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Review

Energy metabolism in islets of Langerhans

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Contents

I. Introduction

All living organisms, in order to survive and perform their many and varied functions, must produce energy continuously. Some specialized organs and cells have unique additional energy requirements to perform one or more specific activities that require larger expenditures of ATP. For example, the heart pumps blood, the liver synthesizes macromolecules and the kidney and brain move ions, while the pancreatic islets produce and secrete hormones. The object of this review is to construct a uniform picture of the energetic properties of pancreatic islets and, in particular, to seek answers to three specific questions: (1) What are the steady-state concentrations of high-energy phosphate compounds and of inorganic phosphate in islets and are these metabolites segregated into various intracellular compartments? (2) What are the key reactions that produce and consume ATP? (3) What are the mechanisms

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that regulate energy production and what is the nature of the processes that are responsible for high-glucose-induced increase in islet energy generation?

There are two points that we wish to raise at the very beginning of this discussion. The first concerns the role of the ATP molecule itself. Although this nucleotide is the primary source of metabolic energy, it can also serve as a regulator of several intracellular events, either by phosphorylating target proteins, and thus modulating their activity, or by acting directly as their modulator. To accommodate these issues, a section at the end of the article provides a brief discussion of these other roles of ATP. The second point is 'technical' and concerns the anatomical nature of pancreatic islets. Islets in some manner resemble organs because they are heterogeneous structures which are composed of several types of cell; 60–80% of those are B-cells that produce and secrete insulin. This proportion increases to over 90% in islets from *ob/ob* mice [1]. The behavior of islets is thus governed to a large extent by events that occur in B-cells. It was the choice of the present authors to limit this review to considerations of the energetic properties of B-cells in islets and not to include results obtained on B-cell-derived clonal cell lines. The reasons for this decision were two-fold. Firstly, these clonal cell lines, like all rapidly dividing cells grown in culture, exhibit alterations in certain aspects of energy metabolism, the most important of which are increased glycolytic activity and changes in patterns of fuel consumption. Secondly, glucose is not always the predominant secretagogue in these cells, which raises the issue of to what extent any knowledge acquired can be extrapolated to the situation in an animal in vivo.

II. Islet energy parameters

II-A. Presentation and analysis of data

One of the objectives of this review is to compare and analyze the vast body of pertinent quantitative information available in the literature. This is not easy because there is no single way of presenting results in a form common to all laboratories. To avoid introducing errors that arise from recalculations, we have preserved, in most cases, the data in the original form in which they were expressed. In a few situations the figures have been normalized to a single, selected unit.

TABLE I

DNA, protein, weight and volume of pancreatic islets

Species	DNA (ng)	Protein (ng)	Wet weight (μg)	Dry weight (μg)	Vol. (nl)	Reference
Rat			8	1.6		Reese et al. [197]
					2.03–2.17	Malaisse et al. [42]
		800		1.0–1.2	2–3	Mailaisse et al. [198]
	28.1			0.64 [a]		Bukowiecki et al. [79]
	25	800	3	0.8	3	Meglasson and Matchinsky [103]
	19.24					Liang et al. [199]
	8.9					Liang and Matschinsky [200]
	27.2				3.2	Ohta et al. [28]
	16.8					Pipeleers et al. [201]
'small' islets	11.5					Michalik (unpublished)
'large' islets	25.2					
'average' islets	16.5	1810 [b], 630 [c]				
female	50	780				Green and Taylor [202]
pregnant female	76	1050				
Mouse				1.2		Hellerström [203]
				0.5	2	Ashcroft et al. [95]
	24					Panten et al. [204]
	16.4			0.67		Hedeskov et al. [205]
male albino-fed	14.8					Hedeskov and Capito [206]
male albino-starved	16.3					
		800		1.0		Sener and Malaisse [207]
ob / ob	55.4					Panten et al. [204]
				11.3		Hellerström [203]
Guinea pig				11.7		
Man		540				Ashcroft et al. [208]

[a] Calculated from Bukowiecki et al. [79]; 43.6 ng DNA/μg dry weight.

[b] Determined according to Lowry et al. [209].

[c] Determined according to Bradford [4].

However, to permit readers to make their own conversions and unbiased calculations, we have summarized in Table I the values of the various units of reference for a single islet, as measured in several different laboratories.

The figures shown in Table I are self-explanatory and we wish to make only a few comments. (i) There are no large differences in the various parameters measured in islets isolated from either rat or mouse. (ii) The mean values for the content of DNA, protein and water, as well as the dry weight per islet vary among the different laboratories or animal species by a

TABLE II

Levels of energy parameters and insulin release in rat pancreatic islets

Conditions	ATP	ADP	AMP	PCr	ATP/ADP	Insulin release	Reference
	(mmol/kg dry weight)					(μunits/ml)	
In vivo	16–20	3.5–4.1		4.4–6.3	4.5–4.6		Matschinsky et al. [25]
Dissected islets perfused for 30 s:							
without glucose	14.1	4.05		1.94	3.5	8.1 [a]	
with 20 mM glucose	12.0	4.83		1.61	2.5	8.7 [a]	
Islets incubated for 30 min with:	(pmol/islet)					(μunits/90 min per islet)	
no substrate	8.26	3.09	1.01			11.0 [b]	Sener et al. [35]
	8.0 [c]	3.5 [c]	2.2 [c]		2.68	11.5 [b]	Malaisse et al. [21]
2.8 mM glucose	13.77	3.79			3.94		Zhou et al. [40]
7 mM glucose	10.92	1.60	0.62		7.01	41.3 [b]	Sener et al. [35]
11 mM glucose	9.50	2.13	1.04		4.46		Malaisse et al. [18]
16.7 mM glucose	9.8 [c]	2.3 [c]	2.2 [c]		5.23		Malaisse et al. [21]
						(pg/min per islet)	
16.7 mM glucose	24.79	3.74			8.02	≈ 125 [d]	Zhou et al. [40]
						(μunits/90 min per islet)	
10 mM L-lysine	9.76	1.83	0.82		5.47	12.4 [b]	Sener et al. [35]
10 mM L-leucine	9.1 [c]	2.5 [c]	3.4 [c]		4.18	51.7 [b]	Malaisse et al. [21]
10 mM L-glutamine	9.1 [c]	3.4 [c]	3.0 [c]		2.91	11.3 [b]	
10 mM L-glutamine + 10 mM L-leucine	8.3 [c]	2.7 [c]	2.6 [c]		3.92	206.7 [b]	
Islets incubated for 2 h with:						(pg/min per islet)	
no substrate	3.95					6.8	Ashcroft et al. [8]
5 mM glucose	7.68					41.5	
20 mM glucose	8.10					324	
5 mM D-glyceraldehyde	7.07					68.9	
2.5 mM L-leucine	7.77					93.3	
Islets perifused with:	(mmol/kg)					(ng/min per 100 islets)	
2.4 mM glucose for 40 min	11.9	5.69	0.935		2.1	1.3	Trus et al. [37]
2.4 mM glucose for 30 min and 14.5 mM glucose for 10 min	14.0	4.46	0.360		3.1	8.1	
	(mM)					(ng/μg DNA per min)	
5 mM glucose for 40 min	1.53	0.27			5.73	0.621	Ohta et al. [28]
5 mM glucose for 40 min and 20 mM glucose for 5 min	1.67	0.22			7.70	2.50	
5 mM glucose for 40 min and 10 mM α-KIC for 5 min	2.25	0.18			8.35	7.18	Ohta et al. [27]
5 mM glucose for 40 min and 40 mM KCl for 5 min	0.81	0.25			3.23	6.24	
Islets cultured for 1–4 days and incubated with:						(pg/μg protein per min)	
3 mM glucose					5.0	≈ 250 [d]	Longo et al. [15]
20 mM glucose					7.0	≈ 670 [d]	

[a] Insulin level in perfusate.

[b] After 90 min of incubation.

[c] Calculated from the percentage of the total adenine nucleotide content.

[d] Determined with perifused islets.

factor of 2–3. Since the diameters of individual islets within preparations range from 20 to 800 μm ([2,3] and references therein) and larger islets are likely to have more DNA, protein and water, the simplest explanation for the inter-laboratory variations is that there are differences in the distribution of islet size. (iii) The data for protein content may depend on the assay method used. The very sensitive procedure of Bradford [4] seems to yield considerably lower values for the amount of protein per islet than other techniques. (iv) Female rats appear to have significantly more DNA per islet than male rats. Since the amount of protein per islet in the same preparations is not abnormally high, the reason for the high DNA content is not immediately apparent. (v) Dry weights of islets from *ob/ob* mice and guinea pig are reported to be substantially greater than of preparations from either albino mice or rats. In the former, this is readily explainable by the much larger average size of islets isolated from the genetically different animals.

In conclusion, preparations of isolated islets of Langerhans contain structures of various sizes in differing proportions. For this reason rather than expressing data on the basis of the number of islets it is preferable to do so in terms of either DNA, protein or dry weight content.

II-B. Concentrations of high-energy phosphate compounds

Pancreatic islets contain nucleotides that yield energy upon hydrolysis of their phosphate bond(s); the most important of these is ATP. In addition, islets may possess another high-energy reservoir, the creatine phosphate/creatine (PCr/Cr) system that is linked to the adenine nucleotides through a rapid equilibration in the creatine phosphokinase reaction:

$$PCr + ADP + H^+ \Leftrightarrow ATP + Cr \quad (1)$$

The apparent equilibrium constant for this reaction at 1 mM free magnesium is 1.66×10^9 [5]. This large value for K_{app} in Eqn. 1 means that at physiological pH (7.0–7.4) creatine phosphokinase favors formation of ATP. Hence, if the enzyme is sufficiently active and maintains near equilibrium, it can rapidly rephosphorylate ADP at the expense of PCr. As a result, creatine phosphokinase can 'buffer' changes in [ATP] and prevent its decrease when energy demand rises [6]. The latter statement holds true if total concentrations of phosphocreatine and creatine are much greater than those of ATP plus ADP.

It is well established that under physiological conditions ATP is present in cells in much larger amounts than ADP or AMP, while the concentrations of PCr and Cr in organs that contain these compounds (skeletal and cardiac muscle, brain) are greater than those of ATP; moreover, PCr exceeds Cr by a ratio of between 3 and 1 to 1. The adenine nucleotides can affect cellular metabolism in one of the following ways: as chemical energy released during hydrolysis of the high-energy phosphate bond (ΔG), (which is a function of [ATP]/[ADP][P*i*]), or as substrates and/or regulators of various processes. In either situation, it is the concentration of the *free* nucleotide (or its Mg^{2+} complex), and more specifically of that located in the cytosol where the overwhelming majority of energy utilizing reactions take place, that is the relevant parameter. This is a crucial point for two reasons. First, any binding of nucleotides and/or their sequestration influences the $[ADP]_{free}$ and $[AMP]_{free}$ much more than $[ATP]_{free}$ because the total amounts of ADP and AMP are considerably smaller. Second, the affinity of ATP for Mg^{2+} is higher than that of ADP [7]; consequently complexation with this cation affects $[ATP]_{free}$ more than $[ADP]_{free}$.

