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Relationships between the Na^+/K^+ pump and ATP and ADP content in mouse pancreatic islets: effects of meglitinide and glibenclamide

[1]Adrian Elmi, [1]Lars-Åke Idahl & *,[1]Janove Sehlin

[1]Department of Integrative Medical Biology, Section for Histology and Cell Biology, Umeå University, SE-901 87 Umeå, Sweden

1 We have previously demonstrated that both D-glucose and glibenclamide stimulate the Na^+/K^+ pump and suggested that this may be part of the membrane repolarization process, following the primary depolarization by these agents. The aim of this study was to investigate whether the non-sulphonylurea meglitinide (HB 699) exerts similar effects as glibenclamide or glucose on the islet Na^+/K^+ pump and if effects of meglitinide or glibenclamide on this pump activity is paralleled by changes in islet ATP content and/or ATP/ADP ratio.

2 The acyl-amino-alkyl benzoic acid derivative, meglitinide, stimulated the islet ouabain-sensitive portion of $^{86}Rb^+$ influx (Na^+/K^+ pump) by 53%, while the ouabain-resistant portion was inhibited by 70%. The stimulatory effect was not additive to that caused by D-glucose, suggesting that both agents may activate the Na^+/K^+ pump *via* the same mechanism.

3 Glibenclamide (10 μM) decreased the islet ATP and ADP content as well as the ATP/ADP ratio at 0 mM glucose. These effects were no longer observed at 10 mM glucose.

4 Meglitinide (10 or 50 μM) lowered the islet ATP and ADP content at 0 mM glucose without affecting the ATP/ADP ratio. At 10 mM glucose, however, 10 μM of the drug reduced the islet ATP content but not the ATP/ADP ratio, while 50 μM of the drug, besides lowering the ATP content, also reduced the ATP/ADP ratio.

5 Diazoxide (0.5 mM) increased the islet ATP content in the absence of glucose, an effect not seen in the presence of 10 mM glucose.

6 The rate of glucose oxidation at 1, 10 or 20 mM of the sugar was not affected by glibenclamide (0.1–10 μM) and at 10 or 20 mM of the sugar not affected by meglitinide (1–100 μM).

7 These results suggest that glibenclamide and meglitinide lower the islet ATP level by indirectly activating the β-cell Na^+/K^+ pump, which is a major consumer of ATP in the islets, while diazoxide increases the ATP level due to inhibition of the pump.
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Keywords: Glibenclamide; meglitinide; HB 699; Diazoxide; β-cell; Na^+/K^+ pump; ATP; ADP

Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; BSA, bovine serum albumin; DAPP, diadenosine pentaphosphate; EDTA, ethylenediaminetetraacetic acid; HB 699, meglitinide = 4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzoic acid; HEPES, N-[2-hydroxyethyl]piperazine-N′-[ethanesulphonic acid]; KRH, Krebs-Ringer-HEPES buffer; PEP, phospho-enol-pyruvate; SUR, sulphonylurea-receptor subunit

Introduction

Sulphonylureas are thought to act mainly by stimulating the pancreatic β-cell secretion of insulin (see Hellman & Täljedal, 1975). This action is mediated by binding to the SUR subunit of the K^+_{ATP} channels (Brown & Foubister, 1984; Zünkler *et al*., 1988; Aguilar-Bryan *et al*., 1998; Ashcroft & Gribble, 1998; Meyer *et al*., 1999; Dörschner *et al*., 1999) and thereby depolarizing the β-cell, causing Ca^{2+} influx and insulin exocytosis (Garcia-Barrado *et al*., 1996). The meglitinide analogues form a new family of oral antidiabetic drugs. Although meglitinide (HB 699) is identical with one major part of the glibenclamide molecule, it is not a sulphonylurea. The mechanism of action of meglitinide is, however, similar to that of glibenclamide (Garrino *et al*., 1985). It is known that meglitinide shows hypoglycaemic effects *in vivo* (Bickel *et al*., 1978; Ribes *et al*., 1981) and stimulates insulin release in perfused pancreas preparations (Geisen *et al*., 1978; Efendic *et al*., 1981) and *ob*/*ob*-mouse pancreatic islets (Norlund & Sehlin, 1984). It has been proposed that glibenclamide and meglitinide bind to the same receptor site (Brown & Foubister, 1984) and later it has been shown that meglitinide binds to SUR1 and SUR2 with the same affinity, whereas glibenclamide has a higher affinity for SUR1 (Meyer *et al*., 1999; Dörschner *et al*., 1999).

