CHARACTERIZATION OF THE ADENINE NUCLEOTIDE TRANSLOCASE OF PANCREATIC ISLET MITOCHONDRIA

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1. Introduction

Insulin secretion from the pancreatic β-cell is known to be an energy-dependent process and can be inhibited by agents such as 2,4-dinitrophenol which block mitochondrial ATP production [1]. Despite this fact, the properties of islet mitochondria have not been studied in detail and little is known about islet mitochondrial bioenergetics.

The exchange of ADP and ATP between cytosol and mitochondrial matrix is generally accepted to be the rate-limiting step in the regulation of oxidative phosphorylation [2]. This exchange is catalysed by the adenine nucleotide translocase (ANT) which is located in the inner mitochondrial membrane. Employing specific inhibitors including atractylate, bongkrekic acid and long-chain fatty acyl CoA esters, that ANT has been extensively investigated in heart and liver mitochondria [3–5]. This communication represents the first report of the existence of an active ANT in islet mitochondria and characterizes the carrier in terms of its sensitivity to specific inhibitors. In addition, the effects of these inhibitors upon rates of islet glucose metabolism to CO_2 and of glucose-stimulated insulin secretion are reported.

2. Experimental

2.1. *Materials*

Collagenase (type V, 320 units/mg) and atractylate were obtained from Sigma and carboxyatractylate from Boehringer. [^{14}C]AMP, [^{14}C]ADP and [^{14}C]-ATP were from New England Nuclear and [U-^{14}C]-glucose and insulin assay kits from Amersham. Unlabelled ADP, ATP and palmitoyl-CoA were from PL Biochemicals (Milwaukee WI). Bongkrekic acid was the kind gift of Dr Berends (Delft University of Technology). Other reagents were of the highest purity available commercially.

2.2. *Isolation and incubation of islets*

Islets were isolated from the pancreas of male Sprague-Dawley rats (250 g) by collagenase digestion [6]. Rates of islet insulin release and of islet glucose metabolism were determined as in [7].

2.3. *Preparation of islet subcellular fractions*

Islets were homogenized at 4°C in medium containing 210 mM mannitol, 70 mM sucrose, 10 mM Tris–HCl and 1 mM EDTA (pH 7.4) using a 2 ml ground-glass homogenizer. Subcellular fractions were then isolated by differential centrifugation as in [8]. Submitochondrial particles were prepared as in [9] except that 5 mM ADP was substituted for ATP in the sonication medium. Protein concentrations were determined as in [10].

2.4. *Assay of adenine nucleotide translocase and cytochrome oxidase*

Cytochrome oxidase activity was measured in subcellular fractions spectrophotometrically at 550 nm as in [11]. ANT activity was measured at 4°C using the forward exchange method [12] with slight modification [13]. Unless otherwise stated, all the experiments reported were carried out at least 3 separate times.

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Table 1
Distribution of cytochrome oxidase and adenine nucleotide translocase activities in subcellular fractions of rat pancreatic islets

Fractions	Cytochrome oxidase (nmol . min^{-1} . mg $protein^{-1}$)	Adenine nucleotide translocase activity (nmol . min^{-1} . mg $protein^{-1}$)
Homogenate	n.d.	0.021
Nuclei + cell debris	0.072	–
Mitochondria	0.273	0.019
Secretory granules	0.105	–

n.d., not determined

Cytochrome oxidase and adenine nucleotide translocase activities were determined as in section 2. The values represent an average of 2 expt

3. Results and discussion

Table 1 compares the distribution of ANT and cytochrome oxidase in the subcellular fractions obtained from the differential centrifugation of the islet homogenate. As shown, the mitochondrial fraction represented ~90% of the ANT and 60% of the cytochrome oxidase enzyme activity. Uptake of [^{14}C]ADP was linearly related to the concentration of mitochondrial protein over the range tested (fig.1). The demonstrated lack of penetration of [^{14}C]AMP under identical conditions supports the presence of a specific translocase for ADP and ATP in the islet mitochondria (fig.1).