The content of adenine nucleotides in pancreatic islets has been measured by several investigators and under a variety of conditions [8–40]. Representative values from several laboratories are summarized in Table II. Two different methods were used to measure the levels of adenine nucleotides, an enzymatic technique [8,15,18,21,25,35,37,40] and HPLC [27,28]. The values, as reported in original papers and displayed in the table, are expressed in non-identical units. They can be interconverted using the information (Table I) that an islet contains about 3 nl of free internal water and about 1 μg of dry weight. Making the necessary conversions and normalizing the data to the unit of concentration one obtains figures of 1.5–8.0 mM for [ATP], 0.2–1.9 mM for [ADP] and 0.1–1.1 mM for [AMP]; these are not markedly different from the amounts reported for other tissues [41]. The numbers at the lower ends of the concentration ranges are those determined by HPLC, which is more sensitive than the enzymatic technique and not subject to a 'drift'. Whether the large, almost 5-fold, variations in the figures reported over the years from some laboratories (e.g., Refs. 16,18,21,22,34–36,42) are caused by technical shortcomings of the enzymatic assays, is not clear. However, based on information in the previous section, such differences are too large to be explained exclusively on the basis of variations in islet size. In our own hands, ATP concentrations in islets perifused in Hepes-containing buffers were twice those in tissues bathed in bicarbonate/CO_2 buffered media [28]. We were unable to explain this discrepancy but the high reproducibility of the phenomenon and the magnitude of the difference made it unlikely that it was an experimental artifact. On the other hand, careful selection of large and uniformly-sized islets, combined with meticulous attention to the details of quick and careful

quenching, decreased variability in the concentrations of the adenine nucleotides observed in our earlier work (compare Ohta et al. [27] and [28]).

Table II also illustrates the effect of various substrates, including those that markedly enhance insulin secretion, on the levels of adenine nucleotides. It can be seen that, in general, the effect is small, although there are indications [8,13] that islets maintained in the complete absence of fuels have a lower content of ATP.

In contrast to the number of studies on the adenine nucleotides, there is very little data on the concentration of creatine phosphate and creatine. In the early 1970's, Matschinsky and co-workers [14,25] reported that PCr was present in an amount equal to 15–25% that of ATP in islets microdissected from both in vivo and in vitro perfused pancreas (Table II). Not much more information had been available until recently, when Ghosh and co-workers [43] presented measurements of both PCr and Cr in islets microdissected from isolated pancreas perfused with a mixture of amino acids and either 4.2 or 8.3 mM glucose, i.e., conditions close to physiological. The content of creatine was slightly greater than of creatine phosphate and again much smaller than that of ATP (about 25%); the ratios of PCr/Cr were less than 1, under all conditions. This ratio is smaller than that in heart (1.2–2.0) and skeletal muscle (2–3) but within the range described for brain (0.8–1.3) [44,45]. The difficulty in measuring PCr and Cr is further underscored by the studies of Panten et al. [30] where PCr could not be found in isolated islets incubated with 10 mM glucose (the detection limit of the assay was 15 pmol/μg of DNA). It is possible that in the latter system both compounds leaked from the cells during lengthy isolation procedures. On the other hand, the authors were able to measure the activity of creatine phosphokinase, which they estimated to be 0.47 μmol/min per mg of protein; this value is 7.4-times less than that of brain homogenates determined under the same conditions. (The value of 3.46 μmol/min per mg of brain protein reported by Panten et al. [30], compares favorably with the figure of 600 μmol/min per g of brain tissue quoted by Veech et al. [45].) Since the activity of creatine phosphokinase in muscle is even higher than that in brain [45] a question arises whether the islet enzyme is active enough to maintain near equilibrium. One has to keep in mind, however, that creatine phosphokinase might also 'leak' from islets during their isolation.

In summary, taking into account difficulties in calculating the actual *concentrations* of various high-energy phosphate compounds in pancreatic islets, the most conservative estimate based on results from many laboratories is that total ATP is 3–5 mM, ADP is 0.8–1.2 mM, while PCr and Cr are present at about equal concentrations of 1 mM each. Thus the total content of the 'auxiliary energy reservoir', [PCr + Cr], is about 2 mM, which is considerably less than that of the adenine nucleotides (4–6 mM). This situation is markedly different than in other PCr + Cr-containing tissues, in which these compounds are present in at least a 2-fold excess over the adenine nucleotides.

II-C. Compartmentation and binding of adenine nucleotides

Measurements of high energy phosphate compounds by analytical methods give their total intracellular levels, whereas the nucleotides are distributed among various intracellular compartments (eg., cytosol, mitochondria, secretory granules) in which their concentrations may, or may not be, the same. It is generally assumed that cytosol is the major location of these compounds and, indeed, in the liver and heart 60–80% of ATP is cytosolic, while the remaining 20–40% is mitochondrial [46–48]. On the other hand, the situation may be quite different in secretory organs such as the pancreas, where not only mitochondria but also intracellular granules may sequester a substantial proportion of the adenine nucleotides. Consistent with this supposition, Leitner et al. [49] found that the granule fraction isolated from pancreatic islets contained both ATP and ADP amounting to 7.94 nmol/mg protein. The content of ATP was higher than that of ADP by a small fraction (4.19 vs. 3.75). Similar, although not identical, results were obtained by Hutton and coworkers [50]. The latter authors found that the content of ATP was 6.6 nmol/mg prot., of ADP 9.8 nmol/mg protein and of AMP, 6.4 nmol/mg protein. If one assumes that the protein of the secretory vesicles constitutes about 15% of the total islet protein [49–52], an inescapable conclusion is that a substantial proportion of the adenine nucleotides, 20–30%, is rather inert and not likely to be available for rapid metabolic interactions. Although this figure may seem large, Ashcroft and co-workers [8] have shown that after addition of an uncoupler, the content of ATP in islets decreased to 30% of the original value within 5 min. The remaining portion took about 40 min to decline. This may indicate that 70% of total islet ATP is accessible to cellular energy consuming reactions and thus is likely to be present, to a large extent, in the cytosol and/or in a compartment (or compartments) which is (are) in rapid equilibrium with it.

To determine the concentrations of the adenine nucleotides in mitochondria, attempts were made [16,36] to separate the cytosolic and mitochondrial fractions directly by treating suspensions of islets for 20 s with 0.5 mg/ml of digitonin and centrifuging the mixture at 5000 × g for 20 s. It was found that ATP and ADP were distributed about evenly between the two fractions. There are, however, serious problems

with this experimental design. (1) The particulate fraction contains not only mitochondria but also cell debris, nuclei etc., while the very shortness of the spin at a relatively low velocity may not be sufficient to pellet all the mitochondria. This constitutes a large potential error and, in our opinion, precludes estimates of the mitochondrial concentrations of the adenine nucleotides with any confidence. (2) Contamination with secretory granules can be another source of error because these organelles represent a large nucleotide-rich compartment. The investigators claimed that in their hands almost all the insulin was recovered in the pellet fraction, therefore, they concluded that the so-called cytosolic fraction was 'pure'. We find this surprising in view of the reports from other laboratories that much higher velocities and much longer centrifugation times were necessary to obtain satisfactory preparations of secretory granules [49,53–56]. (3) The total content of ATP in the study was 2.24 pmol/islet, which is the smallest figure reported from the same laboratory and a number considerably lower than the values given in Table II. This suggests that either there are some inherent, unresolved experimental problems in this approach or that there are uncontrolled variables that contribute large errors. An obvious conclusions from these studies is that although ATP and ADP are undoubtedly present inside mitochondria, the extent of their sequestration remains unknown. However, based on studies with other systems, such as liver, where mitochondria occupy approximately the same total volume of the cell as in islets [46–48], it is not unreasonable to suggest that in the latter 20–30% of the nucleotides is present in these organelles.

An important consequence of the nonuniform distribution of adenine nucleotides is that they may be present in the various compartments at different concentrations. The values for those depend on the water content of the individual compartments and the extent to which the nucleotides are bound. Although the former are reasonably well established, there is almost no information on the extent of ATP (or ADP) binding to various cellular constituents in islets. Some estimates of the free fractions of the adenine nucleotides are discussed below.

In addition to differences caused by the existence of multiple intracellular compartments, it has occasionally been suggested that there are inhomogeneities in the local concentrations of adenine nucleotides. Implicit in such a postulate is the assumption that diffusion of these molecules is not rapid enough to ensure uniform distribution. This mostly concerns compounds that are present at low concentrations and may be caused by very high local rates of either their production or utilization. It was hypothesized that in heart the diffusional fluxes of free ADP are carried by creatine [57] since the latter is present at a much higher concentration and exclusively in the cytosol [48]. In the case of islets, postulates of differences in the local levels of ATP were based on the findings that in a B-cell derived cell line (HIT-T15 cells) the activity of the ouabain-sensitive Na/K pump was inhibited at mM concentrations of the nucleotide [58]. Since in the purified enzyme the K_m for ATP at its catalytic site is below 50 μM [59] it was suggested that the concentration of the nucleotide at the plasma membrane might be in this range of values. However, it should be remembered that there are two sites on the Na/K pump for ATP, the second of which, the regulatory one, exhibits a K_m of 0.5 mM or larger [60]. Hence, the apparently high sensitivity of Na/K ATPase seen in whole cells and also noted in other systems [61] cannot be used as an argument for the existence of local gradients in [ATP].

In conclusion, the adenine nucleotides in pancreatic islets are distributed among various cellular compartments, the most important of which are cytosol, mitochondria and secretory granules; cytosol may contain up to 50% of the total content. The concentrations of adenine nucleotides in the individual compartments remain to be established but they are likely to differ among themselves. At present, there is no solid experimental evidence for the existence of local gradients in the adenine nucleotide concentrations within the cytosolic compartment itself.

II-D. [ATP] / [ADP] and its changes

In addition to listing the levels of the various high energy phosphate compounds, Table II provides values for the $[ATP]_{total}/[ADP]_{total}$. The latter is a dimensionless number and is thus independent of the concentrations of the individual nucleotides. This is of practical consequence because the [ATP]/[ADP] is not related to the amount of material used for analysis, as long as the extraction of the nucleotides is complete (or identical for both). Moreover, because in most metabolic transitions, changes in ATP occur at the expense of ADP and vice versa (i.e., are of the opposite nature), increases and decreases in the nucleotide ratio are larger and intrinsically more sensitive than changes in the individual nucleotides. This is of special importance in pancreatic islets where alterations in [ATP] and [ADP] are expected to occur in the cytoplasm, whereas measurements are made, in most cases, on whole cells. Since in this tissue, a substantial proportion of ATP and ADP is sequestered in a relatively inert and slowly exchangeable vesicular compartment (section above) any rapid alteration in the metabolically active pool is superimposed on (and therefore partially obscured by) a large background.

