The Changes in Adenine Nucleotides Measured in Glucose-stimulated Rodent Islets Occur in β Cells but Not in α Cells and Are Also Observed in Human Islets*

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Glucose metabolism by pancreatic β and α cells is essential for stimulation of insulin secretion and inhibition of glucagon secretion. Studies using rodent islets have suggested that the ATP/ADP ratio serves as second messenger in β cells. This study compared the effects of glucose on glucose oxidation ([(U-14C)glucose]) and adenine nucleotides (lumimetric method) in purified rat α and β cells. The rate of glucose oxidation at 1 mM glucose was higher in β than α cells (4.5-fold, i.e. ∼2-fold after normalization for cell size). It was more strongly stimulated by 10 mM glucose in β cells (9-fold) than in α cells (5-fold). At 1 mM glucose, ATP levels were similar in both cell types, which corresponds to an approximately 2-fold higher concentration in α cells (6.5 mM) than in β cells (∼3 mM). In β cells, glucose dependence increased ATP and decreased ADP levels, causing a rise in the ATP/ADP ratio from 2.4 to 11.6 at 1 and 10 mM, respectively. In α cells, glucose did not affect ATP and ADP levels, and the ATP/ADP ratio remained stable around 7.5. In human islets, the ATP/ADP ratio progressively increased between 1 and 10 mM glucose. In β cells, which often contain human islet preparations, an increase in the ATP/ADP ratio sometimes occurred between 1 and 3 mM glucose. In conclusion, the present observations establish that the regulation of glucagon secretion by glucose does not involve changes in α cell adenine nucleotides and further support the role of the ATP/ADP ratio in the control of insulin secretion.

Glucose homeostasis is largely regulated in the endocrine pancreas through opposite effects of glucose on insulin and glucagon secretion. Pancreatic β cells are fuel sensors that adjust the rate of insulin secretion to the rate at which they metabolize glucose (reviewed in Refs. 1–3). Two major transduction pathways are involved. The first one uses ATP-sensitive K+ channels (K-ATP channels) of the plasma membrane to transduce biochemical into biophysical signals. Thus, glucose metabolism causes closure of these K-ATP channels, which leads to membrane depolarization, opening of voltage-dependent Ca2+ channels, and acceleration of Ca2+ influx. The resulting rise in cytoplasmic Ca2+ concentration ([Ca2+]i) then triggers exocytosis of insulin granules (reviewed in Refs. 4–6). The second pathway, known as the K-ATP channel-independent pathway, increases the effectiveness of Ca2+ on exocytosis by as yet incompletely elucidated mechanisms (7–9). Much less information is available on how glucose inhibits glucagon secretion from α cells (10). Measurements of glucose metabolism in α cell-rich islets (11) and purified α cells (12) and studies using metabolic inhibitors in whole islets or pancreas (13–15) suggest that the inhibition of glucagon secretion is mediated by glucose metabolism in the α cells.

Although early studies reported that glucose increases ATP levels in rodent islets (16, 17), a rise in the ATP/ADP ratio was not a consistent finding (reviewed in Ref. 18). Recently, we demonstrated that glucose causes a large, concentration-dependent increase in the ATP/ADP ratio in mouse islets and that this effect might be involved in the regulation of insulin secretion through both pathways (19, 20). However, the changes measured in whole islets might not exactly reflect those occurring in β cells. It is also not known whether glucose affects adenine nucleotides in α cells. Measurements in islets isolated from animals made diabetic by destruction of most of their β cells with streptozotocin are indeed contradictory (21, 22).

The present study compares the effect of glucose on adenine nucleotides in purified rat α and β cells (23). It also examines whether the changes observed in rodent cells are seen in isolated human islets.

EXPERIMENTAL PROCEDURES

Preparation and Purification of Rat Islet Cells—Rat islet β and α cells were purified from adult male Wistar rats by autofluorescence-activated cell sorting using previously described methods (23).

In a first series of experiments, the islets were cultured overnight in Ham’s F-10 medium supplemented with 2 mM glutamine, 10 mM glucose, 1% bovine albumin, and 5% fetal calf serum. They were then used to obtain three fractions of dispersed islet cells (20% α cells and 65% β cells), purified β cells (>90%), and non-β cells (<70% α cells, 5–10% β cells, and 10–15% other cells). These purities were checked by immunocytochemistry (23). The three fractions were then incubated as described below for measurements of glucose oxidation and adenine nucleotide content.

