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Modulation of islet ATP content by inhibition or stimulation of the Na^+/K^+ pump

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Abstract

High (30 mM) K^+, known to cause β-cell membrane depolarisation, significantly decreased the islet total ATP content, supporting the view that β-cell membrane depolarisation can activate the ATP-consuming Na^+/K^+ pump. Ouabain (1 mM) did not change the islet ATP content after 5–15 min of incubation in the absence or presence of 3 mM glucose but reduced it after 30 min, and in the presence of 20 mM glucose, the reduction by ouabain occurred already after 15 min. Incubation of islets with ouabain for 60 min decreased the islet ATP content in the presence of 3, 10 or 20 mM glucose or 30 mM K^+. Also, the islet glucose oxidation rate was decreased by ouabain. When K^+ deficiency was used to inhibit the Na^+/K^+ pump, no change in ATP content was observed irrespective of glucose concentration, although K^+ deficiency caused a slight inhibition of the glucose oxidation rate. Diazoxide reduced the islet glucose oxidation rate and increased the islet ATP content in the presence of 20 mM glucose. There may exist a feedback mechanism decreasing the flow of glucose metabolism in response to reduced ATP consumption by the Na^+/K^+ pump. © 2001 Published by Elsevier Science B.V.

Keywords: Ouabain; ATP; β-Cell; Islet; Na^+/K^+ pump

1. Introduction

Regulation of the membrane potential of pancreatic β-cells is a central component in the stimulus-secretion coupling process in insulin secretion (for review, see Ashcroft and Rorsman, 1989). It has recently been demonstrated that both nutrient and non-nutrient insulin secretagogues stimulate the islet Na^+/K^+ pump (Elmi et al., 2000a,b,c), and it has been suggested that this stimulation is a consequence of β-cell membrane depolarisation rather than a direct effect of secretagogues on the Na^+/K^+ enzyme activity (Elmi et al., 2000a,b). Further, the non-nutrient and β-cell membrane depolarising insulin secretagogues, glibenclamide and meglitinide, decrease the total islet ATP content, which may be a consequence of the observed stimulation of the Na^+/K^+ pump (Elmi et al., 2000c). The inhibitor of insulin secretion and β-cell hyperpolarising agent, diazoxide, increases the islet ATP content, which may be a consequence of the observed inhibition of the Na^+/K^+ pump (Elmi et al., 2000b,c). Previous results suggest that the Na^+/K^+ pump consumes at least 75% of the basal energy production in β-cells (Malaisse et al., 1978). It therefore seems probable that changes in Na^+/K^+ pump activity could influence the β-cell ATP level, which in turn would affect the β-cell membrane potential through K^+_{ATP} channels (for review, see Ashcroft and Rorsman, 1989). The aim of the present study was to further investigate the relationship between the Na^+/K^+ pump activity and the ATP level in pancreatic islets. Thus, we have investigated the effects of inhibition or stimulation of the Na^+/K^+ pump on islet metabolism measured as islet total ATP content or glucose oxidation.

2. Materials and methods

2.1. Animals and isolation of islets

Non-inbred, 9 months old, female ob/ob mice (Umeå ob/ob) were used in all experiments. Although animals from this breeding stock are metabolically abnormal with hyperphagia, mild hyperglycaemia and peripheral insulin resistance (Stauffacher et al., 1967; Stauffacher and Renold, 1969) due to defective leptin (Zhang et al., 1994), their

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islets show normal regulation of insulin secretion in vitro (Hahn et al., 1974; Hellman et al., 1974; Lindström and Sehlin, 1983). Pancreatic islets from Umeå ob/ob mice contain an exceptionally high proportion of β-cells (> 90%) (Hellman, 1965), which makes it highly probable that the present data on isolated islets are representative of this cell type.

To normalise their blood sugar, food was withdrawn overnight (Sandström and Sehlin, 1988). The pancreata were digested with collagenase to isolate individual islets (Lernmark, 1974).

The medium used for isolation was a Krebs–Ringer medium (KRH) with the following salt composition (mM): NaCl, 130; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; and $CaCl_2$, 2.56. Bovine serum albumin (BSA) at 10 mg/ml and 3 mM D-glucose were added. The medium was buffered with 20 mM HEPES and NaOH to a final pH of 7.4. For incubations, the same medium was used, the concentration of bovine serum albumin was 0 or 1 mg/ml. In the K^+-free experiments, Na^+ was used as substitute for K^+.