Inspection of Table II shows that the [ATP]/[ADP] in islets perifused in the absence of metabolic sub-

strates is between 2 and 4 [21,25,35] and appears to rise to about 5 with non-stimulatory concentrations of glucose (3–5 mM [15,27,28]). A detailed analysis of the effect of glucose and several amino acids on the nucleotide ratio, presented in multiple publications from one laboratory [16,17,19,21,22,31,34–36,62,63] leads, however, to the disappointing conclusion that either the assay technique was not very reproducible or that these substrates induce no systematic and consistent changes in the [ATP]/[ADP]. This is illustrated by a sample of figures displayed in the table. It can be seen that both higher concentrations of glucose (7–16 mM) and several amino acids caused random and small increases in the nucleotide ratios that did not correlate in any predictable manner with insulin secretion.

By contrast, Ashcroft et al. [8] noted a significant positive correlation between ATP level and the extent to which the applied stimulus (sugar or amino acid) was metabolized by islets. In our hands, addition of either high glucose or α-ketoisocaproic acid gave, within 1–5 min, significant and very reproducible increases in the [ATP]/[ADP] that were dependent on glucose concentration [26] and were smaller with 16.7 mM glucose than with α-ketoisocaproic acid; moreover, smaller elevations in the nucleotide ratio also produced a lesser stimulation of insulin secretion [27] which may suggest a positive correlation between the two events. A similarly substantial rise in the [ATP]/[ADP] was reported recently by Longo et al., [15], when glucose concentration was elevated from 3 to 20 mM. It may be important to mention that, as our accuracy in trapping and extracting nucleotides has increased, we have been able to detect not only alterations in the adenine nucleotide ratios but also consistent, albeit small, rises in [ATP] and corresponding reductions in [ADP] after addition of metabolic secretagogues [28].

One of the important questions is whether a rise in the [ATP]/[ADP], seen upon addition of high glucose, occurs in the mitochondria or in the cytoplasm, or in both. The adenine nucleotide translocase that is present in the mitochondrial membrane of islets [64–66] should be able to establish a high [ATP]/[ADP] ratio in the cytosol, notwithstanding that the majority of ATP is produced inside mitochondria. Studies in the partially purified cytosolic and pellet fractions [16,36] seem to indicate that the ratio of the adenine nucleotides in mitochondria changes less than in the cytosol. However, the levels of ATP and ADP and their ratios were very low in both fractions, which indicates either that the separation techniques are fraught with unsolved problems (see discussion above) or that they are not fast enough to preserve the original nucleotide concentrations.

In contrast to metabolic secretagogues, 40 mM KCl (with 5 mM glucose present) does not induce a rise in the [ATP]/[ADP]; it may even reduce it somewhat [27]. Similar behavior has been noted in response to addition of tolbutamide and theophylline [32].

The [ATP]/[ADP] ratios, but not the sums of ATP + ADP, are markedly dependent on external pH (Fig. 4 in Ohta et al. [28]); they are much higher at acid pH and decline when [H^+] is lowered. This behavior would be consistent with the free energy change for ATP hydrolysis (ΔG_{ATP}) decreasing with a fall in proton concentration (see below for further discussion).

In contrast to the results above, no increase in the [ATP]/[ADP] was found in islets microdissected from pancreas perfused in vitro when the glucose level was raised from 4.2 to 8.3 mM; its value remained low at 3–4 [43]. This is somewhat unexpected because in isolated islets a rise in the nucleotide ratio was seen at the latter concentration of the sugar (26]. Although it can be argued that 8.3 mM reflects the physiologically relevant stimulatory level of glucose (as was demonstrated by a substantial rise in insulin release), it is nevertheless surprising that the authors did not administer higher concentrations, in the range 16–20 mM, which should have given larger changes. In isolated perifused islets, infusion of 16–20 mM glucose results in a rise in the [ATP]/[ADP] to 7–9 [15,27,28] and even higher values of 10–12 are observed in the presence of physiological [P_i] [67], i.e., conditions used in the study of Ghosh et al. [43].

In conclusion, if precautions are taken to minimize breakdown of the high-energy phosphate compounds during their extraction and subsequent manipulations and if accurate and sensitive methods are used for quantification of the nucleotides, it can be demonstrated that addition of high [glucose] and other metabolic secretagogues to isolated perifused islets causes an increase in the [ATP]/[ADP] to values as high as 7–12. A concentration of glucose that markedly stimulates insulin secretion does not affect, however, the ratio of the adenine nucleotides in islets microdissected from in vitro perfused pancreas.

II-E. Binding of the adenine nucleotides

As was pointed out in the previous sections, binding of the nucleotides, and in particular of ADP, to various cellular constituents [68] could substantially lower their free cytosolic concentrations. For this reason indirect approaches have been developed to estimate the $[ATP]_{free}$ and $[ADP]_{free}$. The first makes use of the equilibrium in the cytosolic creatine phosphokinase and allows calculation of $[ADP]_{free}$ (or $[ATP]_{free}/[ADP]_{free}$) from the measured values of PCr, Cr and ATP and a knowledge of the equilibrium constant for the CPK reaction. Utilizing this approach, Ghosh et al. [43] concluded that cytosolic free [ADP] was 30–40 μM while $[ATP]/[ADP]_{free}$ was 80–120, which is substan-

tially larger than the values obtained experimentally. Although the derived figures are similar to those in brain and heart [44], in islets activity of CPK and the concentrations of PCr and Cr may be too low to attain near equilibrium. Moreover, sequestration of ATP in secretory vesicles may preclude equating $[ATP]_{total}$ with $[ATP]_{free}$. All these problems could make the use of the equilibrium in the CPK reaction unsuitable for determining the free [nucleotide] in islets. The second approach is to employ ^{31}P-NMR, a non-invasive technique that permits constant monitoring of free concentrations of ATP and PCr over long periods of time. However, in its present state of development this method is not sensitive enough to measure $[ADP]_{free}$ directly owing to its low concentration; moreover because of the low sensitivity it is impossible to apply NMR for use in tissues such as pancreatic islets, which are available in very small quantities.

While discussing binding of adenine nucleotides, one should not fail to mention their complexation with Mg^{2+}. In the CPK reaction, a well as in ATP hydrolysis, the relevant reactants are the Mg^{2+}-complexes of the nucleotides. Since the overwhelming proportion of cellular ATP (over 90% in liver [69,70]) exists as such a complex, the error in approximating cytosolic ATP-Mg^{2+} with cytosolic ATP_{total} is not large. However, this assumption does not hold for a reaction that is dependent on free, uncomplexed ATP, because its concentration may be 10% or less of the total ATP (0.3–0.4 mM as estimated by Taylor et al. [70] for brain).

In conclusion, faced with the lack of appropriate methods, one is forced to conclude that at present we are unable to determine with any confidence absolute values of either $[ATP]_{free}$, $[ADP]_{free}$ or their ratio in pancreatic islets. However, one would expect that a rise in $[ATP]_{total}/[ADP]_{total}$ is reflected in a change in the same direction in the ratio of the free nucleotides. The other prediction we can offer is that in most tissues which contain the PCr/Cr system of sufficient activity, such as brain, heart or skeletal muscle, the $[ATP]/[ADP]_{free}$ is 80 or greater. Thus, if the CPK reaction in islets does perform its usual physiologic function, one would expect an adenine nucleotide ratio in these cells of approximately the same value. This is consistent with the figure of 80–130 reported by Gosh et al. [43] which was calculated neglecting the reservations (see above) concerning the activity of the enzyme and the concentrations of PCr and Cr which may be too low to maintain near equilibrium in the creatine kinase reaction.

II-F. Concentration of inorganic phosphate

Inorganic phosphate is a necessary component in the synthesis of ATP by both oxidative phosphorylation and glycolysis Furthermore, it is an important regulator of several enzymes, as well as the key player in maintaining a proper intracellular balance of calcium. In spite of this crucial role of orthophosphate, there is a dearth of quantitative information on its behavior in pancreatic islets under secretory and non-secretory conditions.

The first observations on the involvement of Pi in islet metabolism and function were made in the middle 1970's by Freinkel and coworkers [71–77]. It was reported that certain metabolic secretagogues (α anomer of *d*-glucose, *d*-mannose, *d*-glyceraldehyde, α-ketoisocaproic acid, leucine) caused a transient release of ^{32}P from islets labeled by preincubation with the radioisotope. The phenomenon was termed 'phosphate flush' and was shown to be suppressed by the membrane stabilizers tetracain and D_2O, but completely unaffected by elimination of calcium from the perifusion medium. The apparent 'K_m' for the glucose-induced ^{32}P release was found to be 1–1.5 mg sugar/ml (5.5–8.3 mM glucose) which is somewhat less than the concentration necessary to enhance insulin secretion by 50% (9–11 mM glucose; Pierce et al. [74]). Non-insulinotropic nutrients (L-glutamine and L-lactate) and non-nutrient secretagogues (arginine, tolbutamide, theophylline) did not induce 'phosphate flush' [78].

These qualitative observations with radioactive phosphate were confirmed by measurements of the total content of the anion in isolated perifused islets and perfused pancreas [37,79,80]. Stimulation with high glucose (14–16 mM) was found to lead to a fall in the tissue level of P_i but not to parallel decreases in the adenine nucleotides [79]. Moreover, the reduction in $[P_i]_i$ occurred both with and without 1.5 mM phosphate in the perifusion medium [37] although the tissue content of this anion, before and after addition of 14.5 mM glucose, was higher when P_i was present in the external medium (Table III). Simultaneous measurements of phosphate efflux (depletion of $^{33}P_i$ content from prelabelled islets) and influx (gain of $^{32}P_i$ by islets

TABLE III

Inorganic phosphate in pancreatic islets

All the data were taken from Trus et al. [37]. Concentrations (mM) were calculated using the following conversion factors: DNA content of 43.6 ng/μg dry weight and 90 pl of intracellular water per ng of DNA.

