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Coupling factors in nutrient-induced insulin release

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Key words. Pancreatic B-cell; nutrient-induced insulin release.

Introduction

In the first report in this series, information was provided on the regulation of nutrient metabolism in islet cells, the view being emphasized that an acceleration of oxidative fluxes may represent an early causal event in the process of nutrient-stimulated insulin release[80]. The present report deals with the coupling of metabolic events to more distal events in the secretory sequence. We wish to make it immediately clear that this funda-

mental question of islet cytophysiology remains, in our opinion, largely controversial. This does not mean, however, that there is a shortage of hypotheses. On the contrary, a number of distinct modalities are currently considered for the coupling of metabolic to distal events in the process of nutrient-stimulated insulin release. It is precisely the aim of the present report to define and discuss these various hypothetical modalities.

One of the difficulties encountered in such a discussion consists in the selection, among a great number of distal events, of those which are likely to be most closely related, in their regulation, to the metabolic events. For instance, the view could be taken that a crucial step in the process of nutrient-induced insulin release consists in a decrease in K^+ conductance, leading to depolarization of the plasma membrane, subsequent gating of voltage-sensitive Ca^{2+} channels, accumulation of cytosolic Ca^{2+} and eventual activation of the effector system controlling the access of secretory granules to their site of exocytosis at the cell boundary[57]. In such a case, the regulation of plasma membrane K^+ conductance should be looked upon as a major target for coupling factors generated by the catabolism of nutrients. However, if it were postulated that the remodelling of ionic fluxes known to coincide with the process of nutrient-stimulated insulin release would be secondary mainly to stimulation of phospholipid turnover, e.g. in the phosphatidylinositol cycle, with coinciding generation of native ionophores, then such an enzyme as phospholipase C could be considered as the essential target for the coupling factors provided by the oxidation of nutrient secretagogues[12]. In other words, any proposal in favor of a given coupling factor is critically dependent on the selection of the major target system supposed to mediate the informational transfer of the input metabolic signal to the output secretory response.

At the first glance, several candidates could act as coupling factors between metabolic and more distal events in the secretory sequence. Indeed, an increase in nutrient oxidation usually coincides with an increase in the generation rate of reducing equivalents (e.g. NADH and NADPH), high-energy phosphate intermediates (e.g. ATP) and protons (H^+), each of which could conceivably act as a coupling factor. Alternatively, one or several metabolite(s) generated in suitable catabolic pathway(s) could play such a role. It is also conceivable that distinct coupling factors act separately to affect distinct distal events and/or in concert to modulate common distal reaction(s)[59]. Moreover, the view should not be ruled out that the modality of coupling between metabolic and distal events is not strictly identical in response to all nutrient secretagogues. For instance, the transport of a given nutrient secretagogue into the pancreatic B-cell may be coupled rather specifically with a given ionic movement, which in turn would somehow participate in the initiation or modulation of the secretory response[75]. Likewise distinct nutrient secretagogues may affect in a different manner both the generation of reducing equivalents in the cytosolic and mitochondrial compartments and the transfer of such reducing equivalents from one to another of these compartments. These considerations obviously call for a prudent and nuanced analysis of the coupling process. Yet, in order to provide a clear presentation of this fundamental problem of B-cell physiology, a rather schematic or simplified approach will here be adopted. Thus, the discussion will be mainly restricted to a few punctual proposals concerning the modality of coupling of metabolic to distal events in the secretory sequence. Nevertheless, we believe that it is essential to keep the present introductory remarks in mind and hence, to realize that the various theories examined in this review may all suffer, for the sake of a cartesian formulation, from undue simplification.

Metabolic intermediates

A few years ago, when it was still a matter of debate whether glucose (and other nutrient secretagogues) needs to be metabolized in the islet cells in order to stimulate insulin release, the view had been taken into consideration that an early metabolite of glucose, e.g. glucose 6-phosphate, may play a crucial role as a signal for insulin release. A compartmentalization of glucose 6-phosphate in islet cells was even proposed to account for differences in the response to extracellular glucose and that derived from endogenous glycogen, respectively[77]. Alternatively, specific reactions in a given metabolic pathway were considered as possible candidates for the regulation of distal events controlling insulin release. This applies for instance to the conversion of glucose to sorbitol[24], the metabolism of glucose 6-phosphate by the pentose phosphate pathway[1,68] or the reaction in the glycolytic pathway catalyzed by glyceraldehyde-3-phosphate dehydrogenase[28]. Now that it seems evident that distinct nutrient secretagogues, which are metabolized by vastly different pathways, owe their insulinotropic capacity to their ability to act as a fuel in islet cells, the idea that a critical metabolite acts as the major coupling factor is received with greater scepticism.