In a second series of experiments, the dispersion of islet cells and their purification immediately followed the islet isolation. Purified β cells were then cultured overnight in Ham’s F-10 medium supplemented with 2 mM glutamine, 10 mM glucose, 2 mM CaCl2, and 1% bovine serum albumin. Non-β cells were first separated from β cells and then purified into α cells (>85% α cells, <5% β cells), which were then cultured overnight in Ham’s F-10 medium supplemented with 2 mM glutamine, 6 mM glucose, and 1% bovine serum albumin. Purified β and α cells were then incubated as described below for measurement of glucose oxidation and adenine nucleotide content.

Measurements of Adenine Nucleotides in Rat Islet Cells—The medium used was a bicarbonate-buffered solution that contained 120 mM...
ATP/ADP ratio from 3.2 to 14.8 between 1 and 10 mM glucose.

Changes were slightly larger, resulting in an increase in the cose. The incubation was stopped by the addition of 20 tide levels and glucose oxidation in purified rat

In islet non-

b

were slightly lower in purified glucose to 12.3 at 10 mM glucose (Fig. 1

A

B

C

D

FIG. 1. Effects of various concentrations of glucose on nucleotide levels and glucose oxidation in purified rat β cells, non-β cells, and islet cells. Nucleotides were measured in cells incubated for 1 h in a medium containing the indicated glucose concentration. Glucose oxidation was measured as 14CO2 production from [U-14C]glucose by cells incubated for 2 h. Values are means ± S.E. for six batches of cells from three separate experiments (nucleotides) or three to six individual experiments (oxidation).

NaCl, 4.8 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, 1 mM Na2HPO4, and 24 mM NaHCO3. It was gassed with O2:CO2 (94:6) to maintain pH 7.4 and was supplemented with 1% bovine serum albumin.

Cell preparations were first washed twice with this medium before batches of 15,000–50,000 cells were incubated for 1 h in 375 μl of medium supplemented with different glucose concentrations. The incubation was stopped by the addition of 125 μl of trichloroacetic acid to a final concentration of 5%. The samples were then processed, and ATP and ADP were measured by a luminometric method exactly as described previously (20).

Measurements of Glucose Oxidation by Rat Islet Cells—Batches of ~100,000 cells from the different cellular fractions were incubated for 2 h in 100 μl of Earle’s-Hepes buffer (24) containing the indicated concentration of glucose and supplemented with 25 μCi/ml [U-14C]glucose. The incubation was stopped by the addition of 20 μl of 1 N HCl and 14CO2 was trapped with hydroxyhyamine. Other details of the method have been published (24).

Experiments with Human Islets—Human pancreata were obtained from organ donors (19–61 years of age) within the framework of β Cell Transplant, a European Concerted Action on islet transplantation. After isolation, the islet preparations were cultured for 2–3 days as described elsewhere (25); their cellular composition was similar to that in our previous studies (25). They were then incubated for 1 h in bicarbonate-buffered medium containing the indicated glucose concentration before being processed for nucleotide measurements.

RESULTS

The nucleotide content of the different cell preparations was measured after 1 h of incubation in the presence of various glucose concentrations (Fig. 1). In dispersed, unsorted, islet cells, ATP levels were 75% higher at 10 than 1 mM glucose (Fig. 1A), whereas ADP levels were 45% lower (Fig. 1B). This resulted in an increase in the ATP/ADP ratio from 3.8 at 1 mM glucose to 12.3 at 10 mM glucose (Fig. 1C). ATP and ADP levels were slightly lower in purified β cells, but their relative changes were slightly larger, resulting in an increase in the ATP/ADP ratio from 3.2 to 14.8 between 1 and 10 mM glucose. In islet non-β cells, the ATP content was similar to that in β cells at 1 mM glucose but did not change when the glucose concentration was raised (Fig. 1A). ADP levels were lower and decreased by 40% between 1 and 10 mM glucose. Thus, as compared with β cells, the ATP/ADP ratio in islet non-β cells was higher at 1 mM glucose but increased much less at 10 mM glucose. In no fraction were ATP or ADP levels different between 10 and 20 mM glucose.