2.2. Measurements of islet ATP content

Pancreatic islets were isolated as described above. Then, batches of four islets were preincubated for 60 min at 37 °C in 1 ml of KRH medium containing 3 mM D-glucose but no bovine serum albumin. After preincubation, islets were incubated for 5–60 min at 37 °C in the same type of basal medium supplemented with test substances and 0, 3,10 or 20 mM D-glucose. The extraction procedure was modified from Lundin and Thore (1975), as follows. After the incubation, the islets were rapidly transferred to a polypropylene micro test tube (Milian Instruments, Geneva, Switzerland) containing 40-μl ice-cold 100 mM KOH with 0.2 mM EDTA. A glass bead was added and the islets were then homogenised by vibrating the test tube at a frequency of 1 kHz for 30 s followed by a short centrifugation. EDTA binds Mg^{2+} and thereby inactivates the kinases. The homogenates were then incubated for 10 min at 60 °C in order to denaturate the enzymes and protect ATP from degradation. The following solutions were used for ATP measurements, A: 2 ml HEPES (0.1 M; pH = 7.5); 2 mg bovine serum albumin; 20 μl $MgCl_2$ (500 mM) and B: HEPES (50 mM; pH = 7.6); KCl (20 mM); $MgCl_2$ (5 mM); bovine serum albumin (0.5 mg/ml); 2.5 μl/ml luciferin (8.5 mM); 2 μl/ml luciferase (1 μM). Fifteen microliters of the homogenate was dissolved in 30 μl of HEPES (0.1 M; pH = 6.0), 5 μl of this solution was mixed in a micro test tube with 5 μl of solution A for ATP measurement. The mixture was incubated for 30 min at room temperature followed by 5 min at 96 °C. One hundred microliters of HEPES (50 mM; pH = 7.6) was added to the tube and 10 μl of the final mixture was added to 75 μl of solution B and was incubated in the dark for 10 min before luminescence was measured in a Packard Tri-Carb liquid-scintillation spectrometer (Model 3310).

Standards of ATP, covering the expected concentration range, were carried through the entire extraction and assay procedure. The method error of a single determination was 9%. Samples of homogenates as well as ATP standards were assayed in triplicates. The islet content of ATP was expressed as pmol/μg protein. The amount of protein was measured spectrophotometrically as previously described (Whitaker and Granum, 1980).

2.3. Glucose oxidation

Islet glucose oxidation was measured as the conversion of [^{14}C]glucose to $^{14}CO_2$, essentially as previously described (Hellman et al., 1974). In brief, batches of three islets were preincubated for 30 min at 37 °C in KRH buffer containing 3 mM glucose. The islets were then incubated for 60 min with uniformly ^{14}C-labelled glucose in 100 μl of the same basal medium as used for preincubation but supplemented with the test substances. The incubations were performed in liquid-scintillation vials equipped with small glass centre wells. The effect of the additives was assessed by parallel incubation in control and test media. Blank values were obtained by incubating media without islets. Metabolism was arrested by the injection of 100 μl 0.1 M HCl into the centre well. The liberated CO_2 was collected in 100 μl of 1 M KOH, which had been placed on the bottom of the outer container vial before incubation. After equilibration for 60 min at room temperature, the centre well was removed, scintillation liquid was added and the radioactivity was counted in a liquid-scintillation spectrometer. After washing, the islets were freeze-dried overnight (−40 °C, 0.1 Pa), and weighed on a quartz-fibre balance. The results are expressed as mmol glucose equivalents/h/kg dry weight.

2.4. Chemicals

Amersham Pharmacia Biotech, Uppsala, Sweden provided D-[U-^{14}C]glucose. Ouabain and diazoxide were from Sigma (St. Louis, MO, USA). Microbial collagenase P (EC 3.4.24.3), firefly luciferase (EC 1.13.12.7), ATP (potassium salt) and electrophoretically homogeneous, lyophilized bovine serum albumin were purchased from Boehringer (Mannheim, Germany), whereas bovine serum albumin (fraction V) was from Miles Laboratories (Stoke Poges, UK). D-Luciferin was purchased from Biothema (Dalarö, Sweden). HEPES was obtained from Calbiochem (La Jolla, CA, USA). Quartz bidistilled water was used throughout. All other chemicals were of analytical grade.