Nevertheless, it is still conceivable that the availability or generation rate of certain metabolites participate in the coupling of metabolic to distal events. For instance, it was proposed that phospho-*enol*-pyruvate may play an important role in controlling either the mitochondrial handling of Ca^{2+}[85] or the activity of such enzymes as adenylate cyclase and protein kinase[15,17]. Likewise, and as already alluded to, the generation of extramitochondrial NADH and ATP in the glycolytic sequence from D-glyceraldehyde-3-phosphate to 3-phosphoglycerate would be well suited to provide regulatory signals for events taking place at the plasma membrane[28,67]. In this respect, it remains to be investigated whether the enzyme glyceraldehyde-3-phosphate dehydrogenase is as closely associated with the plasma membrane in the B-cell as in erythrocytes[51]. In the same perspective, the identification of the reaction catalyzed by the malic enzyme as a major site for the extramitochondrial generation of NADPH[53,82] may be of importance in considering the possible effect of a change in cytosolic redox state upon target systems located at the plasma membrane.

At this point, an important conceptual dilemma should be mentioned. Whenever a potential coupling factor (e.g. a metabolic intermediate or a cofactor generated

by the catabolism of nutrients) is taken into consideration, the question can be raised whether it is the concentration or the generation rate of this coupling factor which matters for the control of distal functional events. When considering the mode of action of a regulatory factor, we are often thinking in terms of the time course and magnitude of changes in its concentration, rather than in terms of changes in its rate of generation. Yet, minor changes in steady-state cellular content (or concentration), at the limit of detection because of either biological variability or assay imprecision, may coincide with major changes in generation rate. For instance, the ATP content of islet cells does not undergo obvious changes in the range of glucose concentration between 2.8 and 27.8 mM[59]. Yet, it is obvious that the rate of ATP synthesis is considerably increased in the same range of hexose concentrations. It is therefore conceivable that an increased availability of a coupling factor coincides with an increase in its rate of utilization, without any major change in its steady-state cellular content (or concentration). In such a case, it could be questioned whether and how the increase in generation rate of the coupling factor may influence its rate of utilization.

Reducing equivalents

There is little doubt that nutrient secretagogues affect the redox state in pancreatic islet cells. It was first shown by Panten and his colleagues that glucose and other nutrient secretagogues augment rapidly the NAD(P)H-fluorescence and decrease the FAD-fluorescence of perifused islets isolated from *ob-ob* mice[71,72]. The latter decrease was interpreted as indicative of a rapid and sustained change in B-cell mitochondrial redox state. However, MacDonald observed that a larger fraction of islet flavin was cytosolic rather than mitochondrial[49]. Further studies have documented that both the NADH/NAD^+ and NADPH/$NADP^+$ ratio increase in a rapid and sustained fashion in glucose-stimulated islets[1,13,19,59]. This was extended to other nutrient secretagogues and the impression was on occasion gained that the NADPH/$NADP^+$ rather than NADH/NAD^+ ratio correlated most closely with the secretory data[41]. Since the ration of reduced to oxidized pyridine nucleotides does not inform on the respective redox state of the cytosolic and mitochondrial compartments and does not specifically refer to the cell content in free nucleotides, the islet content in critical metabolites was then measured to assess specifically the cytosolic redox state for either the NAD or NADP system. These studies have indicated that nutrient secretagogues such as D-glucose, L-leucine and 2-ketoisocaproate indeed augment the cytosolic NADPH/$NADP^+$ and/or NADH/NAD^+ ratio[8,26,63,66,82]. According to Ammon et al.[2], glucose also causes a dose-related increase in the GSH/GSSG ratio from a basal value of 1.0 to a maximal value of 3.3 at glucose 16.7 mM. Anjaneyulu et al.[7] also observed a modest effect of glucose to increase the islet GSH/GSSG ratio from a basal value of 6.7 to a value of 8.4 at high glucose concentration. The latter data are much higher than those reported by Ammon et al.[2] and, as such, much closer to the values found in other tissues. It was also observed that several nutrient secretagogues augment the islet content in thiol groups. For instance, D-glucose (16.7 mM) increases the latter content from a basal value of 119 ± 7 to 170 ± 9 pmol/μg protein[7].