Although the primary action of sulphonylureas is on membrane electrical regulation, it is known that these drugs affect several metabolic parameters in the islets (for review see Gylfe *et al*., 1984). It has been shown that these drugs reduce the islet ATP content (Hellman *et al*., 1969; Kawazu *et al*., 1980; Welsh, 1983; Detimary *et al*., 1998), although the mechanisms by which these drugs affect islet ATP content is poorly understood.

To analyse whether the well-known effects of sulphonylureas on the β-cell membrane potential could be associated with the ATP depression, we have investigated the effects of glibenclamide and meglitinide on islet ATP and ADP content and ATP/ADP ratio in comparison with their effects on the islet Na^+/K^+ pump activity.

*Author for correspondence.

Methods

Animals and isolation of islets

Non-inbred, 9 months old, female *ob*/*ob* mice (Umeå-*ob*/*ob*) were used throughout. Pancreatic islets from Umeå *ob*/*ob* mice contain an exceptionally high proportion of β-cells (>90%) (Hellman, 1965). Although the mice from this breeding stock are metabolically abnormal with hyperphagia, mild hyperglycaemia and peripheral insulin resistance (Stauffacher *et al*., 1967; Stauffacher & Renold, 1969) due to defective leptin (Zhang *et al*., 1994), their islets show normal regulation of insulin secretion *in vitro* (Hahn *et al*., 1974; Hellman *et al*., 1974; Lindström & Sehlin, 1983). The high proportion of β-cells in these *ob*/*ob*-mouse islets makes it highly probable that the present data on isolated islets are representative of this cell type.

All mice were fasted overnight, in order to normalize their blood sugar (Sandström & 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^{-1} and 3 mM D-glucose were added. The medium was buffered with 20 mM HEPES and NaOH to a final pH of 7.4.

Measurements of $^{86}Rb^+$ influx

Pancreatic islets were isolated as described above. Then, batches of five islets were preincubated for 60 min at 37°C in KRH medium containing 3 mM D-glucose and bovine serum albumin (BSA) at 1 mg ml^{-1}. After preincubation, islets were incubated for 5 min at 37°C in the same type of basal medium supplemented with 28 μM $^{86}Rb^+$ and 8 μM [6,6′-^{3}H]-sucrose as extracellular marker, essentially as previously described (Sehlin & Täljedal, 1974). The test substances were dissolved in the same medium. After incubation the islets were collected from the incubation medium using a micropipette and transferred to a small piece of aluminium foil under a stereomicroscope. The excess fluid was removed using a micropipette. The islets on the aluminium foil were then rapidly frozen in liquid nitrogen, the islets were freeze-dried overnight (−40°C, 0.1 Pa), weighed on a quartz-fibre balance and their radioactive contents measured in a liquid scintillation spectrometer.

