NaK-ATPase in Rat Pancreatic Islets*

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Summary. Shifts in the distribution of the monovalent cations Na^+ and K^+ between the extra- and intracellular space seem to be important for the secretory response of the β -cell. An attempt was therefore made to study the enzyme responsible for monovalent cation transport, the (NaK)-activated ATPase. In the presence of NaN_3 as inhibitor of the mitochondrial Mg-ATPase, a NaK-ATPase with a specific activity of $72 \text{ mU} \times \text{mg protein}^{-1}$ could be demonstrated in crude membrane preparations of rat pancreatic islets. The enzyme, which was inactive in the absence of Mg^{++} , needed both Na^+ and K^+ for activation and was inhibited by ouabain and PCMB. The main part of the NaK-ATPase was localized in the microsomal fraction. Glucose, sulphonylureas, somatostatin and diazoxide were without effect on NaK-ATPase.

Key words: Monovalent cations, NaK-ATPase, pancreatic islets, insulin secretion, cation transport.

Insulin secretion in response to secretagogues like glucose, amino acids, glucagon or sulphonylureas is dependent on the presence of extracellular sodium [1, 2]. An increase in intracellular sodium achieved by omission of extracellular potassium or incubation of islet tissue in the presence of ouabain stimulates insulin secretion [2]. The opposite effect was seen

with diphenylhydantoin, in vivo and in vitro [3, 4, 5], which causes a decrease of intracellular sodium. These findings as well as the demonstration of a depolarisation of the β -cell during insulin secretion [6, 7] point to the importance of a sodium-potassium pump for the secretory process.

We have now been able to demonstrate a NaK-ATPase activity in a crude islet membrane fraction comparable to that found in other tissues.

Material and Methods

Pancreatic islets were obtained from male Sprague Dawley rats according to [8]. The animals were pretreated with pilocarpine according to Kuo [9]. About 500 islets were freed from Hanks buffer by 3 washings with 0.2 M sucrose containing 5 mmol/l Tris-buffer pH 7.4. The islet tissue was homogenized by 6 strokes for 20 s in a glass-teflon homogenizer (Dual, Kontes Glass Co., USA). After centrifugation at $6 \times 10^3 \text{ g-min}$ to get rid of cell debris and nuclei, the "crude membrane fraction" was obtained by further centrifugation at $6 \times 10^6 \text{ g-min}$. For the localisation of the ATPase in subcellular fractions the supernatant of the $6 \times 10^3 \text{ g-min}$ centrifugation was spun down at $1 \times 10^5 \text{ g-min}$ to get a "crude mitochondrial fraction". The resulting supernatant was centrifuged at $6 \times 10^6 \text{ g-min}$ in order to obtain the "crude microsomal fraction".

For the ATPase assay, 1–5 μg of membrane protein was incubated in 25 mmol/l Tris-buffer, pH 7.4, in the presence of 5 mmol/l $\gamma\text{-}^{32}\text{P}$ -ATP (specific activity $2\text{--}4 \times 10^5 \text{ dpm}/\mu\text{mol}$ in the assay), 10 mmol/l NaN_3 , 0.1 mmol/l EGTA, 6 mmol/l MgCl_2 , 50 mmol/l NaCl and 5 mmol/l KCl in a total volume of 0.1 ml. Incubations were carried out at 30°C for 60 min and were stopped by addition of 0.2 ml ammonium molybdate (25 g/l in 2.5 N H_2SO_4).

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Table 1. NaK-ATPase activity of a crude membrane fraction in the presence of various inhibitors

	ATPase in the presence of		Significance of difference	n
	Mg ⁺⁺	Mg ⁺⁺ , K ⁺ , Na ⁺		
None	435±62.5	475±82	NS	8
NaN ₃	202.8±21.9	275±26.5	p < 0.05	9
NaN ₃ + Ouabain 0.05 mmol/l	189.4	242.9		2
NaN ₃ + Ouabain 0.5 mmol/l	184.2	220.4		2
NaN ₃ + Ouabain 1.0 mmol/l	161.2±12.3	172.3±17.5	NS	9
NaN ₃ + PCMB 0.1 mmol/l	98±12.9	105±11.2		4
Urea 0.25 M	130±14.2	104.8±11.1		4

For assay conditions see material and methods. Inhibitors were added to the incubation medium. Values are given in mU/mg protein ± SEM

NS: statistically not significant

Table 2. Specific activities and distribution of Mg-ATPase and NaK-ATPase in subcellular fractions from islet tissue