2.5. Statistical analysis

Statistical significance was evaluated by using the two-tailed Student's *t*-test for paired data. Results are expressed as mean ± S.E.M.

3. Results

3.1. Effect of ouabain, K^+ deficiency or excess of K^+ on islet ATP content

Whether in the absence or presence of 3 mM D-glucose, ouabain did not change the islet ATP content after 5 or 15 min of incubation. After 30 min of incubation, the ATP content was reduced (16%, $P < 0.005$ and 12%, $P < 0.01$, respectively). Islets incubated at 20 mM glucose showed a similar pattern but ouabain caused a reduction in the ATP content already after 15 min (13%, $P < 0.005$). In the presence of 3, 10 or 20 mM D-glucose, ouabain (1 mM) significantly reduced the islet ATP content after 60 min of incubation (39%, $P < 0.001$; 21%, $P < 0.001$; and 15%, $P < 0.01$, respectively) (Table 1). When extracellular K^+ was omitted from the incubation medium in order to inhibit the Na^+/K^+ pump, no significant change in ATP content was observed irrespective of incubation time (5–60 min) or glucose concentration (0, 3 or 20 mM) (data not shown). Exposure of the islets to 30 mM K^+, in order to depolarise the β-cells, decreased the islet ATP content both at 3 mM glucose (19%; $P < 0.001$; $n = 11$) and at 20 mM glucose (11%; $P < 0.02$; $n = 9$). The effects of ouabain and excess K^+ on ATP content were partly additive (Table 1). Diazoxide (0.5 mM) increased the islet ATP content after 60 min of incubation in the presence of 20 mM glucose (13%; $P < 0.05$; $n = 9$) (Table 1).

3.2. Effect of ouabain, K^+ deficiency or diazoxide on glucose oxidation

It has been shown that ouabain decreases the islet glucose oxidation (Hellman et al., 1974; Cole and Logothetopoulos, 1974). Table 2 shows that this was also the case in the present experimental situation. Ouabain (1 mM) inhibited the islet glucose (20 mM) oxidation rate (12%, $P < 0.025$). An alternative way to inhibit the Na^+/K^+

Table 1
Effect of ouabain or excess K^+ on islet ATP content

Drug concentration (mM)	Incubation time (min)	ATP content (pmol/μg protein)	
		Primary data	Difference from control
Control (0 mM glucose)	5	6.16 ± 0.26 (9)	–
Ouabain (1)	5	5.95 ± 0.27 (9)	−0.21 ± 0.32, n.s.
Control (3 mM glucose)	5	7.66 ± 0.37 (10)	–
Ouabain (1)	5	7.75 ± 0.28 (10)	0.09 ± 0.27, n.s.
Control (20 mM glucose)	5	8.43 ± 0.28 (12)	–
Ouabain (1)	5	7.87 ± 0.37 (12)	−0.56 ± 0.41, n.s.
Control (0 mM glucose)	15	5.68 ± 0.33 (9)	–
Ouabain (1)	15	4.99 ± 0.43 (9)	−0.69 ± 0.33, n.s.
Control (3 mM glucose)	15	7.87 ± 0.33 (10)	–
Ouabain (1)	15	7.48 ± 0.37 (10)	−0.39 ± 0.27, n.s.
Control (20 mM glucose)	15	7.92 ± 0.18 (12)	–
Ouabain (1)	15	6.90 ± 0.25 (12)	−1.02 ± 0.28, $P < 0.005$
Control (0 mM glucose)	30	5.13 ± 0.30 (9)	–
Ouabain (1)	30	4.29 ± 0.23 (9)	−0.84 ± 0.21, $P < 0.005$
Control (3 mM glucose)	30	7.70 ± 0.39 (10)	–
Ouabain (1)	30	6.75 ± 0.37 (10)	−0.95 ± 0.29, $P < 0.01$
Control (20 mM glucose)	30	7.97 ± 0.31 (12)	–
Ouabain (1)	30	7.10 ± 0.27 (12)	−0.87 ± 0.24, $P < 0.005$
Control (3 mM glucose)	60	7.21 ± 0.55 (11)	–
Ouabain (1)	60	4.40 ± 0.23 (11)	−2.81 ± 0.42, $P < 0.001$
K^+ (30)	60	5.87 ± 0.24 (22)	−1.84 ± 0.40, $P < 0.005$
Ouabain (1) + K^+ (30)	60	5.31 ± 0.17 (22)[a]	−1.97 ± 0.49, $P < 0.005$
Control (10 mM glucose)	60	7.66 ± 0.28 (20)	–
Ouabain (1)	60	6.04 ± 0.28 (20)	−1.62 ± 0.22, $P < 0.001$
Control (20 mM glucose)	60	8.97 ± 0.43 (9)	–
Ouabain (1)	60	7.60 ± 0.31 (9)	−1.37 ± 0.36, $P < 0.01$
K^+ (30)	60	8.01 ± 0.35 (9)	−0.96 ± 0.33, $P < 0.02$
Diazoxide (0.5)	60	10.11 ± 0.49 (9)	1.14 ± 0.43, $P < 0.05$