Glucose (incubation time) (mM)	$[P_i]_c$ (mM)	P_i (internal)	
		(mmol/kg dry wt)	(mM)
0 (freshly isolated)	0	15.4	3.9
2.4 (3 or 30 min)	0	29.7	7.6
2.4 (30 min) + 14.5 (10 min)	0	15.7	4.0
2.4 (30 min) + 14.5 (20 min) + 2.4 (10 min)	0	21.7	5.5
2.4 (40 min)	1.5	39.2	10.0
2.4 (30 min) + 14.5 (10 min)	1.5	21.2	5.4

perifused in the presence of 1 mM [^{32}P]orthophosphate) showed that immediately after administration of high glucose, loss of the anion exceeded its intake. Nevertheless, after 8–10 min the efflux was attenuated and the influx enhanced, which led to phosphate repletion [81]. In the absence of external phosphate, repletion cannot involve uptake from the external medium (unless there has been unrecognized substantial contamination with the anion). Since the latter explanation is unlikely, the observation of Trus et al. [37] that replacement of 14.5 mM glucose with a non-stimulatory concentration (2.4 mM) is followed by a secondary rise in internal [P_i] must mean that there is (are) a source of phosphate within islets that can serve as a storage pool for this anion.

The content of P_i in islets perifused with non-stimulatory concentrations of glucose and in the absence of external phosphate was found to be about 30 mmol/kg dry weight or, assuming appropriate conversion factors, (Table I) about 8–10 mM. (A higher phosphate content, 49.7 mmol/kg of dry weight, was reported from the same laboratory in an earlier study carried out under apparently identical conditions; Bukowiecki et al. [79].) This value increases to 39.2 mmol/kg dry weight (10–13 mM) in the presence of physiological $[P_i]_c$. This is rather high because most other body organs contain less than 3–4 mM orthophosphate. However, using an improved quenching procedure [26–28] we recently obtained a figure of 4.72 mM for [P_i] in islets perifused without this anion and with lactate/pyruvate as the fuel [67]. After stimulation with high [α-ketoisocaproate], phosphate concentration fell to 3.01 mM. These results suggest that $[P_i]_i$ in islets is not much different than in tissues such as brain or liver. Moreover, it is known that secretory granules contain about 130 mg of phosphate per mg of protein [50]. Hence, if a substantial proportion of the anion is sequestered in these organelles, its cytosolic concentration may be significantly lower and close to the 2–3 mM found in other PCr/Cr-containing tissues [44].

In addition to the analytical methods, disappearance of intracellular phosphate has been confirmed by electron probe evaluation of frozen sections of pancreatic islets [82,83]. Histochemical and microprobe examination in an electron microscope [82] revealed specific accumulation of phosphate in the region of the plasmalemma and nucleolus of B-cells. The lead phosphate precipitate did not seem to be related to B-cell granules (i.e., cytoplasmic vesicles or other organelles) and disappeared when high glucose was added to the incubation mixture. This lack of relation to the secretory granules is disconcerting in view of reports on the high content of P_i in these organelles [50]. However, the suggestion from the electron probe studies that exocytosis from the granules was not involved [82] is consistent with the lack of effect of calcium on 'phosphate flush' [72]. In this context it may be important to ask whether or not the release of ATP reported by Leitner et al. [49] upon addition of high glucose is calcium-dependent. If the results of Freinkel et al. [82] do not represent some peculiar experimental artifact, they clearly suggest that there is an accumulation of some kind of inorganic phosphate compound close to the plasma membrane. How a cell would maintain a non-uniform distribution, i.e., a gradient of a diffusible anion, remains a fascinating puzzle.

The mechanism of the 'P_i flush' is unknown and research during the past 10 years has provided no clues to the solution of the mystery. However, we feel that some speculations may be appropriate. In reviewing the literature the present authors were struck by two series of data which may be pertinent to the phenomenon. The first is that high glucose causes a rapid loss of sodium ions from B-cells. This has been demonstrated by both an electron probe analysis [83] and measurements with fluorescent indicators [84]. Since phosphate is carried inwards across the plasma membrane in a co-transport with sodium [85], it is possible that it also exits as a sodium phosphate complex by way of a membrane-bound carrier. A release of P_i by 0.1 mM chloromercuribenzene-*p*-sulfonic acid (2.8 mM glucose present) is consistent with this idea and may indicate that such a carrier requires free SH groups for its operation [86]. Interestingly, tolbutamide, which stimulates insulin secretion, but does not induce phosphate efflux, causes a substantial rise in $[Na^+]_i$.

The second observation of possible relevance is that in other systems certain nutrients, such as amino acids, can induce cell swelling [87]. This, in turn, could activate so-called stretch-controlled channels and alter permeability of the plasma membrane to ions, including phosphate. Whether or not high glucose and other metabolic secretagogues cause swelling of B-cells, is not known at present. The relative insensitivity of high glucose-induced phosphate release to 4-acetamido-4'-isothiocyanostilbene-2,2'disulfonic acid [86], an inhibitor of anion channel function, may argue against such a possibility although not all anion channels are similarly affected by that compound.

Of crucial importance for islet function is the relationship between insulin secretion and $[P_i]_c$. In perifusions in vitro, changes in external phosphate between 0 and 3 mM, which encompass the physiological range, do not seem to affect release of this hormone (e.g., Refs. 88, 89) and several laboratories (e.g., of Matschinsky and of Malaisse) perform their experiments in the nominal absence of phosphate in the medium. On the other hand, Osuna et al. [90] have claimed that in both isolated perfused pancreas and perifused pancreatic islets, total insulin secretion in response to 16.7 mM glucose increases as external phosphate is raised from 0 to 3.6 mM. By contrast, it

has been reported that arginine-induced insulin secretion was enhanced when external phosphate was omitted [91]. Addition of 10 mM phosphate appears to eliminate high glucose-induced hormone release [88]. Whether these changes in secretion are caused by the P_i anion itself, or indirectly by alteration of the concentration of $[Ca^{2+}]_i$, is not clear.

In contrast to the effects in isolated perifused islets, a decrease in intracellular phosphate was not observed when islets microdissected from in vitro perfused pancreas were analyzed after stimulation with 8.3 mM glucose [43]. The content of P_i at 4.2 mM glucose was 16–17 mmol/kg dry weight (about 5 mM) and it increased to almost 20 mmol/kg at the higher [sugar]. If these results are true, one is tempted to speculate that release of orthophosphate seen in isolated islets may be a property of this preparation, and with no relevance to the in vivo situation.

In conclusion, the concentration of inorganic phosphate in pancreatic islets is between 3–4 mM and even less in the cytoplasm, due to partial sequestration of the anion in the secretory granules. In isolated islets, but not in those in perfused pancreas, stimulation with high concentrations of metabolic secretagogues results in phosphate efflux. To what extent this phenomenon occurs in vivo has to be answered by future studies.

II-G. Changes in ΔG_{ATP}

Many anabolic reactions require ATP as the source of energy, which means that, in order to proceed, they must consume the free energy (ΔG) that is liberated during hydrolysis of the high energy phosphate bond. ΔG is the sum of two terms, the standard free energy change, $\Delta G^{0'}_{ATP}$, and the concentration ratio, $RT\ln [ATP]/[ADP][P_i]$. The standard free energy change is dependent on both $[H^+]$ and $[Mg^{2+}]$ because protons and Mg-nucleotide complexes are reactants in the reaction [7,92]. Moreover, in the second term, the concentrations of ATP and ADP are those of free Mg-nucleotides, which are present in the cytosol under cellular conditions. Thus, in order to determine ΔG one should know, with some precision, intracellular pH, magnesium concentration and cytosolic $[ATP]_{free}$, $[ADP]_{free}$ and $[P_i]$. Although the first two do not appear to differ markedly from cell to cell, the free concentrations of the nucleotides do [45]. Unfortunately, as discussed above, there is no reliable method thus far available which would allow calculations of $[ATP]_{free}/[ADP]_{free}$ in islets. For this reason, the value of ΔG in this preparation remains a matter of conjecture. However, based on results obtained in other tissues that contain the PCr/Cr system of sufficient activity, one could expect the free energy change for ATP hydrolysis to be 14–15 kcal/mol.

It is also worth remembering that $\Delta G^{0'}_{ATP}$ is pH dependent and decreases when $[H^+]$ rises [7,92]. On the other hand, the islet [ATP]/[ADP] increases with the rise in proton concentration [28] i.e., it changes in the opposite direction to the $\Delta G^{0'}_{ATP}$. Thus, the reduction in the nucleotide ratio with increasing pH may provide the means whereby the ΔG_{ATP} remains essentially constant. This behavior of pancreatic islets is very similar to that observed previously in isolated hepatocytes [93]. The occurrence of the same phenomenon in diverse systems might suggest that it represents a well-conserved mechanism of cellular homeostasis. The postulate that cellular homeostatic mechanisms tend to maintain a constant ΔG_{ATP}, is also supported by the finding that [ATP]/[ADP] is higher in islets perifused with a physiologic concentration of phosphate [67] when intracellular content of the anion is also larger [37].

Finally, it may be important to point out that the increase in [ATP]/[ADP], which occurs in isolated islets upon addition of metabolic secretagogues, means that ΔG_{ATP} also rises. This rise is further augmented by a simultaneously falling concentration of inorganic phosphate (phosphate 'flush'). Moreover, in islets treated with 2-deoxyglucose, a sugar analogue which ties up orthophosphate, addition of a high concentration of α-ketoisocaproate causes release of insulin that is larger than that without 2-deoxyglucose [67]. Whether this means that hormone secretion is facilitated in some manner by the low internal $[P_i]$ or by a high ΔG_{ATP}, remains to be established.

In conclusion, isolated pancreatic islets, like many other cells, tend to maintain a constant ΔG of ATP hydrolysis under a number of experimental conditions, such as alterations in pH or internal $[P_i]$. Stimulation of insulin secretion by metabolic secretagogues appears to occur in concert with a rise in the free energy change for ATP hydrolysis. Whether this reflects a causal relationship, is not known at present.

II-H. Cyclic changes in nucleotide levels

It has been shown recently that isolated islets perifused with either 10 or 20 mM glucose exhibit oscillations in oxygen consumption, internal calcium levels and insulin secretion [15]. A model was, therefore, proposed that increased glycolytic flux due to increased [glucose] initiates oscillations in glycolysis and the [ATP]/[ADP] ratio. This model was substantiated by studies in permeabilized RINm5F insulinoma cells in which oscillations in $[Ca^{2+}]_i$ were evoked by addition of a cell-free extract of rat skeletal muscle that spontaneously showed oscillatory behavior of glycolysis and linked oscillations in [ATP]/[ADP] [94]. This attractive hypothesis awaits direct demonstration of such cyclic alterations in the islets adenine nucleotide concentration.

In conclusion, cellular [ATP]/[ADP] in islets may undergo cyclic changes. These may be responsible for oscillations in internal calcium and ultimately, insulin release.

II-I. Release of adenine nucleotides to the external environment

As discussed above, secretory granules of pancreatic islets contain ATP and ADP in almost equimolar amounts. These nucleotides are released into the external environment at a rate of 0.09 pmol/islet per min (the number given is for the sum of the two [49]). This value increases to 0.29 pmol/islet per min upon exposure to high glucose concentrations. The release of adenine nucleotides parallels that of insulin, showing a linear relationship between the two mechanisms. Both processes are sustained for long periods in the presence of high glucose concentrations. Despite a rather rapid rate of release, the sum of ATP and ADP inside the islets themselves appears to remain constant under such conditions [26]. This suggests that the rate of adenine nucleotide synthesis must be at least equal to the rate of their loss.

