Anionic Transporters and Channels in Pancreatic Islet Cells

13

Nurdan Bulur and Willy J. Malaisse

Contents

Abstract

After a brief description of the so-called consensus hypothesis for the mechanism of stimulus-secretion coupling in the process of glucose-induced insulin release, the present chapter, which deals with anionic transporters and channels in pancreatic islet cells, concerns mainly a second modality for the control of

N. Bulur • W.J. Malaisse (✉)
Laboratory of Experimental Medicine, Université Libre de Bruxelles, Brussels, Belgium
e-mail: nurdanbulur@hotmail.com; malaisse@ulb.ac.be

insulin secretion by the hexose. In such a perspective, it draws attention to the NBCe1 Na^+/HCO_3^- cotransporters, the volume-regulated anion channel hypothesis, the experimental model of extracellular hypotonicity, the possible role of NAD(P)H oxidase-derived H_2O_2 in the activation of volume-regulated anion channels in β-cells exposed to a hypotonic medium, the identity of the anions concerned by the volume-regulated anion channel hypothesis, the expression and function of anoctamin 1 in rodent and human pancreatic islet cells, the possible role of bicarbonate-activated soluble adenylyl cyclase, the identity and role of aquaporins in insulin-producing cells, and a proposed role for volume-regulated anion channels in glucagon secretion.

Introduction

The so-called consensus hypothesis for the process of glucose-induced insulin secretion postulates that the corresponding mechanism of stimulus-secretion coupling involves a sequence of metabolic, ionic, and motile cellular events.

It had been first proposed that the activation of insulin-producing β-cells in the pancreatic islets in response to a rise in extracellular D-glucose concentration was attributable to the intervention of a stereospecific glucoreceptor possibly located at the level of the β-cell plasma membrane. Such a receptor concept contrasts with the more pedestrian view that the stimulation of insulin release by D-glucose and other nutrient secretagogues is causally linked to their capacity to act as nutrient in the β-cells and, hence, to increase the rate of ATP generation.

The validation of the latter fuel concept emerged inter alia from the following three series of findings. First, the finding that the α-anomer of D-glucose is a more potent insulin secretagogue than its β-anomer, first considered in support of the glucoreceptor theory, was eventually ascribed to the fact that the α-anomer of D-glucose is more efficiently metabolized in isolated pancreatic islets than its β-anomer (Malaisse et al. 1976). Second, a nonmetabolized analog of L-leucine, b(-)-2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH), which was found to duplicate the insulinotropic action of L-leucine itself, was eventually found to activate pancreatic islet glutamate dehydrogenase and, by doing so, facilitate the catabolism of endogenous amino acids (Sener et al. 1981). Third, the insulinotropic potential of 3-phenylpyruvate, first proposed to be attributable to the intervention of a specific β-cell membrane receptor acting as mediator of the insulin-releasing capacity of 3-phenylpyruvate, was eventually found to coincide with an increased

catabolism of endogenous amino acids acting as partners in transamination reactions leading to the conversion of 3-phenylpyruvate into phenylalanine (Sener et al. 1983; Malaisse et al. 1983).

The coupling between the increased catabolism of exogenous or endogenous nutrients and the remodeling of ionic fluxes in insulin-producing cells soon became, within the framework of the fuel concept for insulin release, a further matter of debate (Malaisse et al. 1979a). For instance, changes in the generation rate or content of high-energy phosphates (e.g., ATP), reducing agents (e.g., NADH and/or NADPH), and protons (H^+) were all taken in due consideration. The consensus hypothesis postulates that the nutrient-induced increase of ATP concentration or ATP/ADP ratio in the cytosolic domain provokes the closing of ATP-sensitive K^+ channels (Cook and Hales 1984), this leading in turn to depolarization of the plasma membrane and subsequent gating of voltage-dependent Ca^{2+} channels, eventually resulting in an increase of Ca^{2+} influx into the β-cell, a rise in the cytosolic concentration of Ca^{2+}, and the activation by Ca^{2+} of an effector system for the translocation and exocytosis of insulin secretory granules.