As already mentioned, the changes in redox state are rapid and sustained. In response to glucose administration, they may precede the stimulation of insulin release[52]. Attempts were made to investigate whether the redox state in pancreatic islets may display either a phasic early response to glucose[59] or oscillations during prolonged stimulation[65], as observed for other functional variables of islet function. However, no conclusive results were obtained, so that it remains a matter of speculation whether the rhythmic pattern of bioelectrical and secretory activity in islet cells is ruled by a metabolic or cationic pace-maker. Nevertheless, the changes in redox state evoked by nutrient secretagogues are sufficiently rapid and marked to postulate that reducing equivalents act as a coupling factor between metabolic and distal events in the secretory sequence. The latter view raises questions as to the site of generation of reducing equivalents, their modality of transfer between the cytosol and mitochondria and the nature of the target systems responsive to a change in redox state.

In response to stimulation by certain nutrient secretagogues, e.g. 2-ketoisocaproate, the generation of reducing equivalents is thought to be primarily increased mainly, if not exclusively, in mitochondria. Yet, with other nutrients, e.g. D-glucose, the cytosolic metabolism of the nutrient directly increases the generation rate of extramitochondrial NADH and NADPH. Certain observations suggest that the cytosolic generation of NADPH may play a critical, if not exclusive, role in the coupling of metabolic to functional events. For instance, we recently observed that L-asparagine, which augments leucine-stimulated insulin release[66], is metabolized in the islet cells, in part at least, through the sequence of reactions catalyzed by the cytosolic enzymes, malate dehydrogenase and the malic enzyme[79]. This pathway for the conversion of oxalacetate (derived from exogenous L-asparagine) to pyruvate provides a modality for the coupled conversion of NADH to NAD^+ and $NADP^+$ to NADPH. As a matter of fact, the extramitochondrial conversion of malate to pyruvate may well represent a major site for the cytosolic generation of NADPH in islet cells[53,82].

MacDonald has drawn attention to the possible participation of hydrogen shuttles, i.e. the glycerol phosphate shuttle[48] and the malate aspartate shuttle[50] in the transfer of reducing equivalents into mitochondria of glucose-stimulated islets, as to maintain a low NADH/NAD^+ cytosolic ratio and, hence, a sustained rate of glycolysis. In addition to measuring the activity and subcellular location of enzymes involved in these shuttles, MacDonald reported that aminooxyacetate, an inhibitor of aminotransferase when used in the 5–10 mM range, decreases both (U-^{14}C)glucose oxidation and glucose-stimulated insulin release by the islets[50]. A somewhat different picture was obtained in our laboratory. Thus we observed that aminooxyacetate severely affected the cationic and secretory response of the islets to various nutrient secretagogues, including D-glucose and

2-ketoisocaproate, without causing any obvious alteration in the oxidation of these nutrients[44,63]. Since, aminooxyacetate also lowered the malate/pyruvate ratio in islets stimulated by either D-glucose or 2-ketoisocaproate, we proposed that the integrity of extramitochondrial transamination reactions may be required for the maintenance of a sufficient NADPH/$NADP^+$ ratio. Obviously, further studies are required to assess the extent and mechanism of transfer of NAD(P)H between distinct subcellular locations, as well as the modality of hydrogen exchange between the NAD and NADP systems.

