Interplay between cytoplasmic Ca^{2+} and the ATP/ADP ratio: a feedback control mechanism in mouse pancreatic islets

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In pancreatic β cells, the increase in the ATP/ADP ratio that follows a stimulation by glucose is thought to play an important role in the Ca^{2+}-dependent increase in insulin secretion. Here we have investigated the possible interactions between Ca^{2+} and adenine nucleotides in mouse islets. Measurements of both parameters in the same single islet showed that the rise in the ATP/ADP ratio precedes any rise in the cytoplasmic free-Ca^{2+} concentration ($[Ca^{2+}]_i$) and is already present during the initial transient lowering of $[Ca^{2+}]_i$ produced by the sugar. Blockade of Ca^{2+} influx with nimodipine did not prevent the concentration-dependent increase in the ATP/ADP ratio produced by glucose and even augmented the ratio at all glucose concentrations which normally stimulate Ca^{2+} influx. In contrast, stimulation of Ca^{2+} influx by 30 mM K^+ or 100 μM tolbutamide lowered the ATP/ADP ratio. This lowering was of rapid onset and reversibility, sustained and prevented by nimodipine or omission of extracellular Ca^{2+}. It was, however, not attenuated after blockade of secretion by activation of α_2-adrenoceptors. The difference in islet ATP/ADP ratio during blockade and stimulation of Ca^{2+} influx was similar to that observed between threshold and submaximal glucose concentrations. The results suggest that the following feedback loop could control the oscillations of membrane potential and $[Ca^{2+}]_i$ in β cells. Glucose metabolism increases the ATP/ADP ratio in a Ca^{2+}-independent manner, which leads to closure of ATP-sensitive K^+ channels, depolarization and stimulation of Ca^{2+} influx. The resulting increase in $[Ca^{2+}]_i$ causes a larger consumption than production of ATP, which induces reopening of ATP-sensitive K^+ channels and arrest of Ca^{2+} influx. Upon lowering of $[Ca^{2+}]_i$ the ATP/ADP ratio increases again and a new cycle may start.

INTRODUCTION

Glucose has little influence on the adenine nucleotide concentrations in most tissues [1–4]. The endocrine pancreas is a remarkable exception. Glucose increases the ATP/ADP ratio in islets of Langerhans [5–8], but the magnitude of these changes and the concentration range over which they occur have long been underestimated because a large pool of adenine nucleotides is also present within insulin granules. This pool, in which the ATP/ADP ratio is stable and close to 1, makes up an important background that partially masks the cytoplasmic changes in nucleotides [9]. It is only recently that we could demonstrate that glucose causes a concentration-dependent increase in the ATP/ADP ratio even at concentrations that stimulate insulin release [10,11].

This increase in the ATP/ADP ratio may serve as second messenger at different steps of stimulus-secretion coupling [12,13]. The first, and major, step is the regulation of the ATP-sensitive K^+ channels (K^+-ATP channels) in the plasma membrane [14–16]. The closure of these channels results in membrane depolarization with subsequent opening of voltage-dependent Ca^{2+} channels. The influx of Ca^{2+} then causes an increase in cytoplasmic free-Ca^{2+} concentration ($[Ca^{2+}]_i$) [17,18], which is the triggering signal for the secretion of insulin. The second mechanism by which adenine nucleotides may regulate insulin secretion is an increase of the effectiveness of cytoplasmic Ca^{2+} on the exocytotic process [19,20].

The mechanisms by which glucose accelerates metabolism to increase the ATP/ADP ratio are still incompletely understood [21,22]. Several types of interactions might exist between the changes in $[Ca^{2+}]_i$ and the ATP/ADP ratio. An increase of the latter ultimately leads to a rise of $[Ca^{2+}]_i$ as seen above. In turn, this rise in $[Ca^{2+}]_i$ may activate Ca^{2+}-dependent mitochondrial dehydrogenases and stimulate ATP production [18,23–28]. Finally, the rise in $[Ca^{2+}]_i$ and its functional consequences may be expected to influence ATP consumption. The net impact of Ca^{2+} influx on the ATP/ADP ratio in β cells is difficult to predict and has been investigated here in experiments using normal mouse islets.