The rate of glucose oxidation was similar in β cells and unsorted islet cells; it increased 8-fold (β cells) and 6-fold (islet cells) when the concentration of glucose was raised from 1 to 10 mM (Fig. 1D). The rate of glucose oxidation was lower in non-β cells than in β cells, both at low and high glucose; it increased 4-fold between 1 and 10 mM glucose.

The islet non-β cell fraction is enriched in α cells but may contain up to 15% β cells and up to 20% other cell types (12). The observed changes in glucose oxidation and nucleotide levels might thus take place, at least in part, in cells other than the α cells. We therefore purified the islet non-β cells into a fraction containing over 80% α cells. Fig. 2A shows that the ATP content of α and β cells was similar after incubation in 1 mM glucose and that glucose increased this content more than 2-fold in β cells without affecting it in α cells. In β cells, ADP levels decreased when the glucose concentration was raised (Fig. 2B). The ADP content of α cells was 3-fold lower and did not change with the glucose concentration. As a result, the ATP/ADP ratio in β cells increased more than 4-fold between 1 and 10 mM glucose, whereas the elevated ratio in α cells at low glucose was not modified by a higher glucose concentration (Fig. 2C). All changes occurring in β cells between 1 and 5 mM glucose were significant (p < 0.05 or less), but there was no difference between measurements at 10 and 20 mM glucose.

The rate of glucose oxidation was higher in β than α cells (Fig. 2D). The difference was larger at 10 mM glucose (8-fold) than at 1 mM glucose (4.5-fold) because the oxidation rate was more strongly increased by glucose in β cells (9-fold) than in α cells (5-fold).

In human islets, glucose increased the ATP/ADP ratio dose-dependently (Fig. 3). Because these preparations can contain up to 40% non-endocrine cells (mainly duct cells), we also measured the effects of glucose on the ATP/ADP ratio in duct cells. This ratio increased between 1 and 3 mM glucose and then plateaued; for unknown reasons the increase was observed in only four of seven preparations. The consequence is that the observed rise in ATP/ADP ratio in human islet preparations...
The present study shows that glucose exerts strikingly different effects on adenine nucleotides in insulin- and glucagon-secreting cells. It does not change the ATP/ADP ratio in α cells and increases it in β cells from the rat. The study also establishes that glucose increases the ATP/ADP ratio in human islets.

Two previous studies have used islets obtained from rat (21) or guinea pigs (22) made β cell-deficient and diabetic with streptozotocin to estimate ATP levels in α cells. In one study no difference in islet ATP content was observed between 0 and 10 mM glucose (21), whereas a 25% increase occurred between 1.7–3.3 and 16.7 mM glucose in the other study (22). ADP contents were not reported. The discrepancy between these studies and the increase in ATP levels that insulin caused in these α cell-rich islets of diabetic animals (22, 26) left unanswered the question of whether glucose influences adenine nucleotides in normal α cells. In preparations of islet non-β cells, the composition of which is comparable with that of α cell-enriched islets, glucose slightly increased the ATP/ADP ratio, mainly through a decrease in ADP. However, these changes can be ascribed to contaminating non-α cells, in particular 10–15% of residual β cells with their larger volume (12). Thus, the experiments using purified α cells unambiguously showed that the ATP/ADP ratio is not influenced by glucose (1–10 mM) in glucagon-secreting cells from normal rats.

The observation that the ATP/ADP ratio does not change in glucose-challenged α cells implies that the measurements in whole islets underestimate the actual changes occurring in β cells unless the ratio decreases in δ cells. This is very unlikely owing to the similarities of stimulus-secretion coupling in β and δ cells (15). In practice, because 65% of β cells make up at least 80% of the islet volume in the rat (27) and 80% of β cells make up close to 90% of the islet volume in the mouse (28), the adenine nucleotide measurements in intact islets should satisfactorily reflect the changes occurring in β cells. However, in human islet preparations, the results may be markedly influenced by the degree of contamination with duct cells in which the ATP/ADP ratio is influenced by glucose, at least in the low concentration range (1–3 mM).