There are several modalities by which a change in redox state could affect distal events in the secretory sequence. Hellman and colleagues were the first to propose that a change in the thiol/disulfide balance of membrane-associated proteins may alter their participation in ionic movements, this proposal being based mainly on an extensive study dealing with the influence of sulphydryl reagents upon insulin release[31]. The idea was pursued by several investigators[2,4]. For instance, Henquin[32] and Malaisse et al.[60,64] emphasized the possible role of reduced nicotinamide nucleotides in the regulation of K^+ permeability in islet cells. The change in thiol-disulfide balance of proteins may be mediated by the GSH/GSSG system. Islet homogenates display, in addition to glutathione reductase activity, little glutathione peroxidase activity but high glutathione-cystein transhydrogenase activity[7]. Täljedal also identified thioredoxin in pancreatic islets and proposed that it may participate in electron transport to the B-cell plasma membrane[86]. A recent study dealing with a specific inhibitor of glutathione reductase, 1,3-bis(2-chloroethyl)-1-nitrosourea, supports the view that this enzyme participates in the regulation of distal events in the secretory sequence[22]. An alternative possiblity would be that the mitochondrial redox state controls the efflux of Ca^{2+} from the organelles, an increase in NADH/NAD^+ ratio being reported to lower Ca^{2+} efflux in liver or heart mitochondria[46]. It is also conceivable that the changes in the thiol-disulfide balance of proteins modulate their participation in distal regulatory events, other than ionic movements[5,14]. Last, it should be kept in mind that the reduction of NAD^+ or $NADP^+$ coincides with the appearance of a dihydropyridine ring in the nicotinamide moiety of these nucleotides, this change in structure being possibly directly involved in a regulatory role of these cofactors.

The present remarks certainly do not exhaust all relevant comments which could be offered on the possible role of reducing equivalents in the stimulus-secretion coupling of nutrient-induced insulin release, but at least, clearly indicate that this topic duly merits further attention.

Protons

An increase in the rate of nutrient catabolism is thought to coincide with an increase in the generation rate of protons. It is conceivable, therefore, that protons act as a coupling factor in the insulin secretory sequence[58]. For instance, it was proposed that nutrient secretagogues, by increasing H^+ production, may cause inhibition of both K^+ and Ca^{2+} efflux from the islet cells[58]. It was even documented that, in an artificial membrane model, native ionophores extracted from the islets can mediate both K^+ transport, along an imposed K^+ chemical gradient, and Na^+-Ca^{2+} countertransport[6]. In this model, acidification of the chamber simulating the intracellular (cytosolic) milieu indeed resulted in inhibition of Ca^{2+} uphill translocation. The postulated role of H^+ as a coupling factor should be considered in the light of several findings which are listed below.

Glucose causes a dose-related fall in pH of the incubation medium when the islets are placed in a solution with poor buffering capacity, the pattern of the dose-response curve appearing hyperbolic[59]. Although this observation indicates that glucose increases the net output of H^+ from the islets, it does not inform on the modality of H^+ extrusion. For instance, it is conceivable that the fall in extracellular pH is attributable, in part at least, to the extrusion of organic acids, e.g. pyruvic acid and lactic acid, which could cross the plasma membrane in the undissociated form. Glucose indeed provokes a rapid and sustained stimulation of lactic acid and pyruvic acid output from the islets[56].

Distinct nutrients may affect in a vastly different manner the net generation rate of H^+. For instance, when high concentrations of amino acids are used to stimulate insulin release, the production of NH_4^+ by either deamidation or oxidative deamination might compensate, in part at least, for the production of acidic metabolites and, hence, modify the response of the islets to suitable cationic manipulations[81]. Incidentally, there are also indications that the cationic response of the islet cells to distinct nutrients is not always qualitatively identical[39].

Relatively little information is available on the effect of nutrient secretagogues upon intracellular pH. The two methods of measurement so far used, i.e. the uptake of ^{14}C-labelled 5,5-dimethyloxazolidine-2,4-dione by the islets and the change in fluorescence of islets prelabelled with fluorescein diacetate, do not inform on the heterogeneity of pH between different intracellular compartments. Somewhat unexpectedly, D-glucose causes a rapid, sustained a dose-related increase in the intracellular pH of islet cells[20,42,47]. This finding led to the speculation that the glucose-induced postulated increase in H^+ generation is compensated for by stimulation of such processes as CO_3H^-/Cl^- exchange or Na^+/H^+ countertransport[20,42]. In support of this interpretation, it should be noted that glucose stimulates both the outflow of $^{36}Cl^-$ from prelabelled islets[78] and the inflow of $^{14}CO_3H^-$ into the islet cells[20]. Moreover, in one study, glucose was found to cause an early fall in intracellular pH when the islets were incubated in the absence of extracellular HCO_3^- or in the presence of amiloride, which is thought to selectively inhibit Na^+/H^+ exchange[21]. However, according to Lindström and Sehlin, glucose still causes a late increase in islet cell pH in the absence of bicarbonate[47].