MATERIALS AND METHODS

Materials

Diazoxide was provided by Schering-Plough Avondale (Rathdrum, Ireland); clonidine was from Boehringer-Ingelheim (Ingelheim, Germany); nimodipine was from Bayer (Wuppertal, Germany); fura PE-3 acetoxymethylester was from Mobitec (Göttingen, Germany); ATP, ADP and all the enzymes used for the assays [11] were from Boehringer Mannheim (Mannheim, Germany).

Solutions

The control medium was a bicarbonate-buffered solution which contained 120 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 24 mM $NaHCO_3$. An O_2:CO_2 (94:6) mixture was then bubbled through the solution to maintain pH 7.4, which was supplemented with bovine serum albumin (1 mg/ml). Ca^{2+}-free solutions were prepared by replacing $CaCl_2$ with $MgCl_2$. When the concentration of KCl was increased to 30 mM, that of NaCl was decreased to 94.8 mM to maintain iso-osmolality.

Preparation

Islets were isolated by collagenase digestion of the pancreas of fed female NMRI mice (25–30 g) and then hand-picked. These islets were cultured for 18–20 h at 37 °C in RPMI 1640 medium

Abbreviations used: K^+-ATP channels, ATP-sensitive K^+ channels; $[Ca^{2+}]_i$, cytoplasmic free-Ca^{2+} concentration.

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containing 10 mM glucose, 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin.

Measurement of adenine nucleotides in incubated islets

After culture, the islets were preincubated for 60 min at 37 °C in control medium containing 15 mM glucose, a concentration that causes half-maximum stimulation of mouse islets. They were then distributed in batches of 5 in 0.375 ml medium containing various concentrations of glucose and test substances, and incubated for 60 min at 37 °C. When the effect of test substances was evaluated for shorter periods of time, another 0.375 ml of appropriate prewarmed medium was added after 30, 50 or 55 min. After rapid mixing, half of the incubation medium was removed and the islets were incubated in the remaining 0.375 ml for the last 30, 10 or 5 min. When test substances were applied for only 1 or 2 min, the initial incubation medium was only 0.1875 ml and an equal volume of prewarmed medium was added for the last 1 or 2 min of the 60 min period. Control islets were treated in a similar way. The incubation was stopped by addition of 0.125 ml of trichloroacetic acid to a final concentration of 5%. The sample were then processed, and ATP and ADP were assayed in triplicates by a luminometric method as reported previously [11].

Measurement of insulin release from incubated islets

In one series of experiments cultured islets were preincubated as above before being incubated in batches of 5 in 1 ml of control medium. After 50 min, 0.9 ml was removed and replaced by the same volume of prewarmed medium containing 32.8 mM KCl to reach a final concentration of 30 mM. Ten minutes later, an aliquot of medium was taken for insulin measurement by a double antibody radioimmunoassay using rat insulin as the standard.

Recording of cytosolic Ca^{2+} and measurement of adenine nucleotides in single islets

Cultured islets were incubated for 90–120 min at 37 °C in control medium containing 10 mM glucose and 2 μM fura PE-3 acetoxymethylester. One single islet was then transferred into a small temperature-controlled perifusion chamber, the bottom of which was made of a glass coverslip. The islet was held in place by gentle suction with a glass micropipette and perifused at a flow rate of 1.8 ml/min (the dead space of the system corresponded to 1 min and has been corrected for). The chamber was placed on the stage of a Nikon Diaphot inverted microscope equipped with a 20 X neofluor objective. The tissue was excited successively at 340 and 380 nm, and the fluorescence emitted at 510 nm was monitored by a photomultiplier-based system (Photon Technologies International, Princeton, NJ, U.S.A.). The $[Ca^{2+}]_i$ was calculated as described previously [18]. At selected times, the perifusion was stopped and 0.15 ml of trichloroacetic acid added to the chamber to a final concentration of 5%. The islet and medium were then collected from the chamber, and processed for determination of adenine nucleotide levels.

Presentation of results

Results are presented as means ± S.E.M. for the indicated number of islets or batches of islets, from the given number of preparations. The statistical significance of differences between means was assessed by Student's *t* test for unpaired data, or by analysis of variance followed by a Newman–Keuls test for multiple comparisons.