Measurements of islet ATP and ADP 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 BSA. After preincubation, islets were incubated for 60 min at 37°C in the same type of basal medium supplemented with 0, 10 or 50 μM HB 699 or 0 or 10 μM glibenclamide in the presence or absence of 10 mM D-glucose. The extraction procedure was modified from Lundin & Thore (1975) as follows. After the incubation the islets were rapidly transferred to a polypropylene micro test tube (Milian Instruments S.A., 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 homogenized by vibrating the test tube at a frequency of 1 kHz for 30 s followed by a short centrifugation. The EDTA binds Mg^{2+} and thereby inactivates the kinases. The homogenates were then incubated for 10 min at 60°C in purpose of denaturating the enzymes and protecting the ATP from degradation. The following solutions were used for ATP and ADP measurements: (A) 2 ml HEPES (0.1 M; pH = 7.5); 2 mg BSA; 20 μl $MgCl_2$ (500 mM); (B) 1 ml solution (A); 5 μl PEP (10 mM); 0.1 μl pyruvate kinase (2 mg ml^{-1}) and (C) HEPES (50 mM; pH = 7.6); KCl (20 mM); $MgCl_2$ (5 mM); BSA (0.5 mg ml^{-1}); 2.5 μl ml^{-1} luciferin (8.5 mM); 2 μl ml^{-1} luciferase (1 μM). Fifteen μl 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 and 5 μl was mixed with 5 μl of solution (B) for measurement of the sum of ADP and ATP. Both mixtures were incubated for 30 min at RT followed by 5 min at 96°C. 100 μl of HEPES (50 mM; pH = 7.6) was added to each tube and 10 μl of the final mixture was added to 75 μl of solution C and was incubated in the dark for 10 min before luminiscence was measured in a Packard Tri-Carb liquid-scintillation spectrometer (Model 3310).

Standards of ATP covering the expected 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 contents of ATP and ADP were expressed as pmol μg^{-1} protein. The amount of protein was measured spectrophotometrically as previously described (Whitaker & Granum, 1980).

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^{-1} kg^{-1} dry weight.

Chemicals

Amersham Pharmacia Biotech, Uppsala, Sweden provided $^{86}Rb^+$, D-[U-^{14}C]-glucose and [6,6′-^{3}H]-sucrose. Ouabain was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Microbial collagenase P (EC 3.4.24.3), firefly luciferase (EC 1.13.12.7), ADP (potassium salt), ATP (potassium salt), DAPP (trilithium 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, U.K.). D-luciferin was purchased from Biothema AB (Dalarö, Sweden). HEPES was obtained from Calbiochem (La Jolla, CA, U.S.A.). NaCl, KCl, KH_2PO_4, $NaHCO_3$,

$MgSO_4$ and KOH (Suprapur) were from Merck (Darmstadt, Germany). $MgCl_2$ was from BDH Chemicals (Poole, England). Quartz bidistilled water was used throughout. Svenska Hoechst AB, Stockholm, Sweden, kindly placed HB 699 and glibenclamide at our disposal. All other chemicals were of analytical grade.

Statistical analysis

Statistical significance was evaluated by using the two-tailed Student's *t*-test. Results are expressed as mean ± s.e.mean.

Results

Effect of meglitinide on $^{86}Rb^+$ influx

Previous data have indicated that glibenclamide, at 0.5–10 μM, increases the ouabain-sensitive portion of $^{86}Rb^+$ influx (marker for Na^+/K^+ pump), whereas it decreases the ouabain-resistant portion (Elmi *et al*., 2000b). We now aimed at finding out whether meglitinide, the non-sulphonylurea part of the glibenclamide molecule, exerts any effects on the islet ouabain-sensitive and insensitive $^{86}Rb^+$ fluxes respectively. Meglitinide was used at concentrations ranging from 1–100 μM in the absence or presence of 1 mM ouabain. As shown in Table 1, meglitinide stimulated the ouabain-sensitive $^{86}Rb^+$ influx in a dose-dependent manner. This stimulatory effect was statistically significant and reached its apparent maximum at 25 μM (53%; $P<0.02$; $n=8$). The ouabain-resistant portion of $^{86}Rb^+$ influx was dose-dependently inhibited, at 25 μM of the drug the inhibition amounted to 70% ($P<0.001$; $n=8$) (Table 1). The total influx of $^{86}Rb^+$ was also inhibited at 25 μM meglitinide by 38% ($P<0.001$; $n=8$).