Several attempts were made to affect the intracellular pH of islet cells, independently of any manupulation in their nutritional environment, in order to assess the possible influence of change in cellular pH upon the functional behavior of the pancreatic B-cell. The interpretation of the results obtained in such experiments is

not always easy, and this for at least four reasons. First, a secondary effect of the change in cellular pH upon metabolic variables should not be ruled out. Such a metabolic alteration should be considered in the framework of an important conceptual aspect of B-cell physiology, namely the feedback regulatory role of ionic events upon metabolic events[65]. Second, the tools used to modify intracellular pH may affect islet function independently of the postulated change in pH. To cite only one example, in one study[87], glycodiazin was used at a 5.0 mM concentration as a weak permeant acid in order to lower intracellular pH. Yet, this drug is a potent hypoglycemic sulfonamide and stimulates insulin release when used at a concentration as low as 6.0 μM[62]. Third, in most studies, especially in the extensive work carried out by Pace and colleagues[70, 84, 87] on this theme, the postulated change in cellular pH was not documented. Last, the results of these studies are themselves somewhat equivocal. Most experimental data suggest that a lowering of intracellular pH may facilitate nutrient-induced electrical activity and insulin release. However, there are exceptions to this rule. For instance, it was recently proposed that, at low glucose concentration (1.0 mM), NH_4Cl (5.0 mM) stimulates modestly insulin release[47]. Likewise, the influence of changes in extracellular pH upon nutrient-stimulated insulin release are tightly dependent, for a given nutrient, on the concentration of the nutrient, so that the same change in extracellular pH may either augment or decrease insulin release evoked by two distinct concentrations of the same nutrient[40]. As a matter of fact, the latter observation had been interpreted as an indirect evidence that nutrients indeed solicit the buffering capacity of the islets cells.

With these reservations in mind, several studies[23, 33, 69, 70, 84, 87], suggest that lowering of the cytosolic pH may favor B-cell secretory function. Thus, imidazole (10 mM) and NH_4Cl (20 mM), which are supposed to act as permeable weak bases and to increase cellular pH, inhibit glucose-induced electrical activity and insulin release. The same phenomenon occurs in response to administration of the Na:H ionophore monensin (15 μM). Inversely, 4-4′-diisothiocyano-2,2′-stilbene disulfonic acid which is supposed to act as an inhibitor of HCO_3^-/Cl^- exchange and, hence, to lower cellular pH causes, in the 10–200 μM range, a dose-related increase in glucose-induced electrical activity. A comparable response is seen with amiloride (50–100 μM), which by inhibiting Na^+/H^+ exchange may also cause a decrease in cellular pH. Taken as a whole, these obervations support the view that the K^+ permeability in islet cells is proton sensitive, a fall in intracellular pH causing a fall in K^+ conductance and vice versa[23, 58]. None of these observations, however, establishes that glucose or other nutrient secretagogues affect K^+ permeability through changes in cytosolic pH. Obviously, further work is required to assess the effect of nutrients upon pH in the cytosolic and other cellular compartments.

ATP

Several enzymatic reactions, of which the velocity is increased in stimulated B-cells, use ATP as a substrate. It is generally thought that the secretory activity of the B-cell represents an energy-dependent process. It is conceivable that ATP availability represents not solely a permissive but even a regulatory factor in nutrient-induced insulin release. One proposal postulates that nutrients, by increasing the ATP generation rate, facilitate the active pumping of cytosolic Ca^{2+} into either intracellular organelles or the extracellular milieu, as mediated by suitable ATPases[10]. This would lead to a fall in cytosolic Ca^{2+} activity, with subsequent closing of Ca^{2+}-responsive K^+ channels. The latter modification in K^+ conductance would coincide with a decrease in the outflow of K^+ from the islet cells and depolarization of the plasma membrane. The change in membrane potential could then provoke the gating of voltage-sensitive Ca^{2+} channels, allowing for a facilitated influx of Ca^{2+} into the islet cells and, through an increase in cytosolic Ca^{2+} activity, trigger the exocytosis of secretory granules. Within the restricted scope of the present report, we intend to limit the discussion of such a proposal to the initial events, namely the link between an increase in ATP generation rate and the increase in active Ca^{2+} pumping. More distal steps in the proposed sequence, e.g. changes in K^+ conductance, membrane polarization and Ca^{2+} inflow are considered in later reports in this series. The above proposal is compatible with several experimental findings, as follows.