RESULTS

Temporal sequence of glucose-induced changes in islet $[Ca^{2+}]_i$ and ATP/ADP ratio

A novel technique was developed to record $[Ca^{2+}]_i$ and measure nucleotides in the same single islet. Figure 1 presents the mean

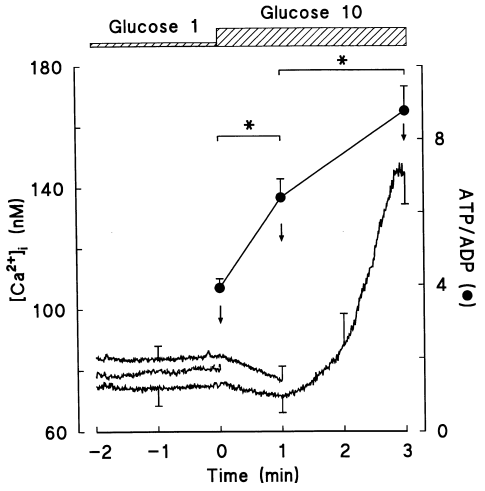

Figure 1 Time sequence of glucose-induced changes in $[Ca^{2+}]_i$ and ATP/ADP ratio in single mouse islets

Single islets loaded with fura PE-3 were perifused with a medium containing 1 and then 10 mM glucose as indicated. While $[Ca^{2+}]_i$ was recorded, the experiments were stopped by addition of trichloroacetic acid before (0), or 1 and 3 min after the stimulation with 10 mM glucose, hence the interrupted traces. Adenine nucleotides were then measured in the same islets, and the corresponding ATP/ADP ratio was calculated. Values of both $[Ca^{2+}]_i$ and the ATP/ADP ratio are means ± S.E.M. for 20 individual islets from five different experiments ($^*P < 0.01$).

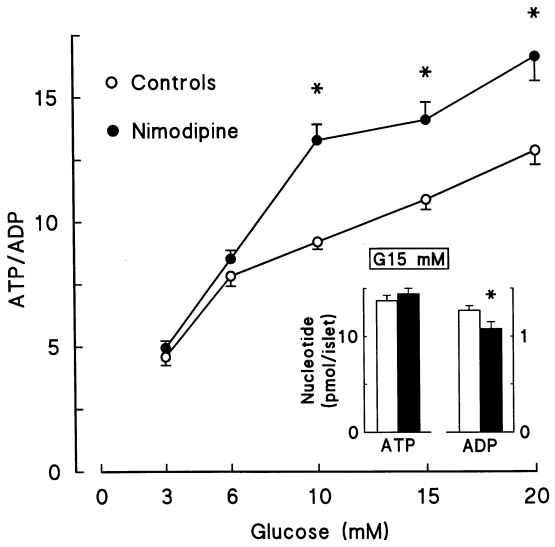

Figure 2 Effects of nimodipine on the ATP/ADP ratio in mouse islets incubated in the presence of various glucose concentrations

Batches of five islets were incubated for 60 min in the presence of the indicated concentration of glucose, and without (○) or with (●) 2 μM nimodipine. The inset shows the effects of nimodipine on ATP and ADP levels in islets incubated in the presence of 15 mM glucose. Values are means ± S.E.M. for 15–20 batches of islets from three or four different experiments ($^*P < 0.001$ for the effect of nimodipine).