In order to investigate whether the stimulatory effect of meglitinide was additive to that of D-glucose, the effect of meglitinide (25 μM), D-glucose (10 mM) (each dose chosen for maximum stimulation) or the combination of these agents on $^{86}Rb^+$ influx was measured. As shown in Table 2, there is no evidence for an additive effect of meglitinide and D-glucose.

Effect of glibenclamide on ATP and ADP content

In the absence of glucose, glibenclamide (10 μM) significantly lowered the islet ATP (28%; $P<0.005$; $n=9$) and ADP (15%; $P<0.02$; $n=9$) contents as well as the ATP/ADP ratio (16%; $P<0.001$; $n=9$). The addition of 10 mM D-glucose alone increased the ATP, decreased the ADP level and markedly elevated the ATP/ADP ratio. In the presence of 10 mM D-glucose, no effects of glibenclamide were observed (Table 3).

Effect of meglitinide on ATP and ADP content

The effect of meglitinide on the islet total ATP and ADP contents and the ratio between them was measured at two drug concentrations (10 or 50 μM). In the absence of D-glucose, meglitinide, at both concentrations, caused a fall in the islet ATP ($P<0.001$; $n=12$) and ADP ($P<0.005$; $n=12$) contents, whereas the ATP/ADP ratio was not significantly affected (Table 3). D-glucose alone, at 10 mM, showed the same effects as described above for the experiment with glibenclamide. In the presence of 10 mM D-glucose, meglitinide (10 or 50 μM), still caused a lowering of the ATP level ($P<0.02$; $n=12$), whereas the ADP was unaffected. At 50 μM, the drug caused a significant reduction in the ATP/ADP ratio ($P<0.005$; $n=12$), which was not observed at 10 μM (Table 3).

Effect of diazoxide on ATP and ADP content

In the absence of glucose, diazoxide (0.5 mM) increased the islet ATP content (20%; $P<0.02$; $n=18$), without significantly affecting the ADP content or the ATP/ADP ratio. The presence of D-glucose alone (10 mM) caused the same effects as described above for the experiments with glibenclamide or meglitinide. In the presence of 10 mM D-glucose the diazoxide effect was no longer apparent (Table 4).

Effect of glibenclamide or meglitinide on glucose oxidation

To investigate whether glibenclamide or meglitinide exert inhibitory effects on the β-cell oxidative metabolism, the effect of the drugs on glucose oxidation in intact islets was studied. As shown in Table 5, no inhibitory effects of glibenclamide (0.1–10 μM) at 1, 10 or 20 mM glucose or meglitinide (1–100 μM) at 10 or 20 mM glucose on islet glucose oxidation could be detected.

Table 1 Effect of meglitinide on $^{86}Rb^+$ influx

Meglitinide concentration (μM)	*$^{86}Rb^+$ influx* (mmol kg^{-1} dry weight) *Ouabain-sensitive* *Primary data*	*Difference from control*	
Control (0)	0.0965 ± 0.013 (8)	–	
1	0.1231 ± 0.012 (8)	0.0266 ± 0.014	n.s.
25	0.1479 ± 0.006 (8)	0.0515 ± 0.015	$P<0.02$
100	0.1415 ± 0.004 (8)	0.0451 ± 0.012	$P<0.01$
	Ouabain-resistant		
	Primary data	*Difference from control*	
Control (0)	0.2830 ± 0.018 (8)	–	
1	0.2392 ± 0.016 (8)	−0.0438 ± 0.010	$P<0.005$
25	0.0863 ± 0.002 (8)	−0.1967 ± 0.019	$P<0.001$
100	0.0779 ± 0.004 (8)	−0.2051 ± 0.021	$P<0.001$

Islets were prepared and preincubated as described in the Methods section and then incubated for 5 min at 37°C in the absence of glucose but presence of different concentrations of meglitinide. Data is expressed as mean values for primary data and difference from control ± s.e.mean for the number of experiments indicated in parentheses. n.s. denotes $P>0.05$ for difference from control.