First, it is well established that nutrient secretagogues augment the rate of ATP generation as judged from either the O_2 uptake[27, 38], the heat production[25] or the measurement of metabolic fluxes in islet cells[80]. However, it should be underlined, as already noted, that the ATP content (or ATP/ADP ratio and adenylate charge) is little affected by glucose in the range of concentrations associated with stimulation of insulin release[59]. The ATP content of pancreatic islets is decreased when they are incubated in the absence of exogenous nutrient[9, 61]. But low concentrations of glucose, which do not stimulate insulin release under normal conditions, are sufficient to maintain an ATP level similar to that seen at higher concentrations of the sugar. This is also true for other nutrient secretagogues[9, 39].

Second, Dawson[18] recently indicated that, in the presence of D-glucose, a transient increase, for 1 min, in the extracellular K^+ concentration (from 5 to 50 mM), which results in depolarization of the islet cell plasma membrane, is followed by a rapid repolarization lasting for about 1–2 min, the duration of this silent phase being positively related to the extracellular Ca^{2+} concentration and negatively related to the glucose concentration. These findings were interpreted as an indication that the silent phase represents a recovery phenomenon and that its duration depends on both the amount of Ca^{2+} entering into the B-cell during the transient depolarization and the capacity of the Ca^{2+}-buffering systems of the B-cell. Since increasing glucose concentrations caused a dose-related shortening of the recovery time, the proposal was made that glucose increases the Ca^{2+}-buffering capacity, e.g. by enhancing the rate of cytosolic Ca^{2+} removal by intracellular organelles.

Third, it has been known for more than 10 years that glucose decreases the fractional outflow rate of ^{45}Ca from prelabelled islets, such a decrease representing a

rapid and sustained phenomenon[55]. It is best seen when the prelabelled islets are perifused in the absence of extracellular Ca^{2+}, whilst it is soon masked by a secondary rise in ^{45}Ca efflux when the islets are perifused in the presence of extracellular Ca^{2+}[36]. The interpretation of the glucose-induced decrease in ^{45}Ca outflow is still a matter of debate. According to some authors, it represents mainly inhibition of a process of Na^+-Ca^{2+} countertransport, which mediates the extrusion of Ca^{2+} from the islet cells against the prevailing electrochemical gradient[37,83]. For other authors, however, the decrease in ^{45}Ca outflow reflects an accelerated uptake of cytosolic Ca^{2+} by intracellular organelles[30]. The latter view would be consistent with the role ascribed to ATP in the above-mentioned proposal. Incidentally, the dose-action relationship for the inhibitory effect of D-glucose upon ^{45}Ca fractional outflow rate displays a hyperbolic pattern with an apparent half-maximal response at a glucose concentration well below that required to cause half-maximal stimulation of Ca inflow into the islet cells[59]. The existence of large differences in the dose-action relationship and apparent 'affinity' for glucose of distinct Ca^{2+} (and other ionic) movements could be attributable to a multifactorial process of coupling between metabolic and ionic events, distinct coupling factors acting on distinct target systems[59].

Fourth, Hellman and his colleagues recently observed that, in tumoral islet cells, glucose indeed lowers the cytosolic Ca^{2+} activity[76]. Glucose is a very weak insulin secretagogue in these tumoral cells and, hence, their cationic response to the hexose may not be representative of the behavior of normal islet cells. Nevertheless, the observation made by the Swedish authors demonstrate that glucose is potentially able to lower cytosolic Ca^{2+} in insulin-producing cells.