In conclusion, ATP and ADP are co-released with insulin from pancreatic islets.

TABLE IV

Rates of lactate production and glucose oxidation by pancreatic islets

Species	Glucose (mM)	Lactate production (pmol/islet per h)	Glucose oxidation		Reference
			Rate	D-[glucose]	
Mouse	1.67		3.8 [a]	[U-^{14}C]	Andersson and Hellerström [53]
Rat	2.8	26.2 [c]	3.2 [b]	[U-^{14}C]	Sener and Malaisse [109]
			7 [b]	[3,4-^{14}C]	
		20.8 [c]	≈ 2 [b]	[6-^{14}C]	Malaisse and Sener [110]
			≈ 7 [b]	[3,4-^{14}C]	
ob/ob mouse	3		7.5, 8.8 [a]	[U-^{14}C]	Hellman et al. [210]
	3.3		7.8 [a]	[U-^{14}C]	Gunnarsson and Hellerström [211]
Mouse	3.3	9	3.5 [b]	[U-^{14}C]	Ashcroft et al. [95]
	3.3 [c]		≈ 10 [b]	[U-^{14}C]	Hellerström et al. [212]
	5.3 [d]		≈ 17 [b]	[U-^{14}C]	
Rat	4.2–5.5	0.15			Matschinsky and Ellerman [134]
		5 [c]	21 [b]	[5-^{3}H]	Pace et al. [107]
		26.2 [c,e]	9.7 [c]	[U-^{14}C]	Sener et al. [108]
		≈ 35			Zawalich and Matschinsky [213]
	11.1	46 [c]	10.4 [b]	[6-^{14}C]	Malaisse et al. [18]
			14.1 [b]	[1-^{14}C]	
		25			Matschinsky and Ellerman [134]
	16.7	50			
		27 [c]	68 [b]	[5-^{3}H]	Pace et al. [107]
		60 [c]	25 [b]	[U-^{14}C]	Sener et al. [108]
		41			Zawalich and Matschinsky [213]
		68.6 [c]	20.2 [b]	[6-^{14}C]	Sener and Malaisse [109]
			37.6 [b]	[3,4-^{14}C]	
		35.7 [c]	24.5 [b]	[U-^{14}C]	Sener et al. [33]
		37.2 [c]	17.2 [b]	[6-^{14}C]	Malaisse and Sener [110]
			45.6 [b]	[3,4-^{14}C]	
Mouse	16.7	29	22 [b]	[U-^{14}C]	Aschroft et al. [95]
			38.1 [a]	[U-^{14}C]	Andersson and Hellerström [53]
	16.7 [d]		≈ 24 [a]	[U-^{14}C]	Hellerström et al. [212]
ob/ob mouse	16.7		29.8 [a]	[U-^{14}C]	Gunnarsson and Hellerström [211]
	18		26.2 [a]	[U-^{14}C]	Frankel et al. [214]
	20		51.4–63.4 [a]	[U-^{14}C]	Hellman et al. [210]
Rat	27.5	78			Matschinsky and Ellerman [134]
Rat	27.5	42.8, 47.3			Zawalich and Matschinsky [213]
	27.8	73 [c]	13.6 [b]	[6-^{14}C]	Malaisse et al. [18]
			19.3 [b]	[1-^{14}C]	
		57 [c]	57 [b]	[5-^{3}H]	Pace et al. [107]

[a] μmol/g dry wt per h.
[b] pmol/islet per h.
[c] As glucose equivalents.
[d] Glucose concentration during islet culture.
[e] Calculated from the percentage of control (16.7 mM glucose).

TABLE V

Rates of O_2 uptake by pancreatic islets

Species	Substrate (mM)	Original units	Oxygen uptake		Reference
			original units	(μmol/g dry wt per h)[a]	
Mouse	endogenous				
		μmol/g dry wt per h	94	94	Hedeskov et al. [205]
		nmol/μg DNA per h	5.6	125	Panten and Klein [215]
	glucose				
	1.7	μl/μg DNA per h	260	259	Welsh et al. [216]
	2.5	μmol/g dry wt per h	132	132	Hedeskov et al. [205]
	3.3	μl/mg dry wt per h	≈ 5	≈ 223	Andersson and Hellerström [53]
	5	nmol/μg DNA per h	7.8–9.0	174–201	Panten et al. [30]
	10		12.0–13.2	268–294	
	15		15.6–16.8	348–375	
	16.7	μl/mg dry wt per h	≈ 9	≈ 402	Andersson and Hellerström [53]
	16.7	μmol/g dry wt per h	239	239	Hedeskov et al. [205]
	16.7	μl/μg DNA per h	490	488	Welsh et al. [216]
	20	nmol/μg DNA per h	12.0	268	Panten and Klein [215]
	30		21.0	468	Panten et al. [30]
Cultured islets:					
for 7 days with					
3.3 mM glucose	none	μmol/g dry wt per h	308	308	Hellerström et al. [212]
5.5 mM glucose	none		317	317	
16.7 mM glucose	none		424	424	
for 6 days with					
27.8 mM glucose	3.3	μl/mg dry wt per h	≈ 14	≈ 625	Andersson and Hellerström [53]
	16.7		≈ 20	≈ 893	
ob/ob Mouse	endogenous				
		μl/mg dry wt per h	4.5–5.7	201–254	Hellerström [115], [212]
			4.2–4.5	188–201	Hellerström et al. [116]
	glucose				
	2.8		5.7	254	Hellerström [115]
	5.6		6.9	308	
	16.7		8.6	384	Hellerström [115], [212]
	33.4		6.8	304	Hellerström [115]
Rat	endogenous				
		pmol/min per islet	8.1–8.5	472–495	Hutton and Malaisse [130]
		μmol/g dry wt per h	≈ 280	≈ 280	Hellerström et al. [212]
		pmol/min per islet	4.16	242	Malaisse et al. [131]
	glucose				
	7.0		6.54	381	
	11		10.4	606	Hutton and Malaisse [130]
	16.7		11.5	670	Malaisse et al. [19]
	16.7	μmol/g dry wt per h	≈ 400	≈ 400	Hellerström et al. [212]
	27.8	pmol/min per islet	12.2	711	Hutton and Malaisse [130]
	3-OH-butyrate				
	10		4.95	289	Malaisse et al. [131]
	10 + glucose 7		7.52	438	
	acetoacetate				
	10		5.20	303	
	10 + glucose 7		6.54	381	
	α-ketoisocaproic acid				
	4		9.7	566	Hutton and Malaisse [130]
	10		11.4	665	
	10 + glucose 27.8		14.8	863	
	leucine				
	10		11.9	694	Malaisse et al. [21]
	40		10.4	606	Hutton and Malaisse [130]
	glutamine				
	10		8.8	513	
	10		11.9	694	Malaisse et al. [21]
	10 + leucine 10		12.5	729	
	pyruvate				
	10		8.9	519	Hutton and Malaisse [130]

TABLE V (continued)

Species	Substrate (mM)	Original units	Oxygen uptake		Reference
			original units	(μmol/g dry wt per h) [a]	
Cultured islets:	glucose				
for 2 days with	1.7	nl O_2/islet per h	3.25	141	Welsh [39]
5.6 mM glucose	5.6		4.09	177	
	11.1		4.66	202	
for 1–4 days with	3	pmol/μg prot per min	≈ 6	≈ 316	Longo et al. [15]
10 mM glucose	20		≈ 13.5	≈ 712	

[a] Calculated assuming: 22.3 g DNA kg dry weight, Panten [217]; 1.03 or 0.84 μg dry weight/islet for rat and mouse, respectively, and 1 μg protein/1 μg dry weight (Table I)

III. ATP turnover in pancreatic islets

III-A. Reactions that produce ATP

Under steady-state conditions the rate of ATP production is exactly balanced by the rate of energy utilization, so that the concentration of ATP within the cell remains constant. Two main processes are responsible for ATP synthesis, glycolysis and oxidative phosphorylation. The former requires glucose (or glycogen) as substrate, the latter can use reducing equivalents from a variety of sources including carbohydrates, fatty acids, amino acids, etc. The rate of glucose oxidation by islets depends on the external sugar concentration ([95–98], Table IV and Refs. therein). Taking the values at 1.67–5.5 mM external glucose as those that reflect basal, i.e., non-stimulated activity (Table IV), one can calculate that the rate of oxidation of this sugar is 11.2 ± 6.7 μmol/h per g dry weight (mean ± S.D., n = 4) in rat islets and 9.3 ± 6 μmol/h per g dry weight (mean ± S.D., n = 6) in mouse islets. The corresponding figures at 16.7–18 mM glucose are 40.4 ± 18 (n = 5) and 28.9 ± 5.6 (n = 5) μmol/h per g dry weight, respectively. Thus glucose oxidation increases between 290 and 360%, which is directly proportional to the rise in the external sugar concentration. This pattern of behavior could reflect the operation of the plasma membrane transporter GLUT-2 [99,100] which has a very high K_m for glucose [99,101]. Although it has been proposed that underexpression of this protein in non-insulin-dependent diabetes impairs glucose-stimulated insulin secretion [102] in healthy individuals transport is not limiting because the transporter has a very high capacity for uptake which is in large excess over intracellular glucose metabolism [103]. Based on the current evidence [103,104], it appears that the rate of glucose oxidation is determined predominantly by changes in the activity of glucokinase, the key regulatory enzyme of islet carbohydrate metabolism (see below).

Metabolism of glucose by islets also produces lactate. Although variations between different laboratories are large, within the same laboratory a relatively consistent pattern is observed; lactate production is greater at higher external glucose concentrations (Table IV) and in the hands of some authors increases almost linearly with the rise in [sugar] [105,106]. Using the same approach as that for glucose oxidation (see above), we calculate a rate of lactate generation of 34.4 ± 18 (n = 6) μmol/h per g dry weight for rat islets treated with 1.67–5.5 mM glucose and of 78 ± 37 (n = 7) μmol/h per g dry weight for those exposed to 16.7 mM glucose; this is a 2-fold increase, which is less than that for glucose oxidation. An interesting conclusion from the analysis of these figures is that, at low external glucose concentrations, almost twice as much of the sugar is converted to lactate as is oxidized to CO_2 and H_2O. However, at 16.7 mM glucose, the two processes are almost equal. These calculations agree well with direct measurements of the rates of glucose utilization. (The rate of glucose utilization in rat pancreatic islets is 29.3 ± 3.1, n = 3, μmol/h per g dry weight at 2.8–5.6 mM glucose and 102 ± 24, n = 6, μmol/h per g dry weight at 16.7 mM sugar [31,33,107–110].)