Islets were prepared and preincubated in the presence of 3 mM glucose as described in the Material and methods and then incubated for 5–60 min at 37 °C in the presence of ouabain, 30 mM K^+ or both or diazoxide and 0, 3, 10 or 20 mM glucose. Data are expressed as mean values and difference from control ± S.E.M. for the number of experiments indicated in parentheses. n.s. denotes $P > 0.05$ for difference from control.

[a] $P < 0.05$ for difference between 30 mM K^+ alone, and 30 mM K^+ plus 1 mM ouabain.

Table 2
Effect of ouabain, diazoxide or K^+ deficiency on glucose oxidation

Drug concentration (mM)	Glucose oxidation (mmol/h/kg dry weight)	
	Primary data	Difference from control
Control (0)	35.44 ± 1.99 (13)	–
Ouabain (1)	31.23 ± 2.30 (13)	−4.21 ± 1.62, $P < 0.025$
Control (0)	35.37 ± 1.67 (12)	–
Diazoxide (0.02)	34.88 ± 1.91 (12)	−0.49 ± 1.71, n.s.
Diazoxide (0.2)	29.18 ± 1.49 (12)	−6.19 ± 1.05, $P < 0.001$
Diazoxide (0.5)	23.96 ± 1.47 (12)	−11.41 ± 1.71, $P < 0.001$
Control (0)	33.52 ± 1.61 (14)	–
K^+ (0)	30.09 ± 1.90 (14)	−3.43 ± 1.01, $P < 0.005$

Islets were prepared and preincubated in the presence of 3 mM glucose as described in the Material and methods and then incubated for 60 min at 37 °C in the presence of ouabain or diazoxide or absence of K^+, and 20 mM D-glucose. Data are expressed as mean values for primary data and difference from control ± S.E.M. for the number of experiments indicated in parentheses. n.s. denotes $P > 0.05$ for difference from control.

pump, K^+ deficiency, also decreased the islet glucose oxidation at 20 mM glucose (10%; $P < 0.005$; $n = 14$) (Table 2). Diazoxide, which decreases the Na^+/K^+ pump activity (Elmi et al., 2000b), dose-dependently inhibited the islet glucose oxidation (17–32%; $P < 0.001$; $n = 12$) (Table 2).

4. Discussion

The Na^+/K^+ pump participates in the generation of the resting membrane potential in pancreatic β-cells and a sudden release of induced Na^+/K^+ pump blockage shifts the membrane potential towards more negative values, indicating the hyperpolarising function of the Na^+/K^+ pump in pancreatic β-cells (Henquin and Meissner, 1982). It has been estimated that this pump consumes as much as 75–80% of the basal energy production in β-cells (Malaisse et al., 1978), and thus is the largest ATP consumer in the β-cells. We have previously provided evidence that the Na^+/K^+ pump is active also in the resting β-cells. Thus, 1 mM ouabain markedly reduced the influx of $^{86}Rb^+$ (K^+ marker) in islet cells, the ouabain-sensitive portion (Na^+/K^+ pump) amounting to more than 60% of the maximum Na^+/K^+ pump activity seen in the presence of 20 mM glucose (Elmi et al., 2000b). The present finding that high K^+, which is known to cause β-cell membrane depolarisation (Meissner et al., 1978), decreases the islet ATP level, supports the view (Elmi et al., 2000a,b,c) that β-cell membrane depolarisation can activate the ATP-consuming Na^+/K^+ pump and thereby decrease the β-cell ATP level. At the same time, this result points at the intricate ATP balance in the β-cell, where increase in ATP is thought to mediate closure of K^+_{ATP} channels followed by depolarisation (for review, see Ashcroft and Rorsman, 1989) in response to nutrient secretagogues and where such depolarisation would also lead to massive ATP consumption by the Na^+/K^+ pump.