Last, it seems evident that islet cells are indeed equipped with Ca^{2+}-responsive K^+ channels. This is supported by an array of observations dealing, for instance, with the bioelectrical, cationic and secretory response to pharmacological agents such as quinine[11,34,35]. The proposal that ATP acts as the major coupling factor, by facilitating active Ca^{2+} pumping nevertheless raises certain reservations. First, it ascribes to a decrease in cytosolic Ca^{2+} activity a key role in coupling the metabolism of nutrient secretagogues to their inhibitory effect upon K^+ conductance. Yet, it is currently assumed that nutrient secretagogues augment cytosolic Ca^{2+} activity. Against the latter objection, it could be argued that the fall in cytosolic Ca^{2+} activity would represent a transient, short-lived phenomenon. However, no initial decrease in cytosolic Ca^{2+} activity was observed in either tumoral cells exposed to suitable nutrient secretagogues, e.g. D-glyceraldehyde, or in normal islet cells stimulated with D-glucose[54,88].

Second, if ATP were indeed to regulate Ca^{2+} active pumping, one may question how the activity of the relevant ATPase(s) may be affected by the availability of ATP in the absence of any detectable change in ATP steady-state concentration (see above). It should be nevertheless kept in mind that the concentration of ATP in the immediate vicinity of postulated target ATPases, located in either the plasma membrane or the endoplasmic reticulum, still remains to be measured. At this point, it should also be mentioned that, when the extracellular concentration of glucose is suddenly increased from 1.7 to 16.7 mM, there is a transient fall in the islet cell ATP content[59]. Such a phenomenon is also observed in other cell types and could be due, in part at least, to a transient imbalance between the ATP-consuming phosphorylation of both glucose and fructose 6-phosphate and the rate of ATP generation in further glycolytic and mitochondrial reactions. Nevertheless, the transient fall in ATP could also point to a stimulation by glucose of other ATP-consuming processes and, hence, suggest that glucose regulates the latter processes by a mechanism different from the actual rate of ATP synthesis.

Third, attempts to favor the sequestration of Ca^{2+} in intracellular organelles, e.g. by exposing the islets to a high concentration of inorganic phosphate have failed so far to result in changes in either ^{86}Rb fractional outflow rate or insulin release comparable to those evoked by nutrient secretagogues[29,45].

Last, when the concentration of glucose is raised from an intermediate to a higher value (e.g. from 8.3 to 16.7 mM), no inhibition of the Ca^{2+}-responsive modality of K^+ extrusion is observed. On the contrary, the increase in glucose concentration provokes an increase in ^{86}Rb fractional outflow rate from prelabelled islets[16]. The latter increase is abolished by quinine or when the islets are perifused in the absence of extracellular Ca^{2+}, suggesting that the increase in ^{86} Rb outflow reflects the gating (and not closing) of the Ca^{2+}-responsive K^+ channels[16,43]. In other words, it is not evident that, within the range of glucose concentrations from about 8.3 mM (or less) to higher values, the enhancing action of the sugar upon insulin release involves inhibition of the Ca^{2+}-responsive modality of K^+ extrusion.

With these reservations in mind, the proposed theory for the role of ATP availability in stimulus-secretion coupling justifies, in our opinion, further investigations. It has certainly the merit to be compatible with the fuel concept for insulin release. Since the majority of ATP is generated in mitochondria, the present hypothesis is also compatible with the view that mitochondria play a key role in the coupling of metabolic to distal events in the secretory sequence.

Role of mitochondria

Certain potent nutrient secretagogues, e.g. 2-ketoisocaproic acid, are metabolized exclusively in mitochondria. Although concomitant changes in extramitochondrial metabolic sequences should not be dogmatically ruled out, the view comes obviously to mind that mitochondria may be the major source of coupling factors connecting the metabolism of nutrients to further functional events. Relatively few experiments were so far carried out on isolated mitochondria prepared from pancreatic endocrine cells. The information so far available indicates, as expected, that mitochondria may participate in the regulation of cytosolic Ca^{2+} concentration[74]. The ion Na^+ stimulates Ca^{2+} efflux from the mitochondria with an apparent half-maximal effective concentration of Na^+ close to 4 mM. Interestingly, the

extramitochondrial steady-state Ca^{2+} concentration is also increased when the pH of the medium is decreased from 7.0 to 6.9.

