Alterations in mitochondrial aconitase activity and respiration, and in concentration of citrate in some organs of mice with experimental or genetic diabetes

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Mouse islets (not used for respiration), kidneys and liver were studied in early and manifest alloxan diabetes, and in genetic diabetes. In these organs the mitochondrial aconitase activity was lower, state 3 respiration with citrate or pyruvate plus malate (but not with succinate) was decreased, and the concentration of citrate was increased, compared with non-diabetic control mice. The alterations suggest a role of lowered activity of mitochondrial aconitase in alloxan diabetes, and probably also in genetic diabetes.

Aconitase *Alloxan* *Citrate* *Diabetes* *Mitochondria* *Respiration*

1. INTRODUCTION

Preceding in vivo and in vitro studies, mainly directed to liver, kidneys and islets, have disclosed that alloxan is taken up in mitochondria [1,2], and alters their structure and function [3–5]. These changes may be due to inhibition of mitochondrial aconitase, since it has recently been reported for the first time that alloxan induces marked decrease of the activity of that enzyme in vitro [6], and since alloxan inhibits mitochondrial respiration, most efficiently when the substrate is citrate or pyruvate plus malate, less efficiently when isocitrate, α-ketoglutarate, glutamate and malate are the substrates, and with least effect when the respiration is supported by succinate [4,5,7]. A similar substrate dependence has been observed for the effect of alloxan on the volume and retention of accumulated Ca^{2+} in isolated mitochondria [7].

The aim of this work was to study whether the activity of mitochondrial aconitase is inhibited in early and manifest alloxan diabetes, and/or in genetic diabetes. In addition to measurement of aconitase activity, the mitochondrial oxygen consumption was studied using different substrates, and the tissue concentration of citrate was determined, since these might be altered if the mitochondrial aconitase activity is decreased.

2. MATERIALS AND METHODS

As in preceding studies [1,5,8], the animals were from local stocks of non-diabetic (C57BL-KsJ-+/+ and *ob/ob*) and genetically diabetic (C57BL-KsJ-*db/db*) mice of both sexes; the *ob/ob* mice being used as controls and for induction of alloxan diabetes for islet studies, and the +/+ mice being used for the same purpose for liver and kidney studies. Alloxan, 200 mg/kg body wt, or saline (controls) was injected i.p. in fasted (21 h) mice, which were allowed to eat again 4 h after the injections. The *db/db* mice were studied in the late stage of the disease [8,9]. Blood glucose was checked in all mice.

The liver, kidneys and islets, isolated by the collagenase technique [1,5,8], were studied. The mitochondrial aconitase activity was determined as previously described (without activation) [6]. The oxygen consumption of isolated mitochondria was determined at 25°C with a Clark-type electrode and 1 mg mitochondrial protein/ml, in a medium containing 200 mM sucrose, 10 mM Hepes,

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50 mM KCl, 4 mM $MgCl_2$ and 3 mM P_i (pH 7.2), in the presence (in combination with succinate) or absence of 1 μM rotenone.

The concentration of citrate was determined enzymatically, using instant freezing in situ for liver and kidneys, and (for technical reasons) using freeze-dried specimens of islets according to [10]. Protein was measured spectrophotometrically.

3. RESULTS

No significant difference in the activity of mitochondrial aconitase was observed between fed and fasted control mice. Compared with non-diabetic control mice, the mitochondrial aconitase activity was significantly lower in the liver, kidneys and islets in early and manifest alloxan diabetes, and a less marked, although significant, decrease in enzymatic activity was found in all organs studied in genetic diabetes (table 1).