As summarized in Table V, islets consume substantial amounts of oxygen in the absence of external glucose or other fuel. In mouse tissue, such endogenous respiration is about 110 μmol/h per g dry weight (1.83 μmol/min per g dry weight) and increases to 200 ± 54 (n = 4) μmol/h per g dry weight (3.33 μmol/min per g dry weight) in the presence of 1.7–5 mM glucose and to 376 ± 126 (n = 4) μmol/h per g dry weight (6.27 μmol/min per g dry weight) with 16.7 mM sugar. In rat islets endogenous respiration is 330 ± 106 μmol/h per g dry weight (5.50 μmol/min per g dry weight) and increases to 594 ± 169 (n = 3) μmol/h per g dry weight (10 μmol/min per g dry weight) with 16.7 mM glucose. In islets isolated from both species of animals, the rise in O_2 consumption upon transition from non-stimulatory to nearly saturating levels of glucose is about 70%.

A comparison of the rates of glucose oxidation and of oxygen consumption shows that, in rat islets at low concentrations of the sugar, its contribution to the overall rate of O_2 uptake is 11.2 × 6 or 67.2 μmol/h per g dry weight, i.e., 20%. (Complete oxidation of 1

mol of glucose requires 6 mol of O_2.) The corresponding figure in mouse islets is 25%. At 16.7–18 mM external glucose, its oxidation accounts for 242 out of 594 μmol/h per g dry weight of O_2 consumed (or 41%) in rat islets and 173 out of 378 μmol/h per g dry weight, 46%, in mouse islets. One can calculate from these figures that between 65% (rat islets) and 75% (mouse islets) of the increment in oxygen uptake which occurs upon transition from the low to high glucose arises from the oxidation of this sugar. These calculations lead to two important and interesting conclusions. The first is that at basal levels, glucose is only a minor fuel for islets. This is in agreements with experiments which show that fatty acids and amino acids are the key supporters of endogenous respiration [111–113]. The second conclusion is that at high external concentrations, glucose and its metabolites provide a major fraction of the reducing equivalents for operation of the respiratory chain. This is consistent with measurements of heat production in pancreatic B-cells [114] which show an increase with a rise in glucose concentration.

Table V summarizes other results which deserve brief comments. (i) O_2 uptake in rat islets is about twice that in the mouse tissue. Since respiration is a function of the content of the respiratory chain proteins and not dry weight, it is not clear whether mouse islets exhibit intrinsically lower activities or contain fewer mitochondria (or respiratory chain components) per islet. (ii) Cultured islets show approximately the same respiration as freshly isolated tissue. (iii) 3-OH-Butyrate and acetoacetate increase respiration by about 25% and high concentrations of leucine, glutamine, pyruvate and α-ketoisocaproate produce large changes. (iv) Not shown in the table is the finding that mannose, fructose and galactose (all at 16.7 mM, [115]) and alanine, valine and arginine (all at 1 mM, [116]) do not affect oxygen consumption.

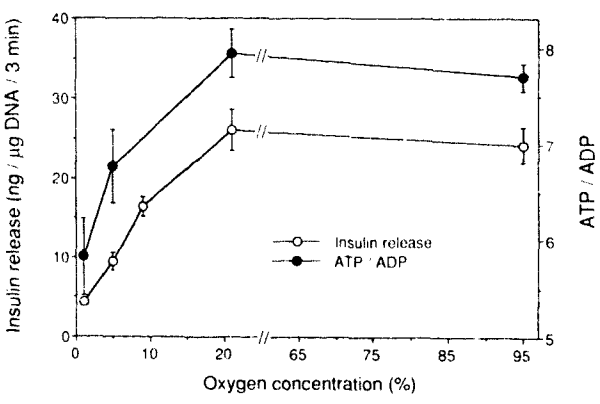

Fig. 1. Relationship between O_2 concentration, ATP/ADP and insulin secretion in isolated pancreatic islets of Langerhans. Results were taken from Ohta et al. [27].

The knowledge of the rates of lactate production and oxygen consumption allows calculations of the amount of ATP produced by glycolysis and oxidative phosphorylation. In rat islets, at low glucose concentration, glycolysis provides 0.57 μmol ATP/min per g dry weight and oxidative phosphorylation, 33.0 μmol ATP/min per g dry weight, i.e., the former accounts for 2% of the total ATP production. (The calculations were performed using stoichiometric factors of 1 mol of ATP formed per mol of lactate generated and 6 moles of ATP produced per mol of O_2 consumed.) The corresponding figures at 16.7 mM glucose are 1.3 μmol ATP/min per g dry weight for glycolysis and 59.4 μmol ATP/min per g dry weight for oxidative phosphorylation, which means that the proportional contribution from glycolysis does not change at high external sugar concentrations.

It may be interesting to compare these numbers with the values for ATP generation in other organs. Brain produces 67–226 μmol ATP/min per g dry weight by oxidative phosphorylation and 0.1–0.7 μmol ATP/min per g dry weight by glycolysis (recalculated from the results presented in Erecińska and Silver, [44] if in brain dry weight constitutes 15–20% of the wet weight [117]). In perfused rat heart, mitochondrial energy production is between 116–248 μmol ATP/min per g dry weight and glycolytic, 3.5–9.0 μmol ATP/min per g dry weight (calculated from Nishiki et al. [118]; Rumsey et al. [119]). These comparisons indicate that all three tissues derive over 95% of their energy supply from mitochondrial metabolism. It is also interesting that during enhancement of brain activity, such as occurs, for example, in seizures, the proportion of energy supplied by glycolysis does not increase even though the rate of lactate generation rises substantially [44]; this situation is similar to that in islets stimulated with high glucose. The reliance on oxidative phosphorylation for support of energy-consuming reactions may be a matter of cellular economy because, during complete combustion of glucose, mitochondrial metabolism provides 17-times as much ATP as does anaerobic glycolysis.

The paramount role of oxidative phosphorylation in cellular energy metabolism means that islet function and ATP concentration may be particularly sensitive to a decrease in the ambient oxygen concentration. Consistent with this supposition it has been shown that a decrease in O_2 level in the perifusion medium reduces insulin secretion as well as the rise in [ATP]/[ADP] induced by metabolic secretagogues, high levels of glucose and α-ketoisocaproate ([27] and Fig. 1). It is, therefore, possible that in pathological states, accompanied by high demands for insulin, such as non-insulin dependent diabetes or obesity, secretion of the hormone may be curtailed by hypoxia, resulting from an inadequate blood supply to the hypertrophied islets

[120]. Confirmation of the effects of lowered oxygen tension comes from experiments with inhibitors of the mitochondrial respiratory chain (antimycin A, amytal, KCN, rotenone) which have shown that these compounds decrease both insulin secretion and ATP levels in islets [8,27,121–123].

In conclusion, mitochondrial oxidative phosphorylation is the key reaction that generates ATP in islets; contribution from glycolysis is only about 2%. At low glucose levels, 5 mM and lower, fatty acids and amino acids are the main sources of reducing equivalents. At higher glucose concentrations, islets utilize proportionally more carbohydrate as a source of energy.

III-B. Reactions that utilize ATP

In view of the correlation between the demand for energy and the rate of its production, it is important to identify the predominant reactions that consume ATP because it is their activities which will be largely responsible for the pattern of metabolic response under physiological and pathological conditions.

The main function of pancreatic islets is production and secretion of protein hormones. One might expect, therefore, that much of the total energy produced by this tissue would be utilized for this purpose. In addition, a variety of other cellular activities are supported by ATP; they include biosynthetic reactions (such as synthesis of other proteins and lipids), ion movements (and especially those that maintain the intra/extracellular balance of Na^+, K^+ and Ca^{2+}), the phosphorylation reactions and maintenance of structural integrity of the cell.

Determination of the amounts of energy normally expended for various processes is not easy. The simplest way is to use an inhibitor of a particular pathway and then measure ATP synthesis under conditions when a given activity is blocked. Using this rationale, cycloheximide has been employed to eliminate protein synthesis. It was found that 0.05 mM of this drug decreased oxidation of D-[6-^{14}C]glucose by only 14% during 120 min incubation [110]. This is a very small reduction for an activity that appears to be of primary significance to islets. However, cycloheximide may either not completely inhibit protein synthesis under the experimental conditions used, or there may be a substantial endogenous pool of precursors which takes much longer to exhaust. Hence, the proportion of ATP used for synthesis and packaging of proteins in islets may be substantially larger than that estimated experimentally.

There are three major cations, Na^+, K^+ and Ca^{2+}, which have to be maintained in electrochemical disequilibrium across the plasma membrane in order to ensure undisturbed cell function and their uphill movements consume energy. The main enzyme responsible for the maintenance of the gradients of the first two ions is the Na^+/K^+ ATPase that moves 3 Na^+ from outside against 2 K^+ in the opposite direction with a concomitant breakdown of 1 ATP [59,60,124,125]. The K_m of this enzyme for Na^+ in various tissues is between 10–30 mM, for K^+, 2–3 mM and for ATP there are two values, one less than 1 μM and the other 0.3–0.5 mM [59,60]. Thus, under physiological conditions, the activity of the Na^+/K^+ ATPase is controlled predominantly by the concentration of intracellular sodium.

An ouabain-sensitive Na^+/K^+ ATPase, inhibited by glucose [126], is also present in islets [126–128] but its activity is 3–4-fold lower than that in the kidney [126]. The enzyme exhibits a single K_m for ATP with a value of 330–350 μM [126]. In 1974 Hellman et al. [98] reported that addition of 0.1 mM ouabain decreased oxidation of 3 mM glucose by islets from 6.1 to 5.2 mmol/h per kg dry weight, i.e., by 15%. Direct measurements of the pump activity in media containing 5 mM K^+, showed that the enzyme moves 14.2 pmol/min per islet of $^{42}K^+$ [129] or 14.8 pmol/min per islet of ^{86}Rb [42]. Since 2 K^+ are transported per each ATP hydrolyzed, the movement of potassium consumes energy at a velocity of 7.1–7.4 pmol of ATP/min per islet. The rate of oxygen uptake under the same conditions is 4.2–8.5 pmol/min per islet (these two values, which differ by a factor of 2, arise from the same laboratory: Hutton and Malaisse, [130]; Malaisse et al., [123,131]) which allows one to estimate that the pump consumes between 14 and 28% of basal ATP generation. The former figure is in reasonable agreement with the value of 15% obtained with ouabain [98]. In the presence of 15 mM glucose, the inhibition of glucose oxidation by the alkaloid was from 45 to 37.6 mmol/h per kg dry weight, i.e., again about 16%. The latter result is consistent with the observation that during stimulation with high glucose, $[Na^+]_i$ does not rise and may even decrease [84], which would prevent activation of the Na^+/K^+ ATPase. The figure of 15% of ATP being used for the movements of Na^+ and K^+ by islets compares with the 5–10% expended by the heart, liver and skeletal muscle enzymes [132] but is much less than the 40–60% consumed by brain [44] and kidney [133]. It is worth mentioning that ouabain substantially decreases production of lactate by islets incubated with either 27.8 mM glucose [134] or with glucose and 8–10 mM L-isoleucine [107] which suggests that glycolytic ATP is also available to support the Na^+/K^+ pump function.

