are interpreted to indicate that the adenine nucleotide-linked enzyme functions as a component of the citric acid cycle whereas the guanine nucleotide-linked enzyme functions in the opposite metabolic direction to produce succinyl-CoA from succinate.

Succinate thiokinase *Guanine nucleotide* *Diabetes* *Streptozotocin* *Ketone body utilization* *(Brain) Metabolism*

1. INTRODUCTION

Succinate thiokinase (succinyl-CoA synthetase) (STK) catalyses the reversible reaction:

ADP/GDP + P_i + succinyl-Coa⇌ATP/GTP + succinate + CoA-SH

It is generally presented in textbooks of biochemistry that animal STKs are linked to the guanine nucleotides (G-STK) whereas bacterial and plant STKs operate with the adenine nucleotides (A-STK). We recently reported [1] the existence of distinct A-STK and G-STK enzymes in mammalian tissues with wide variation in the ratio of the two.

The presence of these two STKs poses the question of their physiological roles. One role for STK lies in the citric acid cycle, where it converts succinyl-CoA into succinate. In mammalian tissues, succinyl-CoA also plays a crucial role in ketone body metabolism, activating acetoacetate to acetoacetyl-CoA, with 3-oxoacid CoA-transferase (OAT). To maintain the flux of ketone body utilization, it is necessary to re-cycle the succinate to succinyl-CoA (see fig.1) again using STK. These different demands on the STK-catalysed reaction may well be accommodated by the action of distinct STKs.

Ketone body meteabolism is perturbed in diabetes; the ability to induce diabetic ketoacidosis, e.g. by treatment with streptozotocin [2], thus offers a means of investigating the participation of STK. The results reported here clearly implicate G-STK in ketone body metabolism and,

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as a corollary, we propose that A-STK may function in the citric acid cycle.

2. EXPERIMENTAL

Female Wistar rats (180–190 g) were fed ad libitum. Diabetes was induced by intravenous administration, under anaesthesia, of streptozotocin (150 mg/kg body wt). After 48 h the animals were decapitated; blood samples were deproteinised and glucose concentrations determined as in [3]. Control rats received no treatment. Tissues (groups of 5 rats) were removed immediately and homogenized in 0.1 M Na/K phosphate buffer (pH 8.0) containing 1 mM EDTA using an Ultra-Turrax homogenizer (5×15 s bursts with cooling). After centrifugation ($30000 \times g$ for 30 min at 4°C) the supernatants were used without further treatment.

STK activity was assayed as polarographically [4] in the presence of 0.5 mM ADP or GDP. Protein concentrations were determined as in [5].

3. RESULTS AND DISCUSSION

Blood glucose levels in the streptozotocin-treated rats were high (5.38 ± 0.77 mg/ml) in comparison with the control rats (0.61 ± 0.05 mg/ml). These elevated levels indicate the diabetic condition of the treated rats and resemble those found by Schein et al. [2] who also reported a 5-fold rise in ketone body concentration.

Diabetes is accompanied by enhancement of ketone body production by the liver and utilization by the brain [6,7]. We therefore examined liver and brain in diabetic and normal rats. Typical results are shown in table 1. In both tissues, A-STK activity doubled in diabetes. In brain, this may be associated with increased ketone body oxidation via the citric acid cycle; enhanced respiration in liver has also been reported to accompany ketogenesis [8].

The G-STK activity of liver fell in diabetes but that of brain increased strikingly (5- to 10-fold in a series of experiments). As a consequence, the ratios G-STK/A-STK changed very significantly. Whereas in the untreated animals A-STK was the dominant form in brain and G-STK in liver, the reverse was the case in the diabetic rats (table 1).

The ability of the brain to use ketone bodies is not believed to be an enzymic adaptation, as the enzymes responsible for ketone body metabolism (hydroxybutyrate dehydrogenase, 3-oxoacid CoA-transferase and acetoacetyl-CoA thiolase) are present in high activities that are unchanged by diabetes [9].

Table 1

Effect of diabetes on the levels of succinate thiokinases

Treatment	Tissue	Activity (nmol/min per mg protein)		Ratio G-STK/A-STK
		G-STK	A-STK	
Control	brain	0.9	1.5	0.6
	liver	4.5	1.5	3.0
Streptozotocin	brain	10.0	2.5	4.0
	liver	1.3	3.0	0.43

Changes in the activity of STK in diabetes have not previously been studied, though it has been suggested that the availability of succinyl-CoA could control ketone body utilization [10,11]. Our finding that G-STK increases in brain suggests that it may produce the succinyl-CoA required for ketone body activation. Fig.1 shows the scheme for ketone body activation and subsequent oxidative metabolism via the citric acid cycle. At two points succinyl-CoA is converted to succinate, by reaction with acetoacetate (catalysed by OAT) and by reaction with nucleoside diphosphate and inorganic phosphate (catalysed by STK). In the latter case, the succinate is further oxidised by the later steps of the citric acid cycle to reform oxaloacetate for condensation with the acetyl-CoA produced from the ketone bodies. In the former case, by contrast, the succinate must be reconverted to succinyl-CoA in order to maintain the flow of acetoacetate to acetoacetyl-CoA. This re-cycling could be achieved either by metabolism of succinate round the citric acid cycle, via oxaloacetate and citrate, or by the action of STK operating in the reverse direction.