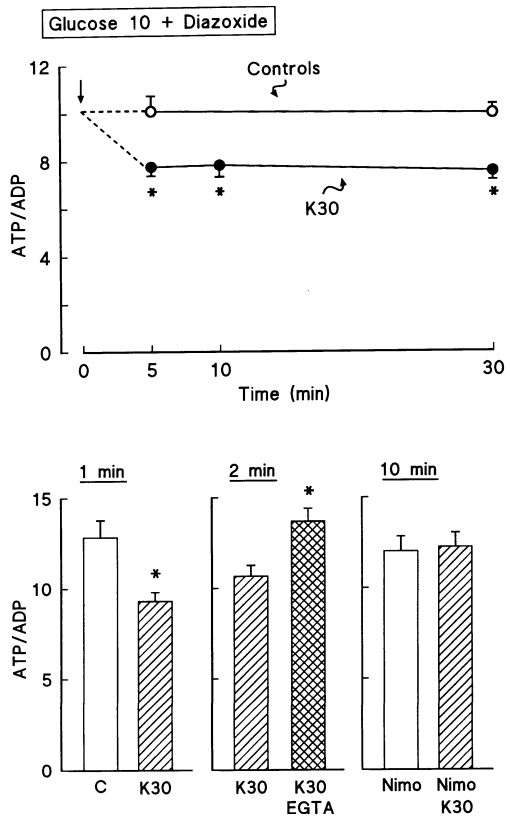

Figure 3 Effects of a depolarization with 30 mM K^+ on the ATP/ADP ratio in mouse islets incubated in the presence of 10 mM glucose and 100 μM diazoxide

Batches of five islets were incubated for 60 min in a medium containing 10 mM glucose and 100 μM diazoxide. Upper panel: the concentration of K^+ was kept at 4.8 mM throughout control experiments and raised to 30 mM during the indicated period of time in test experiments. Lower panels, left: concentration of K^+ was raised to 30 mM for 1 min; middle: 2.6 mM EGTA was added to the high-K^+ medium for 2 min; right: 30 mM K^+ was added for 10 min to a medium containing 2 μM nimodipine (Nimo). Results are means $\pm$ S.E.M. for 15 batches of islets from three different experiments (upper panel) or 20 batches of islets from four different experiments (lower panels). $^*P < 0.01$.

results for 20 islets. When the islets were perifused with a medium containing 1 mM glucose, their $[Ca^{2+}]_i$ was low (86 ± 3 nM) and their ATP/ADP ratio averaged 3.9 ± 0.3. When the glucose concentration was raised to 10 mM, $[Ca^{2+}]_i$ first decreased to a minimum after 1 min. This decrease was observed in 34 out of 40 experiments and averaged 6 nM. At the same time, the ATP/ADP ratio was significantly increased. After 3 min in 10 mM glucose, islet $[Ca^{2+}]_i$ was consistently increased, by a mean value of 69 nM ($P < 0.001$), and the ATP/ADP ratio doubled (Figure 1). Although the increase in the ATP/ADP ratio induced by high glucose clearly precedes the rise in $[Ca^{2+}]_i$, an influence of Ca^{2+} on metabolism remains possible. This was evaluated by measuring adenine nucleotides under various conditions that inhibit or stimulate Ca^{2+} influx.

Influence of an inhibition of Ca^{2+} influx on the ATP/ADP ratio

In control islets, glucose increased the ATP/ADP ratio in a concentration-dependent manner (Figure 2) [11]. Nimodipine, which inhibits voltage-dependent Ca^{2+} channels [29], was used to prevent the depolarization-induced $[Ca^{2+}]_i$ rise in β cells [30]. For instance, in the presence of 10 mM glucose, nimodipine lowered

Table 1 Effects of tolbutamide and nimodipine on the ATP/ADP ratio in islets incubated in a control medium containing 3 or 10 mM glucose

Batches of five islets were incubated for 60 min in a medium containing 3 or 10 mM glucose, with or without nimodipine. Where indicated, tolbutamide was added for the last 10 min of incubation. Values are means $\pm$ S.E.M. for ten batches of islets from two different experiments (glucose 3 mM) or for 15 batches of islets from three different experiments (glucose 10 mM).

Test agents				
Tolbutamide (100 μM)	Nimodipine (2 μM)	ATP (pmol/islet)	ADP (pmol/islet)	ATP/ADP
Glucose 3 mM				
−	−	13.3 ± 0.5	2.8 ± 0.3	5.1 ± 0.4
+	−	$10.7 \pm 0.4^*$	3.0 ± 0.7	$3.7 \pm 0.2^*$
−	+	12.9 ± 0.6	2.6 ± 0.8	5.2 ± 0.5
+	+	13.0 ± 0.4	2.7 ± 0.7	5.2 ± 0.5
Glucose 10 mM				
−	−	15.0 ± 0.6	1.4 ± 0.1	11.6 ± 0.9
+	−	13.9 ± 0.6	$1.8 \pm 0.1^*$	$8.2 \pm 0.4^*$
−	+	14.9 ± 0.5	1.0 ± 0.1	$14.6 \pm 0.9^*$
+	+	14.4 ± 0.4	1.2 ± 0.1	13.8 ± 1.3

* $P < 0.05$ versus controls without test agent.