	Mg-ATPase		NaK-ATPase	
	mU/mg protein	% of total activity	mU/mg protein	% of total activity
Crude mitochondrial fraction	88.2±26.1	27.2	62.7±7.0	35.4
Crude microsomal fraction	155.7±26.5	72.8	89.6±11.5	64.6

For assay conditions see material and methods. Mg-ATPase and NaK-ATPase was determined in the presence of 10 mmol/l NaN₃. NaK-ATPase is expressed as the difference between activities measured in the presence of Mg⁺⁺, Na⁺, K⁺ and Mg⁺⁺ alone. Total activity corresponds to the sum of activity in both fractions. Each value represents the mean from 4 experiments ± SEM

Table 3. ATPase activities under different concentrations of cations

Additions	Mg ⁺⁺ absent	Mg ⁺⁺ 6 mmol/l
None	15.3±0.6 (5)	100 (9)
Na ⁺ 60 mmol/l, K ⁺ 5 mmol/l	25.6 (2)	137.2±4.2 (9)
Na ⁺ 60 mmol/l, K ⁺ 0.5 mmol/l	—	104.4±6.5 (4)
Na ⁺ 10 mmol/l, K ⁺ 5 mmol/l	—	129.1±3.8 (3)

The ATPase activity in the presence of Mg⁺⁺ 6 mmol/l (202.8±21.9 mU/mg protein) was set to 100. All assays were performed in the presence of 10 mmol/l NaN₃. Figures in parentheses give the number of experiments

³²P-phosphate liberated from γ-[³²P]-ATP was extracted by shaking with 1 ml isopropylether/butanol (1:1, v/v) for 20 min at 0° C. The extraction rates were for ³²P-ATP only 0.5–1%, for ³²P-phosphate 99%. An aliquot of 0.5 ml of the organic phase was added to 10 ml scintillation liquid (5.5 g Permablend, 50 g naphthalene, 100 ml ethanol, 20 ml ethyleneglycol added to dioxane to give a volume of 100 ml).

Determination of ³²P-activity was performed in a Packard 3385 tricarb scintillation spectrometer. Protein was determined according to Lowry [10], using a human serum preparation as a standard. γ-[³²P]-ATP was purchased from the Radiochemical Centre, Amersham, England. ATP was from Fluka AG, Switzerland, Permablend from Packard Instruments GmbH, Frankfurt, Germany. All other chemicals were reagent grade from E. Merck, AG, Darmstadt, Germany. If not indicated otherwise, activities are given in mU × mg protein⁻¹ ± S.E.M., 1 mU being equivalent to the formation of 1 nmol ³²P-phosphate × min⁻¹ under the experimental condition. ATPase activities without addition of cations were subtracted in each experiment as blank values. Statistical significance was determined by the Wilcoxon rank sum test as modified by Mann and Whitney [11].

Results

As may be seen from Table 1 ATPase activity in crude membrane fractions from islet tissue could be stimulated by magnesium to 435 ± 62.5 mU/mg protein. The addition of sodium and potassium did not significantly affect the ATPase. An exact determination of NaK-ATPase is therefore difficult unless the Mg-ATPase activity is suppressed by NaN₃ which inhibits the mitochondrial Mg-ATPase [12]. Under these conditions, the Mg-ATPase activity was 202.8 ± 21.9 mU/mg protein. By addition of Na⁺ and K⁺ in a concentration of 60 mmol/l and 5 mmol/l respectively, ATPase activity could be significantly increased to 275.1 ± 26.5 mU/mg protein (p < 0.05), indicating an NaK-ATPase activity of 72.3 ± 9.0 mU/mg protein. Ouabain in a concentration of 0.5 mmol/l inhibited the NaK-ATPase nearly 50%, whilst total suppression was achieved with 1 mmol/l. Parachloromercuribenzoate (PCMB) and urea inhibited both the Mg and the NaK-ATPase very strongly.

The rate of ATP-hydrolysis was linear in respect to time and protein concentration.

Table 2 demonstrates the distribution of ATPase activity among different subcellular fractions.

The persistent Mg-ATPase activity in the presence of NaN_3 had its highest specific activity in the 6×10^6 g-min pellet ("crude microsomal fraction"), the main part of the NaK-ATPase was also found in this fraction, its specific activity being slightly higher than in the 1×10^5 g-min pellet ("crude mitochondrial fraction").

The dependence of ATPase activity on cation concentrations is shown in Table 3. In the absence of Mg^{++} , Na^+ and K^+ are ineffective in stimulating the ATPase. Under our assay conditions the maximal Mg-ATPase activity was found at 6 mmol/l Mg^{++} [13].