Another ion with unequal distribution across the plasma membrane is calcium. There are at least four mechanisms in islets, all dependent directly or indirectly on energy, that maintain low cytoplasmic levels of this cation: the plasma membrane Ca^{2+} pump [126,135], the endoplasmic reticulum pump [136], up-

take by mitochondria (which is driven by membrane potential and thus linked to ATP indirectly) [51,137,138] and the Na^+/Ca^{2+} exchange, fueled by the Na^+ gradient [139,140], which is maintained through the operation of the Na^+/K^+ pump. Moreover, uptake by intracellular granules [51,137] may provide an additional mechanism for lowering cytosolic levels of calcium.

The activity of the plasma membrane Ca^{2+}-ATPase has been measured in islets and shown to be very high (54.8 nmol P_i released/min per mg protein at 37°C, Pershandsingh et al. [135]). However, there is no straightforward relation between the maximum activity of this enzyme and the amount of ATP consumed by islets for the maintenance of the Ca^{2+} gradient. In brain, which continuously removes calcium that enters neurons during action potentials, the plasma membrane pump consumes only 0.3–0.5% of total energy production, whereas the Na^+/Ca^{2+} exchange, which in many tissues is the most prominent reaction for maintaining low cytosolic $[Ca^{2+}]$, consumes 2–3% [44]. Thus, it is unlikely that the figures will be much larger in pancreatic islets.

Yet another reaction which consumes ATP is de novo synthesis of the adenine nucleotides. As mentioned earlier, these metabolites are intrinsic components of the secretory granules [49,50] and are released into the external environment concomitantly with insulin. The rate of nucleotide release may be quite high, in particular during periods of stimulated secretion. Hence, to compensate for the loss, the de novo production must be rapid and thus is likely to consume significant amounts of energy.

In chromaffin cells [141] and synaptosomes [142] exocytosis itself requires ATP and ceases when the concentration of the latter falls below about 1 mM. That this statement also applies to islets is borne out by experiments which show that KCl-triggered insulin release is almost eliminated by addition of inhibitors of the mitochondrial respiratory chain or by lowering oxygen tension in the perifusion medium [27]. However, it is not clear whether the requirement is for ATP itself or for its hydrolysis. Omission of calcium from the perifusion/incubation medium eliminates insulin secretion and concomitantly decreases oxidation of glucose by 20–40% [17,20,98,110]. This might be because exocytosis consumes a measurable fraction of energy. On the other hand, calcium is an ubiquitous intracellular effector and a fall in its concentration could influence other energy consuming reactions with a consequent decrease in the rate of ATP synthesis. Thus, to what extent exocytosis consumes energy cannot be decided at present. It has also been reported that omission of calcium does not decrease oxygen consumption by islets either in their basal state or during stimulation with secretagogues [130]. This result contradicts those on the effect of calcium on glucose oxidation [17,20,98,110] and we have no explanation for the apparent discrepancy in the data.

From the considerations above, it appears that protein synthesis and associated reactions (packaging, nucleotide synthesis, secretion) may consume as much as 40–50% of ATP produced by islets, whereas uphill ion movements utilize about 20%. Assignment of appropriate portions of ATP usage to the individual processes that consume the remaining 30–40% of energy is rather difficult. Lipid synthesis [143,144] and cell maintenance and repair are the definite candidates but, in addition, some proportion is likely to be used in phosphorylation reactions. Several classes of proteins are known to undergo phosphorylation in pancreatic islets including enzymes, regulatory and structural proteins and channels (e.g., [145–149]) and some of these may turn over rapidly and require a significant amount of ATP.

In conclusion, circumstantial evidence indicates that the key energy-consuming reaction in pancreatic islets is the synthesis of proteins. Uphill movements of ions also contribute a measurable fraction although much smaller than in brain or kidney.

III-C. High-glucose dependent increase in ATP synthesis

It has been pointed out above that in steady-state systems an increase in energy transduction occurs in response to an enhancement of the rate of energy utilization. This behavior ensures that the concentration of ATP will remain constant and thus support the consuming reactions at undiminished velocities. Since glycolysis and oxidative phosphorylation are the two pathways that supply ATP, the question of a signal or signals for their activation has to be addressed.

Glycolysis and oxidative phosphorylation are spatially segregated within the cell and are regulated independently, although some of the regulatory factors may be common to both processes. In most tissues, glycolysis is controlled, predominantly, by the activity of phosphofructokinase, the rate limiting step of the pathway [150]. During short-term alterations in cellular function, when the amounts of protein do not change, phosphofructokinase is stimulated by ADP, AMP and P_i and inhibited by creatine phosphate and ATP ([150–152]; see also Refs. 151–154 for a review of regulation by other mechanisms). Since a fall in PCr causes a rise in P_i, while a decrease in ATP results in increases in ADP and AMP, deinhibition of the enzyme is superimposed on its stimulation, which potentiates the activatory response. This allows a very sensitive and powerful augmentation of glycolytic flux when cellular activity rises. However, such a picture is an oversimplification because several homeostatic mechanisms oppose large changes in any single parameter. They include: (i) increased production of ATP at the expense of ADP and P_i by oxidative phosphorylation; (ii) rephosphoryla-

tion of ADP at the expense of PCr; (iii) formation of ATP plus AMP from ADP through the adenylate kinase [155]. All of these processes minimize alterations in [ATP] and [ADP]. Thus an interesting conclusion is that during increased energy utilization, phosphofructokinase may be stimulated to a large extent by both its deinhibition by decreasing [PCr] and activated by increasing [AMP] and [P_i].

However, in pancreatic islets glycolytic flux may not be controlled predominantly by the activity of phosphofructokinase. This tissue contains a powerful sugar-phosphorylating enzyme, glucokinase, which, owing to its high K_m for glucose is not saturated at physiological substrate concentrations [24,103,104,156]. Thus when external glucose is raised, with a consequent increase in its intracellular level, the flux through the pathway rises in proportion, independently from any other changes in the regulatory factors of phosphofructokinase. This may be crucial for enhancement of oxygen uptake, and hence energy production, which occurs in islets exposed to levels of glucose that stimulate insulin secretion.

Mechanisms that stimulate mitochondrial oxidative phosphorylation are much more complex. When oxygen is plentiful, the activity of the respiratory chain is controlled by two variables, the phosphorylation state of the adenine nucleotides, [ATP]/[ADP][P_i] (or an equivalent), and the redox state of the mitochondrial pyridine nucleotides ([NAD^+]/[NADH]) [157,158]. At a constant [ATP]/[ADP][P_i], respiration is inversely related to $[NAD^+]_m/[NADH]_m$. When the latter remains unaltered, O_2 consumption rises with a fall in [ATP]/[ADP][P_i]. During increases in energy utilization [ATP]/[ADP][P_i] falls, however transiently, which activates respiration and leads to oxidation of the intramitochondrial pyridine nucleotides. Such an oxidation has been described on addition of ADP to isolated mitochondria [159]. A rise in the $[NAD^+]_m/[NADH]_m$ has two opposing effects on respiration: it decreases the activity of the respiratory chain, because NADH is a substrate for this multienzyme complex, and it stimulates key mitochondrial dehydrogenases (pyruvate, isocitrate and 2-oxoglutarate; Hansford [160]) with a consequent increase in the production of NADH. Since a decline in [ATP]/[ADP][P_i] also activates the same dehydrogenases [160], transient oxidation of the pyridine nucleotides is likely to be followed by their reduction and an additional increase in O_2 uptake.

It follows from the above considerations that an increase in energy production, triggered by a rise in ATP usage, can be explained by alterations in both [ATP]/[ADP][P_i] (or equivalent) and mitochondrial pyridine nucleotides, which in turn, stimulate glycolysis and oxidative phosphorylation. However, the same mechanism does not seem to account for the 70–80% enhancement in energy production that occurs upon addition of high glucose to pancreatic islets for the following reasons: (1) there is no obvious primary reaction that consumes ATP. Phosphorylation of glucose in the glucokinase reaction is an intrinsic part of sugar metabolism; (2) glycolytic flux can be enhanced directly by an increase in concentrations of the pathway intermediates; (3) while a rise in energy utilization usually involves a fall in the energy state, an opposite result is seen with high glucose [15,27]. Even, if one assumes that there is an early, transient fall in [ATP]/[ADP][P_i], an occurrence that has still to be demonstrated experimentally, one has to explain how the sustained period of enhanced energy production is maintained in the presence of an increased level of ATP (or [ATP]/[ADP][P_i]). The rise in the latter may even be accentuated by simultaneously declining intracellular [P_i] [67, 71,72,80].

The simplest explanation is that the mitochondrial redox state must rise, i.e., the substrate input into the respiratory chain has to increase, independently and beyond that which normally accompanies a fall in the energy state. There are at least three possible ways in which this could happen. The first involves participation of the mitochondrial glycerol-3-phosphate dehydrogenase, a flavin-dependent enzyme known to be present in islets and capable of high activities [161,162]. This protein could facilitate rapid transfer of NADH from the cytoplasm to the respiratory chain. The second involves activation of the rate-controlling mitochondrial dehydrogenases by calcium [163] the level of which has been reported to rise in islets treated with high glucose [164]. Such an activation raises [NADH] independently of changes in [ATP]/[ADP][P_i] (or equivalent) and thus leads to an increase in ATP synthesis. It has been observed that islet pyruvate [165], 2-oxoglutarate [36] and 3-glycerol-phosphate dehydrogenases [162,166] are sensitive to alterations in calcium concentration and that glucose activates the first two enzymes [36,165]. Finally, Halestrap [167] has postulated that there may be some Ca^{2+}-dependent alterations in the mitochondrial volume that activate the respiratory chain directly. It should be emphasized that the latter two mechanisms require that a rise in cytosolic $[Ca^{2+}]_i$ precedes that in [ATP] or [ATP]/[ADP]. Although the need for well-designed and controlled experiments is obvious, it may be important to mention that our unpublished results (Ohta and Erecinska, unpublished) show that, in the presence of calcium channel blockers, which inhibit stimulated insulin secretion over 80% and prevent the rise in intracellular calcium [168], the increase in [ATP]/[ADP] induced by a high concentration of glucose remains unaltered.