It has been well documented [3,5,13,14] that atractylate, bongkrekic acid and long-chain acyl CoA esters inhibit the transport of adenine nucleotides

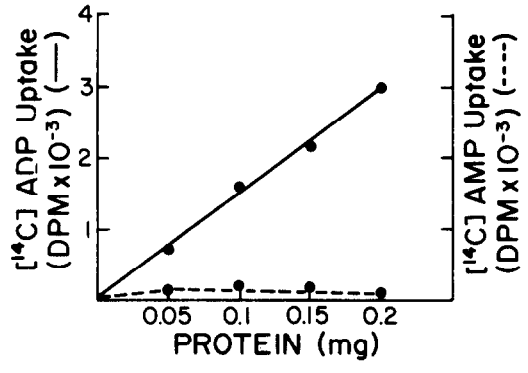

Fig.1. Adenine nucleotide translocase in islet mitochondria. The activity was measured by the forward exchange method and stopped by rapid filtration [12,13]. Each point is an average of 3 expt.

across the inner membrane of mitochondria isolated from heart and liver. It was therefore of importance to investigate the effects of these inhibitors upon [^{14}C]ADP uptake by islet mitochondria. Fig.2 shows the sensitivity of the islet ANT to inhibition by atractylate, bongkrekic acid and palmitoyl CoA. From fig.2, it is apparent that the islet mitochondrial translocase was inhibitable by these agents although the sensitivity of the carrier appeared to be somewhat less than is the case with that of heart and liver mitochondria. With increase in the concentrations of the inhibitors, however, qualitatively similar, dose-related inhibition of [^{14}C]ADP transport was observed with all 3 agents (fig.2A). Thus, ~75% inhibition of [^{14}C]-ADP transport was seen in the presence of 10^{-5} M atractylate or palmitoyl-CoA and of 10^{-4} M bongkrekic acid. Since atractylate acts to inhibit the ANT only at the outer surface of the inner mitochondrial membrane, the effect of this inhibitor was next examined employing mitochondria pre-inverted by sonication. The expected abolition of the atractylate effect confirmed in this experiment (fig.2B) further supports the conclusion that the [^{14}C]ADP transport observed is a function of an islet mitochondrial ANT. By contrast, the inhibitory effects of bongkrekic acid and palmitoyl-CoA were essentially unaltered when these agents were tested using inverted submitochondrial particles (fig.2B).

An alternative mechanism of adenine nucleotide transport has been described in liver mitochondria from perinatal rats [15,16]. This so-called Mg^{2+}-dependent, atractylate-independent uniport system does not appear to be present in islet mitochondria since, in the presence of atractylate, no stimulatory

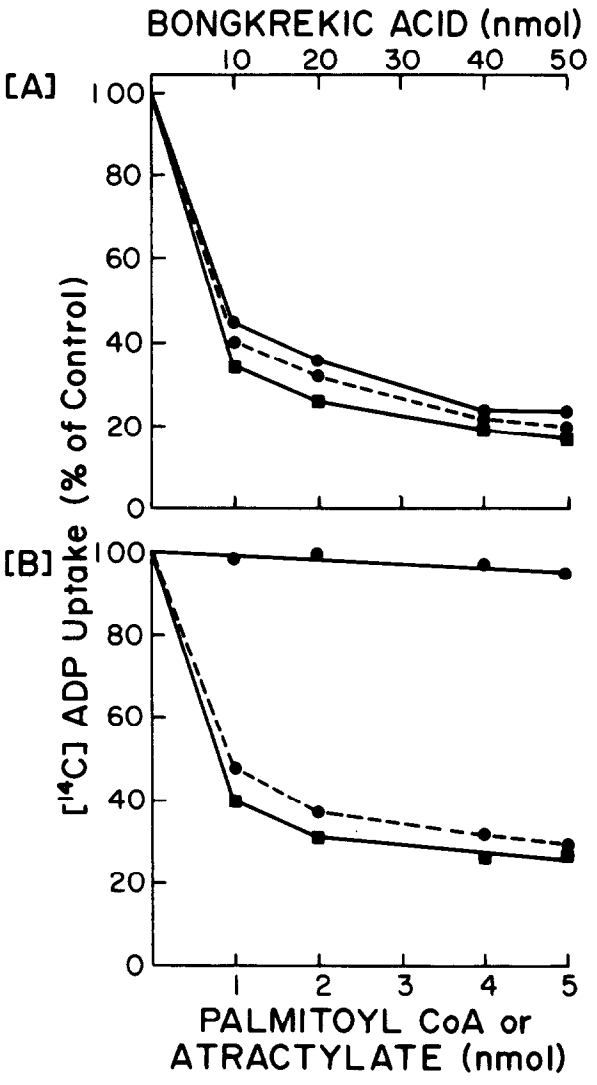

Fig.2. Sensitivity of adenine nucleotide translocase to inhibition by increasing concentrations of atractylate (●——●), palmitoyl-CoA (■——■), and bongkrekic acid (●– – –●) in rat islet mitochondria [A] and submitochondrial particles [B]. The indicated amounts of each inhibitor were added to an incubation mixture of total volume 0.5 ml. Each point is an average of 3 expt.