Panten et al.[73] recently speculated that enhanced hydrogen supply to the respiratory chain governs B-cell function by driving both Ca^{2+} influx and efflux in the mitochondria. However, these authors were careful not to decide whether the predominant effect, in stimulus-secretion coupling, consists in the stimulation of mitochondrial Ca^{2+} efflux (with a subsequent rise in cytosolic Ca^{2+} concentration) or influx (with a resulting fall in cytosolic Ca^{2+} activity indirectly causing plasma membrane depolarization).

At this point, it should be mentioned that, for a given increase in O_2 uptake above basal value, the rate of insulin release appears higher when glucose, as distinct form L-leucine or 2-ketoisocaproate, is used as the secretagogue[38]. A greater proportion of the reducing equivalents formed in the metabolism of glucose, however, pass via pyridine rather than flavin nucleotides than in the case of 2-ketoisocaproate, thus giving a higher P/O ratio when glucose is the substrate. Furthermore, glucose, by stimulating glycolysis in islet tissue, allows for the extramitochondrial generation of ATP. When these qualitative and quantitative differences are considered, it appears that the rate of ATP generation is virtually identical at equivalent insulinotropic concentration of D-glucose and 2-ketoisocaproate, respectively[38]. Thus, it could be argued that the rate of ATP generation would indeed appear as the crucial variable regulating the secretory activity, whatever the site (cytosolic vs mitochondrial) of such a generation.

Conclusions

In the process of nutrient-stimulated insulin release, the mechanism for the coupling of metabolic to distal events in the secretory sequence remains a matter of debate. Although specific metabolic intermediates may participate in such a coupling procedure, current hypotheses emphasize the apparent role of an increase in the generation rate of high-energy phosphate intermediates, protons and reducing equivalents. These coupling factors could affect such variables as plasma membrane K^+ conductance or the activity of enzyme involved in the regulation of other distal events in the secretory sequence. It seems evident that, at least in response to certain nutrient secretagogues, mitochondria may act as the source of such coupling factors. A reasonable, even if apparently too easy or somewhat irresponsible, concluding proposal could be that several coupling factors generated by the catabolism of nutrients act in concert to regulate a number of discrete cytophysiological events in the pancreatic B-cell. Each of such events may either represent a necessary step or play a modulating role in the informational transfer of the input metabolic signal to the output secretory response. The participation of feedback loops, e.g. regulation of metabolic events by ionic variables, in this informational transfer and its possibly rhythmic time course should not be overlooked.

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Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic B-cells

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Key words. Pancreatic B-cells; ions; insulin release; membrane potential.

Introduction

It has been known for more than fifteen years that ions play a crucial role in the stimulus-secretion coupling in pancreatic B-cells. Their importance was first demonstrated by the influence of extracellular cations on insulin release[35, 91], by the stimulatory effect of glucose on $^{42}K^+$ uptake by islet cells[57], and by the appearance of electrical activity in B-cells stimulated with insulin secretagogues[23]. Since then, numerous investigations have addressed various aspects of the complex role of ions in the B-cell function. Many of them have already been reviewed in articles dealing with ionic fluxes in islets and with the importance of ions for insulin release[37, 42, 64, 71, 72, 74, 109, 111], or with the regulation of the membrane potential in B-cells[2, 76, 77, 82, 86, 87].

This report is an attempt to summarize and to integrate our current knowledge of the mechanisms controlling ionic fluxes in islet cells and membrane potential in B-cells. Emphasis will be put on certain aspects of the question that were studied recently, that were not discussed previously or that remain controversial. The significance of these phenomena for insulin release will also be considered. The review, however, will be almost totally restricted to the events occurring during glucose stimulation and will not deal with Ca^{2+} fluxes, that are discussed in another chapter of this series.

1. Membrane potential of B-cells or of non-B-cells?

Despite their limitations and possible artifacts, high resistance microelectrodes are the most sensitive and reliable means to measure the membrane potential of islet cells. Most, if not all laboratories currently use a technique of long-lasting impalements in single cells of partially microdissected islets[89]. The electrical activity induced by glucose in certain islet cells is so typical that it is now used to identify insulin-secreting B-cells. To what extent, however, are we sure to record the membrane potential of B-cells? The confidence of electrophysiologists rests on three types of considerations: statistical, anatomical and functional. First, the probability to impale B-cells is the highest simply because they constitute 70–80% of the mouse islet. Second, PP-cells are very rare in the splenic part of the pancreas that is used for the experiments and the recordings are usually