The oxygen consumption could not be adequately measured in islet mitochondria. No significant difference was seen between fed and fasted control mice. State 3 respiration with pyruvate plus malate (table 2) or with citrate (not shown in table) was significantly decreased in the liver and kidneys in

Table 1

Mitochondrial aconitase activity in mice with genetic diabetes, and in those with experimental diabetes in which the tissues were sampled at different intervals after the injection of alloxan

Animal group; interval after injection	$10^3 \times$ spec. act. ($\Delta A_{240} \cdot$ mg protein$^{-1} \cdot$ min^{-1})		
	Liver	Kidneys	Islets
Controls	29.5 ± 2.3 (14)	45.5 ± 2.7 (14)	39.4 ± 4.4 (3)
Alloxan, 10 min	8.9 ± 4.0 (4)[c]	21.0 ± 5.1 (4)[c]	–
Alloxan, 1 h	10.1 ± 3.9 (6)[c]	18.4 ± 5.1 (6)[c]	11.0 ± 5.6 (3)[b]
Alloxan, 2–4 days	14.7 ± 3.7 (6)[b]	26.5 ± 4.3 (6)[b]	23.5 ± 4.7 (3)[b]
Controls	33.4 ± 3.7 (7)	49.0 ± 4.2 (7)	46.0 ± 3.7 (3)
db/db	19.8 ± 4.8 (7)[a]	24.2 ± 6.7 (7)[a]	28.0 ± 6.1 (3)[a]

The substrate is 1 mM citrate, the figures are means ± SE and the number of observations is given in brackets. Statistical difference from corresponding control values by Student's *t*-test: [a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$. Details are given in the text

Table 2

Oxygen consumption in liver and kidney mitochondria of mice with genetic diabetes or alloxan diabetes

Animal group; interval after injection	Substrate	ngatom O$\cdot$min$^{-1}\cdot$mg protein^{-1}			
		Liver		Kidneys	
		State 3	State 4	State 3	State 4
Controls	P + M	70.9 ± 5.1 (9)	18.3 ± 3.3 (9)	62.5 ± 4.8 (8)	14.9 ± 3.8 (8)
Controls	S	145.1 ± 23.4 (8)	34.7 ± 4.9 (8)	131.4 ± 18.1 (7)	35.4 ± 6.1 (7)
Alloxan, 1 h	P + M	25.1 ± 5.1 (4)[c]	20.6 ± 4.5 (4)	17.4 ± 4.8 (3)[c]	10.0 ± 4.4 (3)
Alloxan, 1 h	S	123.5 ± 18.9 (4)	41.2 ± 4.7 (4)	100.5 ± 10.1 (3)	40.9 ± 5.6 (3)
Alloxan, 2–4 days	P + M	47.1 ± 4.3 (5)[b]	17.8 ± 4.0 (5)	27.6 ± 5.1 (5)[c]	12.8 ± 3.9 (5)
Alloxan, 2–4 days	S	132.0 ± 24.9 (4)	39.0 ± 5.1 (4)	107.7 ± 9.5 (4)	33.3 ± 8.1 (4)
Controls	P + M	65.0 ± 5.5 (6)	21.0 ± 4.0 (6)	58.4 ± 5.1 (3)	15.7 ± 4.4 (3)
Controls	S	138.9 ± 18.5 (6)	32.4 ± 4.3 (6)	139.8 ± 21.0 (3)	21.4 ± 2.1 (3)
db/db	P + M	31.3 ± 4.0 (6)[c]	18.0 ± 3.9 (6)	21.8 ± 4.0 (3)[b]	12.5 ± 3.8 (3)
db/db	S	125.4 ± 19.9 (6)	36.4 ± 3.8 (6)	131.1 ± 17.5 (3)	38.4 ± 6.5 (3)

The substrates are 1 mM pyruvate (P), malate (M) and succinate (S). Details as in table 1 and in the text

Table 3

Concentration of citrate in liver, kidneys (μmol/100 g wet wt) and islets (μmol/100 g dry wt) of mice with genetic or alloxan diabetes