Ottaway and co-workers [12–14] suggested that ketone body utilisation requires the partitioning of succinyl-CoA (formed by oxoglutarate dehydrogenase) between OAT and STK; as the K_m for succinyl-CoA is very much lower for STK than for OAT, they proposed that inhibition of STK is

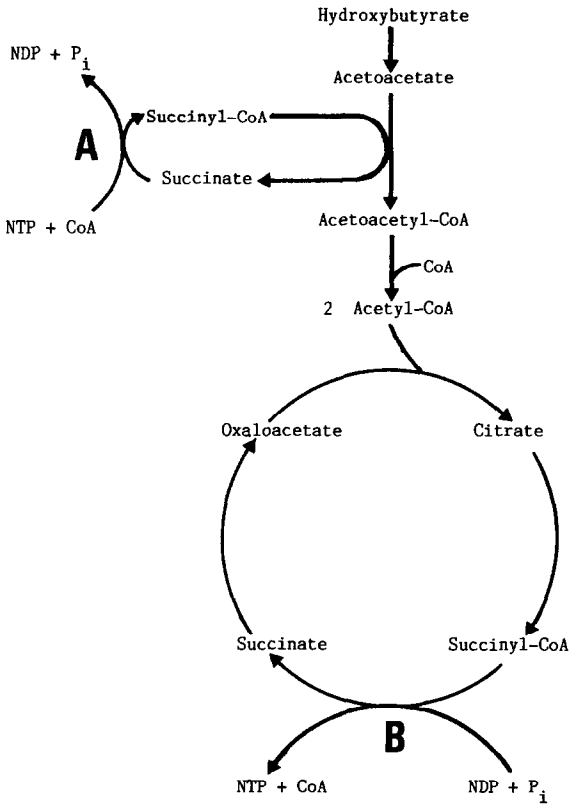

Fig.1. Scheme of ketone body metabolism. NDP and NTP represent nucleoside diphosphate and triphosphate respectively. A and B are two points at which the succinate thiokinase reaction occurs.

necessary to permit ketone body activation. Such inhibition might be effected by maintaining a high NTD/NDP ratio. In support of this proposal they cited [14] mammalian mitochondrial values of ATP/ADP ~1 and GTP/GDP ~100. The proposed inhibition of STK to permit ketone body metabolism, when that very metabolism requires citric acid cycle flux, presents a difficulty, but our discovery of the existence of two STK enzymes, each specific for its nucleotide substrate, offers a solution. The two enzymes allow metabolic compartmentation of the two distinct roles of STK. As part of the citric acid cycle, the A-STK can function to phosphorylate ADP to ATP at an ATP/ADP ratio of ~1, while the G-STK can catalyse the opposite reaction (succinate to succinyl-CoA) at a high GTP/GDP ratio. Our finding that the level of G-STK in brain increases dramatically with increased ketone body utilization supports this proposal and also suggests that re-cycling of succinate to succinyl-CoA is indeed achieved directly by the action of G-STK. It is conceivable that the two STKs are differentially organised within the mitochondrion.

It has been commented above that diabetes leads to increased ketogenesis in the liver. The reduction in the level of liver G-STK which we have observed following streptozotocin treatment may be related to the recent report [15] of the succinylation and inactivation of hydroxymethylglutaryl-CoA synthase by succinyl-CoA. This enzyme functions in ketone body formation; its inactivation by succinyl-CoA may therefore be a control mechanism in ketogenesis. Stimulation of ketogenesis, e.g. by glucagon, appears to be accompanied by reduction in the concentration of succinyl-CoA in the liver [16]; this reduction might lead to de-inhibition of hydroxymethylglutaryl-CoA synthase and hence to increased ketogenesis [15]. Our observation of a decreased level of liver G-STK is therefore consistent with a reduction in succinyl-CoA formation and may be associated with these other processes.

It was recently reported [17] that streptozotocin has a direct inhibitory action on mouse liver G-STK in vitro (50% inhibition at 10 nM streptozotocin), though no in vivo alteration of the activity of this enzyme was observed. We have tested the effect of 100 nM streptozotocin on rat liver and brain G-STK but failed to observe any inhibition. Hence our finding of reduced G-STK in rat liver in vivo following streptozotocin treatment is unlikely to be due to inhibition of activity but rather to a reduction in the level of the enzyme.

In conclusion our results indicate that A-STK and G-STK play distinct metabolic roles within animal tissues. Moreover, as succinyl-CoA is not only involved in citric acid cycle and ketone body metabolism but also acts as a precursor of porphyrins, it may be that STK performs a further function in providing the succinyl-CoA for that biosynthetic process. This is being investigated.

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