It was recently shown that the stimulation of the Na^+/K^+ pump by nutrient (glucose) or non-nutrient (glibenclamide) insulin secretagogues could be reversed by diazoxide (Elmi et al., 2000b), which is known to hyperpolarise the β-cell membrane by opening K^+_{ATP} channels (Trube et al., 1986; Dunne et al., 1987). This strengthens the hypothesis that the activation of the Na^+/K^+ pump is secondary to membrane depolarisation (Elmi et al., 2000b). It was also shown that diazoxide reduced the basal activity of the pump (Elmi et al., 2000b) and elevated the islet ATP content (Elmi et al., 2000c), suggesting that reduced Na^+/K^+ pumping leaves more ATP available in the β-cells. That diazoxide increases the islet ATP is confirmed in the present study. However, a previous study showed that diazoxide moderately reduced the islet ATP content (Grimmsmann and Rustenbeck, 1998). At present, there is no ready explanation for this discrepancy. In the present study, we used two other ways of inhibiting the Na^+/K^+ pump, i.e. exposure to ouabain or K^+ deficiency. The results show that ouabain either was without effect or reduced the ATP level depending on incubation time, whereas K^+ deficiency had no effect irrespective of incubation time. In the case of K^+ deficiency, it has to be considered that the islet cells contain large amounts of K^+ (Sehlin and Täljedal, 1974), the release of which may counteract K^+ deficiency in the islet extracellular space. Thus, it is difficult to estimate the degree of inhibition of the Na^+/K^+ pump resulting from K^+ deficiency. As concerns the effect of ouabain, one would have expected that sudden inhibition of the Na^+/K^+ pump by ouabain would elevate the islet ATP content due to reduced ATP consumption. The present observation of ouabain not changing the ATP content in short-term incubations does not conform with previous observations, showing inhibitory effects of ouabain within a few minutes (Tsuura et al., 1998). It is noteworthy that the delay in the ouabain action found in the present data is present irrespective of glucose concentration (0, 3 or 20 mM). However, ouabain decreased the islet ATP content after 15–60 min of incubation in accord with previous observations (Tsuura et al., 1998). The mechanism causing this decrease is not clearly understood. Grapengiesser et al. (1993) showed that ouabain strongly increased the levels of Na^+ in intact islets and the cytoplasmic Ca^{2+} in isolated β-cells. They also found that the drug reduced the activity of K^+_{ATP} channels and suggested that this effect could be due to an increased ATP activity in the submembrane space due to the inhibition of the ATP-consuming Na^+/K^+ pump (Grapengiesser et al., 1993). The present data cannot confirm this idea, but it could be expected that ouabain affects other cellular mechanisms and/or metabolic steps apart from inhibiting the Na^+/K^+ pump, for example, by directly or indirectly changing cellular ion concentrations (Grapengiesser et al., 1993), which after 30–60 min of

incubation may result in decreased ATP production. This is, at least in part, supported by the observed inhibition of glucose oxidation after 60 min of ouabain treatment.

It has been shown that ouabain (Hellman et al., 1974; Cole and Logothetopoulos, 1974; Pace et al., 1975), K^+ deficiency (Henquin and Lambert, 1974) or diazoxide (Hellman et al., 1974) inhibit glucose metabolic flux in islets. The present observation that these three distinctly different ways of inhibiting the Na^+/K^+ pump slightly reduced the glucose oxidation is compatible with the existence of a feedback mechanism decreasing the flow of glucose metabolism in response to the reduced ATP consumption. However, this interpretation is not entirely in line with the present data on total ATP content but the discrepancies could be explained by regional differences in free ATP activity in the β-cells. The present results point at the important position of the ATP-consuming Na^+/K^+ pump in the β-cell function. Further studies are required to show whether a changed function of the pump can explain defective β-cell secretory responses in diabetes models.

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