Inhibition by streptozotocin of the activity of succinyl-CoA synthetase in vitro and in vivo

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The activity of succinyl-CoA synthetase from mouse liver and kidney was inhibited by streptozotocin in vitro. Streptozotocin behaved essentially as a non-competitive inhibitor, and the following kinetic values were obtained (in the presence of 10 nM streptozotocin): apparent K_m 1.7 mM, apparent K_i 10 nM, and k_{cat} 440 nkat·kg^{-1}. Compared with non-diabetic control mice, the succinyl-CoA synthetase activity was significantly decreased in the islets and kidneys of mice with early (1 h) and manifest ($\geq$ 2 days) streptozotocin diabetes, whereas the activity in the liver was not significantly altered. Inhibited succinyl-CoA synthetase activity is believed to play a prominent role in the cellular effects of streptozotocin.

Diabetes *Enzyme inhibition* *Streptozotocin* *Succinyl-CoA synthetase*

1. INTRODUCTION

Streptozotocin, which chemically is a 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose, possesses antibiotic, diabetogenic, antitumoral and tumor-inducing properties, and, besides alloxan, represents the most commonly used compound for experimental production of diabetes. The diabetogenicity is related to necrosis of islet B-cells, the nitrosourea moiety providing the cytotoxic effect and the glucose moiety facilitating the entry of the drug into the B-cells [1].

The mechanism underlying the cellular effects of streptozotocin remains as yet unknown. According to one current hypothesis, the diabetogenicity is due to induction of DNA strand breaks and stimulated poly(ADP-ribose) synthetase activity leading to intracellular NAD depletion [2]. The same hypothesis has been proposed for alloxan diabetes [2]. However, the biochemical changes in alloxan diabetes are not identical with those in streptozotocin diabetes, although there are considerable similarities. According to our hypothesis, alloxan diabetes is dependent on structural and biochemical alterations in the mitochondria of alloxan-sensitive organs, with inhibited activity of citric acid cycle enzymes [3–5] and disturbed Ca^{2+} homeostasis [6–10] as prominent features. Our finding of alloxan-induced Ca^{2+} release from isolated mitochondria has recently been verified by others [11].

Alloxan inhibits the activity of succinyl-CoA synthetase [3], and in our studies of different aspects of experimental diabetes we have observed that streptozotocin is a potent inhibitor of the activity of the same enzyme. Since this enzymatic inhibition might be of importance for the cellular effects and the diabetogenicity of streptozotocin, description is given here for the first time.

2. MATERIALS AND METHODS

Enzyme activities were determined in sonically disrupted, isolated mitochondria from liver and kidneys of 3–4-month-old C57BL–KsJ– +/+ mice of either sex. For in vivo experiments, streptozotocin (200 mg/kg body wt) or saline (controls) was injected i.p. in KsJ mice, and for islet studies in *ob/ob* mice [4,5,7,10], and the organs were removed 1 h or 2–7 days later. Blood glucose measurements with the glucose oxidase method disclosed hyperglycemia in the initial stage of diabetes development, and more marked hyperglycemia

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(only animals with ≥18 mmol/l used here) in the stage of manifest diabetes. All control mice were normoglycemic. The activity of succinyl-CoA synthetase was determined at 235 nm [12], that of citrate synthetase was followed at 232 nm [13], and that of aconitase was measured as described in [3].

3. RESULTS AND DISCUSSION

The injection in experimental animals of either alloxan or streptozotocin results in manifest diabetes, usually within 2 days, and the islet B-cells are structurally altered and blood glucose concentration changed already during the first hour following treatment, the earliest morphological alteration being localized to the mitochondria and the pattern of Ca^{2+}-containing pyroantimonate precipitation 10 min after alloxan injection [6]. Although the development and manifestations of alloxan diabetes are in many ways similar to those of streptozotocin diabetes, there are differences in other respects [1], and therefore it is probable that the effects of these drugs on the cellular level are similar, but not identical.

The rapid development of morphological and biochemical changes in animals treated with alloxan or streptozotocin points to a possible role of inhibition of essential enzymes. A preceding study disclosed that alloxan inhibits the activity of most citric acid cycle enzymes, including citrate synthetase, aconitase and succinyl-CoA synthetase [3], and in vivo and additional in vitro data supported the finding of inhibition of mitochondrial aconitase [4,5].