$[Ca^{2+}]_i$ from 157 ± 5 to 96 ± 3 nM ($n = 13$). It was without effect on the ATP/ADP ratio in islets incubated in the presence of glucose concentrations (3–6 mM) which do not stimulate Ca^{2+} influx. In contrast, the Ca^{2+}-channel blocker augmented the increase in the ATP/ADP ratio brought about by glucose concentrations which normally raise $[Ca^{2+}]_i$. The inset of Figure 2 shows that the increase in the ratio brought about by nimodipine was due to a small (6%) increase in ATP and larger (15%) decrease in ADP. Diazoxide, which prevents glucose-induced Ca^{2+} influx and $[Ca^{2+}]_i$ rise by opening K^+-ATP channels and holding the membrane hyperpolarized [31], similarly increased the ATP/ADP ratio in the presence of 10 mM glucose (12.9 ± 0.6 versus 10.3 ± 0.4; $n = 15$; $P < 0.01$).

Influence of a stimulation of Ca^{2+} influx on the ATP/ADP ratio

Islets incubated in the presence of 10 mM glucose and 100 μM diazoxide, in which $[Ca^{2+}]_i$ was thus low, were depolarized by 30 mM K^+ to promote Ca^{2+} influx and raise $[Ca^{2+}]_i$ [31]. This resulted in a significant decrease in the ATP/ADP ratio that was maximal within 5 min (Figure 3, upper panel). In separate experiments, a significant decrease in the ATP/ADP ratio was already observed after 1 min (Figure 3, lower left panel), which was owing to an 8% decrease in ATP and a 20% increase in ADP ($P < 0.05$). Conversely, the ATP/ADP ratio of islets incubated in the presence of high K^+ increased within 2 min of Ca^{2+} influx blockade by addition of an excess EGTA (Figure 3, lower middle panel). The lowering of the ATP/ADP ratio caused by high K^+ was also prevented by nimodipine (Figure 3, lower right panel), and can therefore be attributed to the stimulation of Ca^{2+} influx. Nimodipine was without effect in control islets incubated with diazoxide (data not shown), which is expected from the ability of the latter to block Ca^{2+} influx.

The islets were also depolarized by blocking K^+-ATP channels with tolbutamide. This is known to result in a rise of $[Ca^{2+}]_i$ [18] that can be prevented by Ca^{2+}-channel blockers. For instance, in the presence of 10 mM glucose, the increase in $[Ca^{2+}]_i$ brought about by 100 μM tolbutamide (from 157 ± 5 to 254 ± 8 nM) was suppressed (105 ± 3 nM; $n = 13$) by 2 μM nimodipine. As shown in Table 1, in both low and high glucose, tolbutamide caused

Table 2 Effects of clonidine on the ATP/ADP ratio, cytoplasmic $[Ca^{2+}]_i$ and insulin secretion in islets stimulated by 30 mM K^+

In all experiments the medium contained 10 mM glucose and 100 μM diazoxide. For measurements of adenine nucleotides and insulin secretion (in separate experiments), batches of five islets were incubated for 50 min with or without 1 μM clonidine. Most of the medium was then removed and replaced by prewarmed medium to increase the concentration of K^+ to 30 mM. The incubation was then stopped 10 min later (see Materials and Methods). Cytoplasmic $[Ca^{2+}]_i$ was measured both in islets perifused with a medium containing clonidine and with a medium without clonidine. $[Ca^{2+}]_i$ was integrated over the last 5 min of a 10 min stimulation with 30 mM K^+. Values are means $\pm$ S.E.M. for 15 batches of islets from three experiments (ATP/ADP), 14 islets from two experiments ($[Ca^{2+}]_i$) or 25 batches from five experiments (insulin secretion).