Animal group; interval after injection	Fed (F)/ starved (S)	Liver	Kidneys	Islets
Controls	S	61.4 ± 7.4 (5)	44.5 ± 5.1 (5)	59.8 ± 6.3 (3)
Alloxan, 1 h	S	98.9 ± 9.6 (5)[a]	126.7 ± 8.4 (5)[c]	173.5 ± 17.1 (3)[c]
Controls	F	31.3 ± 4.8 (7)	41.3 ± 4.4 (7)	63.6 ± 6.9 (3)
Alloxan, 2–4 days	F	70.2 ± 9.0 (5)[b]	94.7 ± 8.4 (5)[c]	108.7 ± 11.5 (3)[c]
Controls	F	36.2 ± 5.9 (6)	49.8 ± 5.2 (6)	68.4 ± 8.8 (3)
db/db	F	67.0 ± 6.1 (7)[b]	104.5 ± 9.0 (7)[c]	115.0 ± 12.8 (3)[b]

Details as in table 1 and in the text

early and manifest alloxan diabetes and in genetic diabetes. In contrast, no significant difference was seen in state 3 respiration with succinate, or in state 4 respiration with pyruvate plus malate, citrate or succinate.

Since the concentration of citrate in the liver of fasted control mice was significantly ($p < 0.05$) higher than that in fed controls, differentiation has been made in table 3 between fed and fasted mice. No such difference was found in the kidneys or islets. The concentration of citrate in liver, kidneys and islets was significantly increased in early and manifest alloxan diabetes and in genetic diabetes.

4. DISCUSSION

Preceding in vitro studies indicated that alloxan inhibits the activity of mitochondrial aconitase in liver, kidneys and islets [6,7], and the present observation of lower activity of this enzyme in the same organs both in the early and the manifest stages of alloxan diabetes, than in non-diabetic control mice, suggests that alloxan also inhibits mitochondrial aconitase in vivo. Interestingly, diabetic symptoms have been reported in animals poisoned with the aconitase inhibitor fluorocitrate [11,12], although symptoms from the nervous system and heart usually predominate [13].

A consequence of in vivo inhibition of mitochondrial aconitase, e.g., by fluorocitrate, is accumulation of citrate in different organs, including liver, kidneys and whole pancreas [14–16]. The present finding of increased tissue concentration of citrate therefore lends support to the view of inhibition by alloxan of mitochondrial aconitase. It should also be noted that there are reports on citrate accumulation in diabetic conditions, although the cause has not been elucidated. Thus, increased concentration of citrate has been found, e.g., in liver and whole pancreas in alloxan diabetes [16,17], and in endocrine pancreas in obese hyperglycemic mice [10].

The in vitro effects of alloxan on oxygen consumption, transport and retention of ions, membrane potential and volume of liver, kidney and islet mitochondria are highly dependent on the substrate used [4,5,7], and the present data indicate that this also pertains to the mitochondrial oxygen consumption both in mice treated with alloxan in vivo and in mice with genetic diabetes. Thus, state 3 respiration with citrate or with pyruvate plus malate was decreased in liver and kidney mitochondria, compared with those from non-diabetic controls, whereas state 3 respiration with succinate was not significantly altered. Using higher concentrations of alloxan in vitro, than those supposed to result from in vivo administration, some alterations were also observed in state 3 respiration with succinate and in state 4 respiration with different substrates [4,5,7]. Interestingly, fluorocitrate has been reported to be a potent inhibitor of the oxidation of citrate in liver mitochondria, with marked effect on state 3, but only slight effect on state 4 [18], and depressed state 3 respiration with pyruvate plus malate, but unaffected state 3 respiration with succinate, has been found in heart mitochondria of genetically diabetic mice [19].

The occurrence of a substrate dependence, with highest sensitivity to alloxan in mitochondria respiring on citrate or on pyruvate plus malate, is consistent with inhibition of mitochondrial aconitase. Moreover, the existence of similar alterations in aconitase activity, oxygen consumption and concentration of citrate in mice with experimental diabetes and in those with the genetic disease, suggests similar, although not necessarily identical, diabetogenic mechanism(s) in these kinds of diabetes.

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