Table 2 Effect of meglitinide and D-glucose on $^{86}Rb^+$ influx

	$^{86}Rb^+$ influx (mmol kg^{-1} dry weight)		
	Ouabain-sensitive		
Drug concentration	*Primary data*	*Difference from control*	
Control (0)	0.0792±0.013 (7)	–	
10 mM glucose	0.1316±0.006 (8)	0.0534±0.016	$P<0.02$
25 μM meglitinide	0.1228±0.004 (8)	0.0447±0.015	$P<0.05$
Glucose + meglitinide	0.1357±0.009 (8)	0.0578±0.015	$P<0.01$
	Ouabain-resistant		
	Primary data	*Difference from control*	
Control (0)	0.2717±0.024 (7)	–	
10 mM glucose	0.1502±0.004 (8)	−0.1203±0.025	$P<0.005$
25 μM meglitinide	0.0754±0.003 (8)	−0.1965±0.025	$P<0.001$
Glucose + meglitinide	0.0959±0.002 (8)	−0.1766±0.023	$P<0.001$

Islets were prepared and preincubated as described in the Methods section and then incubated for 5 min at 37°C in the presence of meglitinide, glucose or both. Data is expressed as mean values for primary data and difference from control±s.e.mean for the number of experiments indicated in parentheses.

Table 3 Effect of glibenclamide or meglitinide on islet ATP and ADP content

	Primary data			*Difference from control*		
	ATP	*ADP*	*ATP/ADP*	*ATP*	*ADP*	*ATP/ADP*
Drug concentration		(pmol μg^{-1} protein)				
0 mM glucose (control)	5.897±0.232	2.527±0.107	2.351±0.089 (9)	–	–	–
0 mM glucose + 10 μM glib.	4.219±0.280	2.149±0.131	1.967±0.073 (9)	−1.678±0.337 P<0.005	−0.378±0.129 P<0.02	−0.384±0.064 P<0.001
10 mM glucose (control)	6.414±0.276*	1.841±0.089***	3.564±0.250 (9)***	–	–	–
10 mM glucose + 10 μM glib.	6.012±0.388	1.790±0.114	3.484±0.315 (9)	−0.402±0.254 n.s.	−0.051±0.062 n.s.	−0.080±0.241 n.s.
0 mM glucose (control)	6.095±0.299	2.500±0.162	2.480±0.088 (12)	–	–	–
0 mM glucose + 10 μM meglit.	5.019±0.286	2.115±0.124	2.403±0.113 (12)	−1.076±0.145 P<0.001	−0.385±0.069 P<0.005	−0.077±0.090 n.s.
10 mM glucose (control)	7.153±0.415**	1.788±0.130***	4.125±0.234 (12)***	–	–	–
10 mM glucose + 10 μM meglit.	6.440±0.373	1.730±0.142	3.904±0.281 (12)	−0.713±0.239 P<0.02	−0.058±0.062 n.s.	−0.222±0.165 n.s.
0 mM glucose (control)	6.309±0.350	2.691±0.143	2.352±0.068 (12)	–	–	–
0 mM glucose + 50 μM meglit.	5.514±0.308	2.384±0.167	2.348±0.081 (12)	−0.795±0.143 P<0.001	−0.307±0.083 P<0.005	−0.005±0.060 n.s.
10 mM glucose (control)	7.713±0.462***	1.823±0.132***	4.359±0.276 (12)***	–	–	–
10 mM glucose + 50 μM meglit.	6.644±0.399	1.879±0.143	3.724±0.225 (12)	−0.921±0.320 P<0.02	0.056±0.063 n.s.	−0.534±0.142 P<0.005

Islets were prepared and preincubated as described in the Methods section and then incubated for 60 min at 37°C in the presence of different concentrations of meglitinide or glibenclamide and D-glucose. Data is expressed as mean values for primary data and difference from control±s.e.mean for the number of experiments indicated in parentheses. n.s. denotes $P>0.05$ for difference from control, (*$P<0.05$, **$P<0.02$, ***$P<0.001$, for difference from 0 mM glucose).