Test agents				
K^+ (mM)	Clonidine	ATP/ADP	Cytoplasmic $[Ca^{2+}]_i$ (nM)	Insulin secretion (ng · islet^{-1} · 10 min^{-1})
4.8	—	12.9 $\pm$ 0.6	94 $\pm$ 2	0.33 $\pm$ 0.05
30	—	8.3 $\pm$ 0.3*	264 $\pm$ 7	2.16 $\pm$ 0.24*
4.8	+	13.2 $\pm$ 0.9	93 $\pm$ 2	0.35 $\pm$ 0.05
30	+	8.5 $\pm$ 0.4*	258 $\pm$ 7	0.55 $\pm$ 0.05

* $P < 0.001$ versus 4.8 mM K^+.

a decrease in the ATP/ADP ratio that was prevented by nimodipine which, when used alone, influenced the ratio in the presence of 10 mM glucose only. The effects of tolbutamide were rapidly reversible: in islets incubated in the presence of 10 mM glucose and 100 μM tolbutamide, the ATP/ADP ratio increased from 7.6 $\pm$ 0.3 to 9.6 $\pm$ 0.6 ($P < 0.001$) 2 min after addition of nimodipine and diazoxide. In the absence of external Ca^{2+}, the ATP/ADP ratio was 13.8 $\pm$ 1.5 and was not significantly affected by the addition of tolbutamide (12.9 $\pm$ 0.7), nimodipine (12.4 $\pm$ 1.8) or a combination of the two drugs (13.0 $\pm$ 0.5).

Influence of changes in insulin secretion

We next evaluated whether the decrease in the ATP/ADP ratio induced by a rise in $[Ca^{2+}]_i$ could be owing to the stimulation of insulin secretion. When islets were incubated in the presence of diazoxide and normal K^+, $[Ca^{2+}]_i$ was low and insulin secretion was not stimulated [31]. Activation of α_2-adrenoceptors by clonidine did not influence the ATP/ADP ratio, $[Ca^{2+}]_i$ or insulin secretion under these conditions (Table 2). In the presence of 30 mM K^+, $[Ca^{2+}]_i$ was raised, insulin secretion was stimulated and clonidine blocked secretion without affecting $[Ca^{2+}]_i$ [32]. Table 2 also shows that clonidine did not attenuate the fall in the ATP/ADP ratio caused by high K^+.

DISCUSSION

Glucose-induced changes in the ATP/ADP ratio precede $[Ca^{2+}]_i$ changes

Recordings of the mitochondrial membrane potential in islet cell clusters [33], and of the redox state of pyridine nucleotides in single rat islet cells [27], suspensions of HIT-T15 cells [34] and intact mouse islets [18], have indicated that an acceleration of metabolism precedes the rise in $[Ca^{2+}]_i$ induced by glucose stimulation. By combined measurements in single mouse islets, we show here that raising the glucose concentration from 1 to 10 mM in the perifusion medium increased the ATP/ADP ratio before $[Ca^{2+}]_i$ increased. This temporal sequence is compatible with a role of adenine nucleotides as second messengers in the closure of K^+-ATP channels and subsequent depolarization and Ca^{2+} influx. A similar conclusion has been reached in studies of the early metabolic events occurring in suspensions of HIT-T15 cells [34] and *ob/ob* mouse islet cells [35] incubated in a glucose-free medium and suddenly stimulated by addition of the sugar to the cuvette.

A small and very transient decrease in the ATP/ADP ratio has been measured in HIT-T15 cells 3 s after stimulation with glucose [34]. A much larger and more delayed (1 min) decrease was also observed in rat islets [6]. In contrast, no initial drop in the ATP/ADP ratio was detected in islet cells from *ob/ob* mice in spite of frequent measurements [35]. In the present study, the first measurement was made after 1 min of stimulation and showed a clear increase in the ATP/ADP ratio coinciding with the initial decrease in $[Ca^{2+}]_i$ that glucose produces. This decrease in $[Ca^{2+}]_i$ depends on glucose metabolism [36] and is generally attributed to a sequestration of Ca^{2+} into the endoplasmic reticulum [37]. It is plausible, therefore, that the rise in energy state promotes Ca^{2+} sequestration, as shown in permeabilized RIN m5F cells [38]. The glucose-induced increase in ATP/ADP ratio might thus exert dual effects on Ca^{2+} handling by β cells: a stimulation of Ca^{2+} sequestration in the endoplasmic reticulum and a depolarization-mediated stimulation of Ca^{2+} influx from the extracellular space.