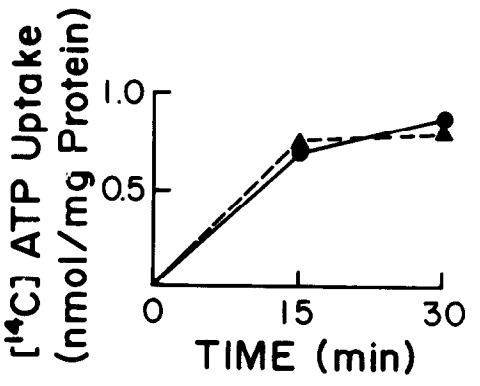

Fig.3. Assay for a functional adenine nucleotide uniport system in islet mitochondria. The experiment was done as in [15]. Mitochondria were depleted of adenine nucleotides by treatment with pyrophosphate. The depleted mitochondria (0.01–0.05 mg protein) were then incubated with 0.1 mM [^{14}C]ATP in the presence of 10^{-5} M atractylate in the presence (●——●) or absence (▲– – –▲) of 10 mM $MgCl_2$ and ATP uptake was determined as in [15]. At the times indicated the suspension was filtered through millipore filters, and rapidly washed with 10 ml 150 mM NaCl. Radioactivity on filters was determined by liquid scintillation counting.

effect of Mg^{2+} upon ATP uptake by adenine nucleotide-depleted islet mitochondria was seen (fig.3).

Finally, the effects of inhibitors of ANT were investigated in intact islets. Atractylate and its carboxy derivative at 1.2×10^{-4} M were both without effect upon rates of islet insulin release or glucose metabolism (table 2). By contrast, bongkrekic acid at 2×10^{-5} and 2×10^{-4} M led to significant inhibitions of both glucose-stimulated insulin release and, at the higher concentration, of metabolism of [U-^{14}C]glucose to $^{14}CO_2$ (table 2). In light of the inhibitory effects seen with bongkrekic acid, it seems likely that the failure of effect of atractylate and carboxyatractylate is attributable to poor penetration of these agents across the islet cell membranes as has been reported for intact heart tissue [17]. In table 2, the effects of 2,4-dinitrophenol (2×10^{-4} M) upon the rate of insulin release and of iodoacetamide (5×10^{-4} M) upon rates of glucose metabolism are included as positive controls.

We have demonstrated the presence in islet mitochondria of an adenine nucleotide translocase which exhibits sensitivity to inhibitors similar to that observed in mitochondria from other tissues. In the intact islet, bongkrekic acid in the concentration range shown to inhibit the translocase of isolated mitochondria, was shown to inhibit glucose-stimulated insulin release. Since phosphoenolpyruvate, which is known to be transported by the ANT in other tissues [18, 19], has been suggested as a possible 'signal' for glucose-stimulated insulin release by inducing Ca^{2+} egress from the mitochondria [20,21], we are now investigating the possible significance of these findings in relation to stimulus–secretion coupling in the islets.

Table 2
Effects of inhibitors on rates of islet insulin release and glucose metabolism

Additions to medium	Rate of insulin secretion ($\mu U \cdot 2$ islets$^{-1} \cdot h^{-1}$) Glucose concentrations in medium		Rate of [U-^{14}C]glucose metabolism to $^{14}CO_2$ (pmol . 5 islets$^{-1} \cdot h^{-1}$)
	2 mM	20 mM	
Control	13 ± 1 (6)	238 ± 19 (10)	70 ± 4 (8)
Atractylate (1.2×10^{-4} M)	14 ± 4 (6)	254 ± 25 (12)	64 ± 6 (8)
Control	12 ± 2 (6)	150 ± 11 (10)	70 ± 4 (8)
Carboxyatractylate (1.2×10^{-4} M)	9 ± 1 (6)	173 ± 14 (10)	77 ± 7 (8)
Control	14 ± 4 (6)	232 ± 28 (8)	109 ± 6 (12)
Bongkrekic acid (2×10^{-5} M)	–	134 ± 22 (10)[a]	–
Bongkrekic acid (2×10^{-4} M)	18 ± 1 (6)	50 ± 8 (10)[b]	60 ± 5 (12)[b]
2,4-Dinitrophenol (2×10^{-4} M)	–	12 ± 10 (4)[b]	–
Iodoacetamide (5×10^{-4} M)	–	–	15 ± 2 (8)[b]

[a] $p < 0.02$ *vs* corresponding control; [b] $p < 0.001$ *vs* corresponding control

Rates of islet insulin release and glucose metabolism were determined as in section 2. Results are expressed as means ± SEM for (no. obs.)

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