Table 4 Effect of diazoxide on islet ATP and ADP content

	Primary data			*Difference from control*		
	ATP	*ADP*	*ATP/ADP*	*ATP*	*ADP*	*ATP/ADP*
Drug concentration		(pmol μg^{-1} protein)				
0 mM glucose (control)	6.089±0.273 (18)	2.743±0.171 (10)	2.477±0.084 (10)	–	–	–
0 mM glucose + 0.5 mM diaz.	7.326±0.514 (18)	3.015±0.307 (10)	2.406±0.073 (10)	1.236±0.478 P<0.02	0.272±0.172 n.s.	−0.071±0.068 n.s.
10 mM glucose (control)	7.461±0.398 (18)***	1.955±0.172 (10)***	4.250±0.311 (10)***	–	–	–
10 mM glucose + 0.5 mM diaz.	8.134±0.465 (18)	2.136±0.165 (10)	3.780±0.193 (10)	0.673±0.444 n.s.	0.181±0.122 n.s.	−0.471±0.215 n.s.

Islets were prepared and preincubated as described in the Methods section and then incubated for 60 min at 37°C in the presence of 0.5 mM diazoxide and/or 10 nM D-glucose. Data is expressed as mean values for primary data and difference from control±s.e.mean for the number of experiments indicated in parentheses. n.s. denotes $P>0.05$ for difference from control, (***$P<0.001$, for difference from 0 mM glucose).

Discussion

Previous studies have indicated that D-glucose as well as glibenclamide cause activation of the Na^+/K^+ pump (Elmi & Sehlin, 1999; Elmi *et al*., 2000a,b). Both agents are known to stimulate the β-cells by creating a rhythmic pattern of membrane depolarizations and repolarizations (Meissner & Atwater, 1976; Henquin & Meissner, 1982a; Henquin & Meissner, 1982b). It has been suggested that activation of the Na^+/K^+ pump by membrane depolarizing agents, like

Table 5 Effect of meglitinide or glibenclamide on glucose oxidation

Drug concentration (μM)	*D-glucose conc.* (mM)	*Glucose oxidation* (mmol h^{-1} kg^{-1} dry weight) *Primary data*	*Difference from control*	
Control (0)	20	35.42±2.58 (10)	–	
Glibenclamide 0.1		35.55±2.82 (10)	0.12±1.98	n.s.
Glibenclamide 1		32.03±1.86 (10)	−3.39±2.24	n.s.
Glibenclamide 10		31.95±2.07 (10)	−3.47±1.74	n.s.
Control (0)	10	25.62±1.21 (10)	–	
Glibenclamide 0.1		28.09±1.86 (10)	2.46±1.61	n.s.
Glibenclamide 1		27.23±1.67 (10)	1.60±0.96	n.s.
Glibenclamide 10		24.07±1.78 (10)	−1.55±1.88	n.s.
Control (0)	1	2.64±0.21 (12)	–	
Glibenclamide 0.1		2.70±0.15 (12)	0.06±0.16	n.s.
Glibenclamide 1		2.59±0.19 (12)	−0.06±0.14	n.s.
Glibenclamide 10		2.66±0.18 (12)	0.02±0.15	n.s.
Control (0)	10	28.87±2.04 (13)	–	
Meglitinide 1		26.86±2.17 (13)	−2.01±1.24	n.s.
Meglitinide 10		26.04±1.62 (13)	−2.83±1.66	n.s.
Meglitinide 100		26.34±1.85 (13)	−2.53±1.37	n.s.
Control (0)	20	24.47±3.80 (10)	–	
Meglitinide 1		25.99±2.29 (10)	1.53±2.06	n.s.
Meglitinide 10		25.58±5.05 (10)	1.11±3.01	n.s.
Meglitinide 100		22.25±2.34 (10)	−2.21±2.00	n.s.