Addition of 60 mmol/l Na^+ and 5 mmol/l K^+ resulted in an increase of 37.2% over the activity with Mg^{++} alone. A reduction of K^+ from 5 to 0.5 mmol/l almost completely abolished the activity. It was not possible to test Na^+ concentrations below 10 mmol/l due to the presence of 10 mmol/l NaN_3 .

Discussion

NaK-ATPase activity in crude islet membranes amounted to 72 mU/mg protein. This value is only slightly lower than NaK-ATPase activities in liver (130–230 mU/mg protein), heart muscle (170–250 mU/mg protein), skeletal muscle (90–170 mU/mg protein) or brain (200–1700 mU/mg protein) [14]. Considering the fact that the latter activities were related to purified plasma membrane fractions, we can assume that NaK-ATPase activity in islet tissue is indeed comparable to that in other mammalian tissues.

In the crude membrane fraction studied, a very high Mg-dependent ATPase masked the NaK-ATPase activity. Part of this Mg-stimulated ATPase can be assumed to be identical with the mitochondrial Mg-ATPase described in other tissues (for review see [15]). The fact that this enzyme can be suppressed by NaN_3 , as has been shown by Vigers and Ziegler [12], provided a tool to uncover the NaK-ATPase. According to our findings (Table 1), NaN_3 seems not to inhibit NaK-ATPase, which was found to be even more active in the presence of this compound.

Several data support the interpretation that the NaK-ATPase found in islets is identical with the transport ATPase for monovalent cations of several tissues described in the literature (for review see [16]).

The inhibition by ouabain (a characteristic of these enzymes) could be demonstrated as well as the suppression by PCMB, indicating that this enzyme from islets also contains a SH-group essential for its activity (Table 1).

The enzyme studied was dependent on the presence of Mg^{++} (Table 3). As we had observed an inhibitory action of higher amounts of Na^+ , the final concentration of Na^+ in the assay was kept at 60 mmol/l. Under these conditions K^+ in concentrations between 5 mmol/l and 20 mmol/l gave optimal stimulation. Higher concentrations of K^+ resulted in a reduction of NaK-ATPase activity. The importance of the second monovalent cation is demonstrated by the fact that a reduction of K^+ to 0.5 mmol/l resulted in a complete loss of activity (Table 3). Our data are compatible with the idea that the NaK-ATPase of islets is located in the plasma membrane, since two thirds of the total activity was found in the "microsomal fraction". Very recently Lernmark et al. [17] found high activities of NaK-ATPase in a purified plasma membrane fraction from isolated islets.

The existence of a NaK-ATPase in microsomes from mouse pancreatic islets was reported by Formby et al. [18, 19]. These authors used pretreatment with urea (2.5 M) in order to uncover the NaK-ATPase, according to Skou and Hilberg [20], with a final concentration of 0.25 M urea in the test. In our hands the addition of urea to the test (Table 1) or the pretreatment with this substance inhibited strongly Mg-ATPase and NaK-ATPase; in the presence of NaN_3 , urea had no influence on the enzymes (data not shown). These observations could explain why the NaK-ATPase activity described by Formby et al. was very low (2 mU/mg protein) when compared to our data.

From their experiments on the Na^+ uptake of islets Sehlin and Täljedal [21] calculated a relatively high intracellular Na^+ concentration (95 mmol/l) and concluded that the Na^+ extrusion mechanism should be of low activity; correspondingly the authors were not able to find a NaK-ATPase in the islet tissue. This interpretation was also supported by measurements of Dean and Matthews [6] who found a resting membrane potential of only -20 mV in islets. More recent experiments from Meissner et al. [7, 22] indicate that the islet membrane potential is as high as -47 mV, which can be sustained only by an active NaK-ATPase.

The observed effect of ouabain and PCMB on the NaK-ATPase might explain their effect on insulin secretion [2, 23] and sustains the concept that an increase of intracellular Na^+ leads to a stimulation of the secretory process [1, 2, 3, 4, 5]. The intracellular Na^+ concentration might secondarily influence the cytosolic Ca^{++} level, as has been proposed repeatedly (for review see [24]). Despite these considerations, the general role of the NaK-ATPase in the regulation of the secretory process is doubtful. Our

efforts to demonstrate an influence of important effectors of insulin secretion such as glucose, sulphonylureas, somatostatin and diazoxide on NaK-ATPase have so far been in vain.

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