An interesting, and very seldomly recognized consequence of stimulation of oxidative metabolism, which is accompanied by an increase in the cell ATP level, is

transient intracellular alkalinization. This occurs because ATP synthesis is the major reaction that consumes H^+ and, therefore, a rise in [ATP] means that production of energy and thus consumption of protons exceeds the rate of energy utilization (i.e., the major pathway of proton generation) [169]. Consistent with this prediction, it has been shown that addition of high glucose leads to a rise in islet pH_i [170,171].

In conclusion, addition of high glucose concentrations to perifused islets causes a marked increase in the rate of oxygen consumption, which is accompanied by a rise in the $[ATP]/[ADP][P_i]$. The primary activation of substrate influx into the respiratory chain could explain the increase in O_2 uptake but without sustained increases in the $[ATP]/[ADP][P_i]$. Persistent enhancement of respiration, concomitant with a rise in [ATP] [26], is an anomaly because it suggests that some energy-consuming reaction(s) must be turned off while cellular homeostatic mechanisms fail to respond appropriately with a decrease in energy generation. Whether islets truly behave in an unorthodox manner, which at present eludes our understanding, or whether we are still missing some vital facts about islet energy metabolism remains a challenge for future work.

IV. Other roles of adenine nucleotides

In order to offer a relatively complete picture of intracellular functions of the adenine nucleotides, we felt that it was necessary to discuss very briefly their roles other than those as the units of energetic currency. In these other roles, the adenine nucleotides present themselves as effectors of both channels and receptors and, hence, influence islet behavior secondarily either via alterations in ion fluxes or synthesis of second messengers.

IV-A. Nucleotides as effectors of K^+ channels

It has to be pointed out at the very beginning of this discussion that, in contrast to the preceding sections, where abundant information was available on energetic properties of isolated pancreatic islets, in the case of the role of the adenine nucleotides as effectors of channel function, the situation is rather different; most of the knowledge comes from studies on clonal cell lines. These will be quoted below with the proviso that B-cells exhibit a very similar behavior.

Pancreatic B-cells and clonal-derived cell lines possess ATP-dependent K^+-channels in their plasma membranes (Refs. 172, 173, for review see Refs. 174, 175). It is believed that increased metabolism of glucose (and of other metabolic secretagogues) raises the intracellular concentration of ATP and causes closure of these normally open channels, thereby resulting in depolarization of the cell membrane and enhancement of Ca^{2+} influx. An ensuing rise in the cytosolic $[Ca^{2+}]_i$ leads, in turn, to insulin secretion.

The existence of the ATP-controlled K^+ channels in islets and clonal cell lines is beyond doubt; however, the mechanism of their regulation by ATP under in vivo conditions and consequently their role in islet physiology is still a matter of some dispute. To simplify the analysis, we shall first summarize the most salient experimental results and then indicate the points of obscurity and/or controversy.

The ATP-controlled K^+ channel is influenced by ATP as well as ADP ([174,175] and references therein); ATP affects its activity by interacting at two pharmacologically distinct sites, both of which are accessible to the internal, i.e., intracellular nucleotide. (These sites might be either on the channel itself or on a closely associated protein.) One site is inhibitory and leads to a decrease in channel activity, ie., its closure. In membrane patches, closure of the channel occurs with K_i values for ATP of between 10–70 μM. By contrast, in RINm5F cells, half-maximal inhibition of the glibenclamide-sensitive ^{86}Rb efflux takes place at intracellular $[ATP]_{total}$ of 0.8 mM [176]. Phosphorylation does not appear to be associated with the inhibitory action of the nucleotide because its non- and partially hydrolyzable analogues are effective in channel closure [177,178]. Moreover, the more potent inhibitor of activity seems to be free ATP and not its Mg^{2+}-complex [177].

ATP interaction with the other site, which requires concentrations of the nucleotide higher than 0.1–0.2 mM, as well as the presence of Mg^{2+}, leads to an increase in channel availability [179–181]. Non-hydrolyzable analogues are ineffective, which indicates that ATP and channel phosphorylation are necessary for this function. It has been postulated [149] that a cAMP-dependent protein kinase may be involved. The presence of ATP at this site prevents (or reverses) the rundown of K^+ channel activity, an effect also sometimes referred to as 'refreshment' [180], which is commonly seen in excised patches.

ADP (100–500 μM) activates the K^+ channels when added to the bath solution in contact with the inside of the plasma membrane in saponin-permeabilized RINm5F cells [182]. Moreover, when ADP is added in the presence of ATP, it shifts the dose-response curve for ATP to higher values [13,178,180,182]. This 'competition' between the two nucleotides led to the suggestions [174,175,180] that it may not be solely the concentration of ATP but also the cytoplasmic [ATP]/[ADP] that controls channel activity.

An interesting observation with respect to the role of ADP has been made recently during studies on the purification of the glibenclamide receptor from a hamster pancreatic B-cell line, HIT T15 [183,184]. It has been shown that this receptor is an ADP-binding pro-

tein and that the diphosphonucleotide competes with glibenclamide for binding. It was, therefore, suggested that 'ADP and sulphonylureas share common biding sites on the extracellular side of B-cell plasma membranes, where they inhibit the activity of the K-channel, resulting in an increase in intracellular Ca^{2+} concentration and insulin release' [183]. Unless there are two sites for ADP, one external and one accessible from inside, ie., at the same side at which ATP interacts, it is not clear to the present authors how the cytoplasmic [ATP]/[ADP] could contribute to the regulation of the channel activity.

Direct extrapolation of our knowledge on properties of the ATP-controlled K^+ channels, obtained to a large extent with the patch-clamp technique, to the behavior of such channels in vivo has met with two types of difficulties. The first concerns differences in the sensitivity of the channel to the adenine nucleotides measured in vitro, as compared to concentrations that are likely to exist under in vivo conditions. Based on considerations above, it appears that total cytoplasmic ATP is 3–4 mM whereas ADP is at least 10-fold lower. In in vitro measurements, the K^+ channel is closed at [ATP] of less than 100 μM, i.e., at the concentration of the nucleotide at least an order of magnitude smaller than the in vivo level. Several explanations have been put forward to explain the discrepancy between the two numbers, including local variations in [ATP] and its binding to the cellular constituents (see Refs. 174, 175 for discussion). In the opinion of the current authors, the easiest explanation is that free, cytoplasmic [ATP], i.e., that not complexed with Mg^{2+}, may be low in islets, 0.3–0.4 mM or less, and thus the changes that occur under various conditions are larger than those evaluated from measurements of total cellular [nucleotides]. Free ADP could be lower than 0.1 mM and complexed with Mg^{2+} to a smaller extent than is ATP. Consequently, the ratio of $[ATP]_{free}/[ADP]_{free}$ could be much smaller than that of their respective Mg-complexes and be closer to the values at which it operates effectively as the putative controlling factor of channel activity [175].

The second type of controversy concerns the question of whether or not increased glucose metabolism induces a rise in $[ATP]_{free}$ or [ATP]/[ADP], which is of sufficient magnitude to close the K^+ channels. Based on the recently observed rather large increases in the nucleotide ratio [15,28,67], which occur upon administration of high concentrations of metabolic secretagogues to in vitro perifused islets, our answer is positive. Moreover, a good correlation between the decrease in the ability of high glucose and α-ketoisocaproate to induce a rise in [ATP]/[ADP] and a reduction in the amount of insulin secreted by islets perifused under various oxygen tension [27] further strengthens this answer. However, recent studies on isolated pancreas seem to throw serious doubts on this postulate. Under conditions when the glucose concentration was increased from a basal level of 4.2 mM to 8.3 mM, i.e., to a physiological postprandial concentration, and insulin release rose 10-fold, there was no apparent change in either the content, or ratio, of the adenine nucleotides (or PCr and Cr) in islets microdissected from a frozen organ. If this were true, the present form of the hypothesis on direct involvement of the ATP-controlled K^+ channels in insulin secretion in vivo should be reconsidered.

In conclusion, pancreatic B-cells possess K^+ channels which are controlled by the adenine nucleotides. The nature of the regulatory factor(s) in vivo remains, however, elusive. It appears that results in islets perifused in vitro are consistent with direct involvement of these ATP-controlled in insulin secretion. There is no evidence that this is happening in islets either in in vitro perfused pancreas or those in vivo.

IV-B. Nucleotides as effectors of purinergic receptors

It has been known for almost 30 years [185] that ATP stimulates insulin secretion from the pancreas. Detailed examination of this phenomenon, and, in particular, its nucleotide specificity, has established that B-cells possess on their surface P2 purinergic receptors [186,187]. Stimulation of these receptors causes activation of protein kinase C, increased synthesis of inositol 1,4,5-triphosphate and mobilization of calcium from internal stores [188–190]. A consequent rise in cytosolic $[Ca^{2+}]_i$ causes insulin release. It has been calculated [190] that the amount of ATP released with insulin from the secretory granules during administration of secretagogues is high enough (μM) to stimulate the purinergic receptors. It has to be pointed out that such an activation of P2 receptors will further enhance insulin release via a rise in internal calcium concentration.

An interesting observation was made recently by Arkhammar et al. [188]. These authors have noted that in RINm5F cells extracellular ATP inhibits whole cell K_{ATP} currents. Since formation of inositol triphosphate was seen both with and without external calcium it was postulated that the effect of ATP on channels was mediated through protein kinase C. This study may suggest that P2 receptors might be closely associated with the ATP-dependent K^+ channels.

In conclusion, ATP co-secreted with insulin from B-cells, can activate P2 receptors, which, via activation of protein kinase C and inositol-triphosphate synthesis, leads to a rise in $[Ca^{2+}]_i$. This mechanism allows potentiation of insulin release.

IV-C. ATP and oscillations in $[Ca^{2+}]_i$

ATP applied to the outside of B-cells, in the presence of glucose, induces oscillation in intracellular calcium [191]. This effect is exerted through ATP action on P2 receptors discussed above. However, ATP at 3 mM injected into isolated B-cells through a patch clamp pipette also induces oscillatory changes in $[Ca^{2+}]_i$ [192]. It was suggested that this effect of physiological concentration of ATP occurs through activation of the G-proteins. More work is needed, however, to characterize these phenomena.

In conclusion, both internal and external ATP can induce oscillatory patterns in $[Ca^{2+}]_i$. The physiologic function of these phenomena, and, in particular, their relevance to insulin secretion, remains to be established.

V. Concluding remarks

There have been several recent reviews that deal with various aspects of islet function and mechanisms of insulin secretion (e.g., Refs. 170, 193–196). The objective of this review was somewhat different. It was our deliberate choice to concentrate on constructing a uniform picture of the energetic properties of pancreatic islets, that would incorporate a spectrum of data from different laboratories. It has been our hope that a careful analysis of the behavior of cellular energy transduction has facilitated understanding of the role of ATP not only as a source of metabolic energy but also as a possible direct modulator of secretory events.

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