Islets were prepared and preincubated as described in the Methods section and then incubated for 60 min at 37°C in the presence of different concentrations of meglitinide or glibenclamide and D-glucose. Data is expressed as mean values for primary data and difference from control±s.e.mean for the number of experiments indicated in parentheses. n.s. denotes $P>0.05$ for difference from control.

glucose (Elmi *et al*., 2000a) or glibenclamide (Elmi *et al*., 2000b) follows as a consequence of the membrane depolarization and that it may contribute to membrane repolarization and that these secretagogues do not exert any direct effect on the Na^+/K^+ ATPase activity in islet homogenates (Elmi *et al*., 2000a,b). This is further supported by the observation that the effects of D-glucose as well as glibenclamide were abolished by diazoxide (Elmi *et al*., 2000b), known to hyperpolarize the β-cell membrane by opening K^+_{ATP} channels (Trube *et al*., 1986; Dunne *et al*., 1987).

The present results indicate that the non-sulphonylurea, meglitinide, stimulates the islet Na^+/K^+ pump activity, as indicated by the increase in ouabain-sensitive $^{86}Rb^+$ influx, in the same manner as previously shown for glibenclamide (Elmi *et al*., 2000b). However, the effect of glibenclamide became evident and statistically significant already at 0.5 μM (Elmi *et al*., 2000b), whereas the effect of meglitinide was seen at 25–100 μM (present work). This difference in potency between the drugs resembles the different potencies of glibenclamide and meglitinide to induce insulin secretion in isolated islets (Norlund & Sehlin, 1984). Meglitinide and glibenclamide exert their primary effect on the β-cells by binding to the sulphonylurea-receptor subunit (SUR) of the K^+_{ATP} channels (Brown & Foubister, 1984; Zünkler *et al*., 1988; Aguilar-Bryan *et al*., 1998; Ashcroft & Gribble, 1998; Meyer *et al*., 1999; Dörschner *et al*., 1999) causing the closure of these channels, which in turn leads to depolarization of the β-cells (Sturgess *et al*., 1985; Trube *et al*., 1986), Ca^{2+} influx and insulin exocytosis (Henquin, 1980; 1998; Garcia-Barrado *et al*., 1996). The present finding that meglitinide, like glibenclamide (Elmi *et al*., 2000b), dose-dependently inhibits the ouabain-resistant portion of the $^{86}Rb^+$ influx conforms well to the view that this drug closes K^+_{ATP} channels. It is well established that glucose stimulation of pancreatic β-cells is mediated by closure of K^+_{ATP} channels (for review, Ashcroft & Rorsman, 1989). The present results showing that the effects of meglitinide and D-glucose on ouabain-sensitive $^{86}Rb^+$ influx, like previously shown for glibenclamide and D-glucose (Elmi *et al*., 2000b), are not additive suggest that all three agents may be acting through the same mechanism to produce activation of the Na^+/K^+ pump.

The Na^+/K^+ pump participates in the generation of the resting membrane potential (Henquin & Meissner, 1982a). 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 β-cells. As meglitinide and glibenclamide appear as stimulators of the islet Na^+/K^+ pump, it was of interest to analyse whether these drugs affect islet ATP content and the ATP/ADP ratio. The results demonstrate that both glibenclamide and meglitinide decreased the ATP content and the ATP/ADP ratio in β-cells in the absence of glucose. That this decrease was counteracted by the presence of 10 mM glucose can probably be ascribed to the ATP production from glucose metabolism. In order to further characterize the effects of glibenclamide and meglitinide on islet nucleotide content, the effects of these drugs on islet glucose oxidation rate was studied. The finding that the glucose oxidation rate was not significantly decreased by glibenclamide or meglitinide indicates that the ATP-lowering effect of these drugs is not primarily due to inhibition of glucose metabolism in intact islets. This is in accord with previous observations regarding effects of glibenclamide and related substances on nutrient metabolism (for review see Gylfe *et al*., 1984).