Here, streptozotocin (100 μM) did not alter the activity of mitochondrial aconitase or citrate synthetase (not shown). In contrast, streptozotocin markedly inhibited the activity of succinyl-CoA synthetase. 50% inhibition of enzyme activity was found at approx. 10^{-8} M streptozotocin with enzyme from both mouse liver and mouse kidney. Streptozotocin behaved essentially as a non-competitive inhibitor as demonstrated in fig.1 for enzyme from mouse liver. In the presence of 10 nM streptozotocin the apparent K_i was 10 nM. The apparent K_m was 1.7 mM, and the k_{cat} 440 nkat · kg^{-1}. The values represent the means of data obtained from Lineweaver-Burk and Eadie-Hofstee plots and direct computerized calculation.

Thus, the preceding [3] and the present data indicate that the activity of syccinyl-CoA synthetase is inhibited by the two most well-known diabetogenic compounds detected so far. Because of the central role of the citric acid cycle for cellular function, this kind of inhibition might lead to or contribute to pathological alterations, including the manifestations of diabetes. If this is the case, it ought to be possible to demonstrate inhibited enzyme activity in diabetic animals also under in vivo conditions, not only in the manifest stage of the disease, but also in the initial stage, shortly after the injection of the diabetogenic compound.

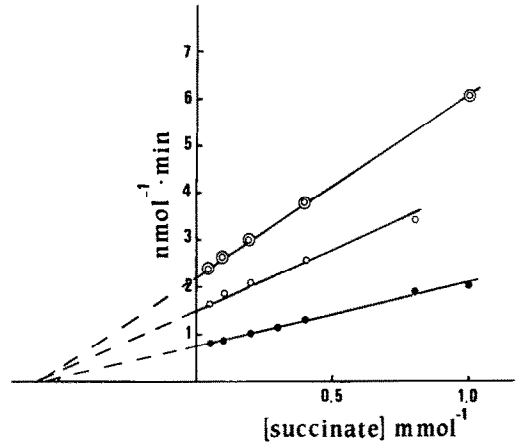

Fig.1. Lineweaver-Burk plot of succinyl-CoA synthetase activity in mouse liver mitochondria in the absence (●—●) and presence of 10 nM (○—○) and 100 nM (◎—◎) streptozotocin.

Consequently we determined the activity of succinyl-CoA synthetase in different stages of streptozotocin diabetes, using organs (islets and kidneys) with 'high sensitivity', and one organ (liver) with 'lower sensitivity' to diabetogenic drugs. The data in table 1 show that the activity of succinyl-CoA synthetase was significantly decreased in the islets and kidneys of mice, in both the early and manifest stages of the disease. Although the enzyme activity in the liver was lower in the diabetic mice than in the control animals, the decrease was not statistically significant.

The occurrence of significant in vivo inhibition of enzyme activity in the islets and kidneys, but not in the liver, is consistent with differences among the organs in the sensitivity to diabetogenic compounds. Factors appear to be present in vivo which make the liver more resistant to diabetogenic in-

Table 1

Succinyl-CoA synthetase activity in mice injected with streptozotocin (STZ) or saline (controls)

Animal group; interval after injection	$10^2 \times$ spec. act. ($\Delta A_{240} \cdot$ mg protein$^{-1} \cdot$ min^{-1})		
	Liver	Kidneys	Islets
Controls	29.1 ± 0.9 (8)	36.6 ± 1.2 (8)	32.8 ± 1.4 (5)
STZ; 1 h	25.9 ± 1.0 (6)	11.1 ± 0.8 (6)[a]	8.5 ± 0.9 (3)[a]
STZ; ≥2 days	26.5 ± 1.1 (6)	3.5 ± 0.4 (6)[a]	2.1 ± 0.3 (4)[a]

The substrate was 10 mM succinate. Values are means ±SE with the number of observations given in parentheses. Statistical difference from control values by Student's t-test: [a]$p < 0.001$

fluences than the islets and kidneys. It should also be noted that there are organ differences in the in vivo uptake of diabetogenic compounds [7]; higher concentrations of such compounds may be reached in organs which are freely permeable to glucose than in those which are dependent on insulin.

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