Pancratic α and β cells have different sizes that must be taken into account in a comparison of their nucleotide contents. The intracellular space is about 2.3-fold larger in β than in non-β cells from the rat (620 versus 270 pl/10^3 cells) (12, 29). From these values it can be calculated that the ATP concentration increased from ~3 to ~7 mM in β cells and remained around 6.5 mM in α cells when the concentration of glucose was raised from 1 to 10 mM. Conversely, the concentration of total ADP decreased from ~1.2 to 0.6 mM in β cells and was stable around 0.9 mM in α cells. It is important to realize that these values do not exactly correspond to the nucleotide concentrations in the cytoplasm. ATP and ADP, in a ratio close to 1.0, are present in insulin-containing granules (30–32). It is likely that adenine nucleotides are also present in glucagon-containing granules, but this has not been directly demonstrated. Another possible confounding factor is the degree of granulation of the two cell types. If one postulates that the relative granular pool of adenine nucleotides is similar in both cell types, α and β cells would seem to have similar cytoplasmic concentrations of ATP in the presence of high glucose. The peculiar feature of β cells appears to be an inability to maintain these high values when the concentration of glucose decreases, but the fact that substantial changes in the ATP/ADP ratio occur between 10 and 5 mM glucose clearly indicates that they do not express mere suffering of fuel-deprived cells.

The metabolic organization of α and β cells has recently been compared (12, 24). After normalization for the differences in cell volume, the rate of glycolysis is similar probably because of the presence of a glucokinase in both cell types (33). However, the ratio of lactate dehydrogenase/mitochondrial glycerol-3-phosphate dehydrogenase is lower in β cells than in α cells, β cells express much more pyruvate carboxylase than α cells, and glycolysis is largely aerobic in β cells and anaerobic in α cells (24). In the present study basal glucose oxidation (at 1 mM glucose) was lower in non-β and purified α cells than in β cells, but this difference is largely attenuated after correction for the difference in cell size. Upon stimulation with 10 mM glucose, the relative increase in oxidation was larger in β cells (8–9-fold) than in non-β or α cells (4–5-fold). A tight coupling of glycolysis with pyruvate oxidation and ATP production in mitochondria may explain why the ATP/ADP ratio changes so much with the glucose concentration in β cells. These variations and a high anaplerosis of glucose-derived carbons (24) may be the two major metabolic features distinguishing α and β cells.

The lack of effect of glucose on the ATP/ADP ratio in α cells is a general metabolic feature shared by many cell types (34–36), whereas the large variations occurring in β cells are exceptional from a metabolic standpoint. It is therefore tempting to speculate that these variations have a functional significance. The changes in the ATP/ADP ratio induced by glucose may regulate insulin secretion via the K^+-ATP channel-dependent and -independent pathways (20, 37). K^+-ATP channels have not been identified in guinea pig α cells (38) but are present in rat α cells (39, 40). However, the data presented here and the characteristics of the α cell electrical activity (38, 40) do not support a role for these channels in the regulation of glucagon secretion by glucose. Mechanisms other than changes in adenine nucleotides should be sought to explain how glucose inhibits glucagon secretion.

The current model of stimulus-secretion coupling in the pancreatic β cell has been built on experiments with rodent islets. Studies with human islets have shown that it can largely be extrapolated to the human β cell. In particular, the key role of

![Diagram](http://www.jbc.org/)

**Fig. 3. Effects of various concentrations of glucose on nucleotide levels in human islets and human pancreatic duct cells.** Nucleotides were measured in cells incubated for 1 h in a medium containing the indicated glucose concentration. Values are means ± S.E. for 20–23 batches of islets or duct cells from six (islets) and seven (duct cells) separate preparations. The dotted line shows calculated results for islet cells after correction of a contamination by 20% duct cells.
glucose metabolism in the control of K+-ATP channels, membrane potential, and cytoplasmic [Ca$^{2+}$], has been verified (41, 42). The present study validates an additional important extrapolation: the metabolism of glucose in human islets is followed by an increase in the ATP/ADP ratio, which may thus play a universal role of second messenger in the regulation of insulin secretion.

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