An increase in $[Ca^{2+}]_i$ lowers the ATP/ADP ratio

Although the glucose-induced increase in ATP/ADP ratio is not secondary to the rise in $[Ca^{2+}]_i$, it is possible that the latter potentiates glucose metabolism by activating mitochondrial dehydrogenases [24–28]. Some reports support this proposal, but others do not. Thus, inhibition of the $[Ca^{2+}]_i$ rise abolished the preferential mitochondrial oxidation of glucose [39] and attenuated the increase in NAD(P)H levels [18], but it did not affect O_2 consumption by isolated islets [40]. The impact on adenine nucleotides is also controversial. Omission of extracellular Ca^{2+} was reported not to affect ATP content of mouse islets (ADP was not measured) [23,41], whereas Ca^{2+} channel blockers increased the ATP/ADP ratio in both non-stimulatory and stimulatory concentrations of glucose [42]. Our results show that blockade of Ca^{2+} entry and subsequent $[Ca^{2+}]_i$ rise did not prevent the glucose-dependent increase in ATP/ADP ratio, which reinforces the conclusions drawn from the time course study. On the contrary, nimodipine increased the ATP/ADP ratio, but did so only in the presence of glucose concentrations ($\geqslant$ 10 mM) that normally raise $[Ca^{2+}]_i$. Since a similar effect was observed with diazoxide, the data suggest that the rise in $[Ca^{2+}]_i$ in β cells tends to lower the ATP/ADP ratio. This was further evaluated with non-nutrient secretagogues.

In the presence of glucose, sulphonylureas increase O_2 consumption and the NAD(P)H level, provided extracellular Ca^{2+} is present [18,23], but do not promote the preferential mitochondrial metabolism of the sugar [39]. The drugs have also been found not to affect [43] or to decrease islet ATP [5,23,44], and one study has reported a fall in the ATP/ADP ratio in the presence of a very high concentration of tolbutamide (740 μM) [45]. Our results show that depolarization of β cells with 100 μM tolbutamide or 30 mM K^+ caused a rapid fall in the ATP/ADP ratio that can be ascribed to the rise in $[Ca^{2+}]_i$ because it was abrogated by several measures known to prevent this rise. Altogether the data, therefore, indicate that a rise in $[Ca^{2+}]_i$ stimulates ATP breakdown more than ATP synthesis. The effects of Ca^{2+} on β cell metabolism may thus involve increased provision of ADP to the mitochondrial adenine nucleotide translocase in addition to the stimulation of mitochondrial dehydrogenases. How Ca^{2+}

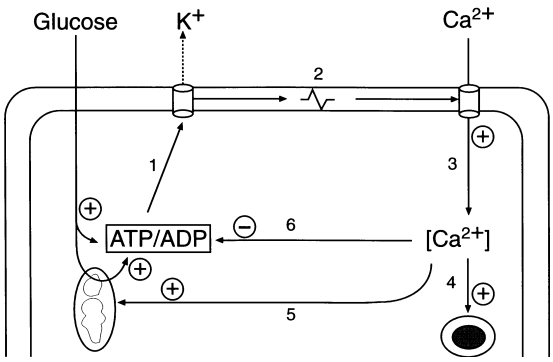

Figure 4 Model of the feedback control of the ATP/ADP ratio by cytoplasmic Ca^{2+} in pancreatic β cells

Glucose metabolism through glycolysis and the tricarboxylic acid cycle increases the ATP/ADP ratio in the cytoplasm. This leads to closure of K^+-ATP channels (1) with depolarization of the plasma membrane (2) and opening of voltage-dependent Ca^{2+} channels. Ca^{2+} influx (3) then causes a rise in $[Ca^{2+}]_i$ which, in addition to triggering exocytosis of insulin granules (4), exerts effects that influence the ATP/ADP ratio in opposite directions. Through activation of mitochondrial dehydrogenases $[Ca^{2+}]_i$ promotes ATP production (5), and through yet unidentified mechanisms it increases ATP consumption (6). The net result is a decrease in the ATP/ADP ratio, leading to reopening of K^+-ATP channels, with partial membrane repolarization, arrest of Ca^{2+} influx, lowering of $[Ca^{2+}]_i$ and eventual restoration of a high ATP/ADP ratio, which initiates a new cycle.

accelerates ATP consumption has not been extensively studied. However, the observation that inhibition of insulin release by clonidine did not prevent the decrease in ATP/ADP ratio evoked by high K^+ indicates that mechanisms other than exocytosis must be involved. These may include activation of ATPases involved in the maintenance of Ca^{2+} and Na^+ homoeostasis in β cells, or secretory steps upstream of the site of blockade by clonidine.