It is noteworthy that diazoxide, an opener of the K^+_{ATP} channels (Trube *et al*., 1986; Dunne *et al*., 1987) and inhibitor of insulin secretion by hyperpolarizing the β-cells (Henquin & Meissner, 1982b), increased the islet ATP content in the absence of glucose. This effect may, at least in part, be attributed to the inhibition of the Na^+/K^+ pump activity caused by diazoxide in resting β-cells (Elmi & Sehlin, 1999; Elmi *et al*., 2000b). The present observation that the effect of diazoxide was counteracted by 10 mM glucose taken together with previous data, showing that diazoxide causes a modest decrease in glucose oxidation and increase in fructose 1,6-diphosphate in the islets (Hellman *et al*., 1974), suggests that a primary elevation of the ATP level by diazoxide due to inhibition of the Na^+/K^+ pump may exert a modest inhibitory effect on glycolysis.

We have previously provided evidence that the Na^+/K^+ pump is active also in the resting β-cells in the absence of glucose. This basal activity amounted to more than 60% of the maximum Na^+/K^+ pump activity seen in the presence of glucose or glibenclamide (Elmi *et al*., 2000b). It has been suggested that the increase in $[Ca^{2+}]_i$ resulting from closure of K^+_{ATP} channels, caused by metabolism of glucose, tends to lower the ATP/ADP ratio (Detimary *et al*., 1998). The same authors have shown that depolarization of β-cells with 100 μM tolbutamide or 30 mM K^+ causes a rapid fall in ATP/ADP ratio that can be ascribed to the rise in $[Ca^{2+}]_i$ (Detimary *et al*., 1998). They therefore proposed that the increase in $[Ca^{2+}]_i$ causes a larger consumption than production of ATP, inducing reopening of K^+_{ATP} channels and a new cycle of secretion. However, the mechanism causing this larger consumption is not clearly understood and may include activation of ATPases involved in the maintenance of Ca^{2+} and Na^+ homeostasis in β-cells (Detimary *et al*., 1998). Based on the present results we propose that stimulation of the Na^+/K^+ pump activity as a result of membrane depolarization by glibenclamide or meglitinide may, at least in part, explain the larger ATP consumption and decreased ATP content. Also, this could explain the previous findings of negative effects on islet function after long-term exposure to glibenclamide in islet culture (Borg & Andersson, 1981). Stork *et al*. (1969) showed that glibenclamide increases oxygen consumption by islets incubated at a low glucose concentration and suggested that endogenous metabolism was increased. However, the present finding of reduced ATP levels would suggest that the increase in endogenous metabolism is not sufficient to compensate for the increased ATP consumption.

A large portion of the islet ATP is sequestered in insulin granules (Detimary *et al*., 1996). However, it is not likely that the decrease in islet ATP content by glibenclamide and meglitinide could be explained by co-secretion of insulin and ATP stored in the granules. The fractional secretion of insulin from *ob*/*ob*-mouse islets during 1 h of maximum stimulation is about 2% of the total islet insulin content (Sandström & Sehlin, 1988). Thus, even if the total islet ATP pool were located in insulin granules, the present data could only to a minor extent be explained by such ATP discharge. The drug-induced changes in insulin secretory activity is not likely to be responsible for the shifts in ATP levels demonstrated here. The effects of diazoxide on the ATP level cannot be explained by inhibition of secretion, as ATP was significantly increased only in the absence of glucose, when there is no secretion. In stimulated islets (10 mM glucose), diazoxide did not significantly elevate the ATP level.

In conclusion, the decrease in islet ATP content caused by glibenclamide and meglitinide is not due to inhibition of islet metabolism or increased secretion. These drugs increase the islet Na^+/K^+ pump activity, which is suggested to be associated with the depolarization of the β-cells. We therefore suggest that the islet ATP level is lowered due to increased ATP consumption by the Na^+/K^+ pump.

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