Functional significance of the changes in the ATP/ADP ratio induced by Ca^{2+}

We have recently shown that the islet ATP/ADP ratio increases when ambient glucose is raised from a threshold (6 mM) to a submaximal (20 mM) concentration, and that this increase correlates well with insulin secretion [11]. The changes in ATP/ADP evoked here by agents stimulating or inhibiting Ca^{2+} influx have a similar magnitude to those occurring over that range of glucose concentrations (Figure 2 and [11]). On average ATP and ADP contents varied by 6–7 and 15–20 % above and below control values. As approximately 20 % of total ATP and 50 % of total ADP of cultured islets are present in the non-diffusible, granular pool [9], cytosolic ATP and ADP are expected to change markedly when $[Ca^{2+}]_i$ fluctuates between a low and a high level. Such variations should influence the activity of K^+-ATP channels [46–48]. Our observations that changes in Ca^{2+} influx produce large and rapid changes in the ATP/ADP ratio in the opposite direction suggest that the following feedback loop could be involved in the generation of the oscillations of membrane potential and $[Ca^{2+}]_i$ in β cells (Figure 4). The metabolism of glucose through glycolysis and the tricarboxylic acid cycle leads to an increase in the cytoplasmic ATP/ADP ratio. This causes closure of K^+-ATP channels with depolarization of the β cell membrane to the threshold potential where Ca^{2+} channels open and Ca^{2+} influx starts. In addition to triggering insulin secretion, the ensuing rise in $[Ca^{2+}]_i$ stimulates metabolism by activating mitochondrial dehydrogenases, and increases ATP consumption, the net effect being a decrease in the ATP/ADP ratio. This leads to reopening of K^+-ATP channels with repolarization, arrest of Ca^{2+} influx and lowering of $[Ca^{2+}]_i$. The following increase in the ATP/ADP ratio closes K^+-ATP channels and a new cycle starts.

The present model differs from others in several respects. It has previously been proposed that intrinsic properties of glycolysis induce oscillations of ATP production, which lead to oscillations in K^+-ATP channel activity, membrane potential and Ca^{2+} influx [49]. The oscillations in $[Ca^{2+}]_i$ are not supposed to influence metabolic oscillations in any feedback control. Another, theoretical, model proposed that the rise in $[Ca^{2+}]_i$ is followed by Ca^{2+} uptake in mitochondria, decrease in mitochondrial membrane potential and inhibition of ATP synthesis. This could indeed trigger oscillations of the ATP/ADP ratio through feedback inhibition by Ca^{2+} of mitochondrial ATP production [50]. However, experimental data do not support this hypothesis [33]. A third model suggested that Ca^{2+} exerts a feedback control of K^+-ATP channels via changes in ATP consumption and hence of the ATP/ADP ratio [51]. This hypothesis was based on the Ca^{2+}-induced changes in β cell membrane potential. The present study provides direct experimental support for this feedback loop. A decisive demonstration would be provided by measurements of oscillations of the ATP/ADP ratio during the course of spontaneous $[Ca^{2+}]_i$ oscillations induced by glucose. However, this remains a challenging goal that may require novel technologies to monitor ATP changes in the submembrane compartment of the β cell.

We thank Professor H. G. Hers for comments on an early version of the manuscript and M. Nenquin for editorial help. This work was supported by Grant 3.4525.94 from the Fonds de la Recherche Scientifique Médicale (Brussels), grant ARC 95/00-188 from the General Direction of Scientific Research of the French Community of Belgium, and by the Interuniversity Poles of Attraction Programme (P4/21) – Belgian State, Prime Minister's Office – Federal Office for Scientific, Technical and Cultural Affairs. P.G. is Chercheur Qualifié of the Fonds National de la Recherche Scientifique, Bruxelles.

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Received 2 February 1998/6 April 1998; accepted 16 April 1998