The third and last step in the stimulus-secretion coupling of glucose-induced insulin release was indeed ascribed to motile events leading to the intracellular translocation of insulin-containing secretory granules and their eventual access to an exocytotic site at the β-cell plasma membrane. The participation of a β-cell microtubular-microfilamentous system in these motile events is supported by a series of ultrastructural, biochemical, functional, and pathophysiological observations (Malaisse and Orci 1979). For instance, the study of motile events in pancreatic endocrine cells by time-lapse cinematography documented that secretory granules underwent back-and-forth saltatory movement along oriented microtubular pathways. A rise in extracellular D-glucose concentration from 2.8 to 16.7 mM resulted in a twofold increase in the frequency of saltatory movements. The second type of motile events consisted in the formation of outward expansions which extend from the cell boundary and, thereafter, retract more or less rapidly. Secretagogues, such as D-glucose, increased the ruffling of the cell membrane in terms of frequency, speed of expansion, duration, and amplitude. Cytochalasin B also dramatically increased the frequency and amplitude of the bleb-like outward expansions of the cell (Somers et al. 1979). These findings support the view that the microtubular apparatus serves as guiding cytoskeleton for the oriented translocation of secretory granules, whereas the microfilamentous cell web may control the eventual access of the granules to exocytotic sites. At these sites, the exocytosis of secretory granules entails the fusion and fission of membranes, followed by the dissolution of the granule core in the interstitial fluid. A chemoosmotic hypothesis was proposed to account for the fission of membranes at the exocytotic site (Somers et al. 1980). It was also proposed that anionic transport at exocytotic sites may account for the phenomenon of chain release, in which two or more secretory granules are discharged, in a row, at the same exocytotic site (Orci and Malaisse 1980).

A Second Modality for the Control of Insulin Secretion by Glucose

More than 20 years ago, Carpinelli and Malaisse (1981) documented the relationship between $^{86}Rb^+$ fractional outflow rate from prelabeled and perifused rat pancreatic islets and the concentration of D-glucose at values of zero, 1.7, 2.8, 4.4, 5.6, 8.3, and 16.7 mM. A rise in D-glucose concentration up to about 6–8 mM decreased ^{86}Rb outflow, indicating a decrease in K^+ conductance, itself attributable to the closing of ATP-sensitive K^+ channels. However, no further decrease in ^{86}Rb fractional outflow rate and, on the contrary, a modest but significant increase was observed at higher glucose concentrations, namely, in the range of glucidic concentrations provoking the most marked stimulation of insulin release. Thus, it was considered that the progressive increase in K^+ conductance recorded when the concentration of D-glucose is decreased below 5–6 mM is well suited to prevent undesirable insulin secretion in situations of hypoglycemia, but that another series of cellular events may be responsible for enhancing insulin secretion at high concentrations of D-glucose.

Such a view is supported by later findings, documented among others by Henquin and colleagues, and proposing that, in addition to the closing of ATP-sensitive K^+ channels located at the plasma membrane of β-cells, a second site may participate in the control of insulin secretion by D-glucose (Henquin et al. 1994). Thus, it was observed that in mouse pancreatic islets exposed to diazoxide (0.2 mM) in order to prevent glucose-induced β-cell plasma membrane depolarization, no increase in cytosolic Ca^{2+} concentrations and no stimulation of insulin release occurred when the concentration of extracellular D-glucose was raised from zero to 6.0 or 20.0 mM. In the presence of diazoxide, depolarization of the plasma membrane was nevertheless provoked by raising the extracellular K^+ concentration from 4.8 to 30.0 mM. Even in the absence of D-glucose, this resulted in a dramatic increase in cytosolic Ca^{2+} concentration and a modest increase in insulin output. Most importantly, however, under the same experimental conditions, i.e., in the presence of diazoxide and a high extracellular K^+ concentration (30.0 mM), the rise in D-glucose concentration from zero to 6.0 and 20.0 mM caused a concentration-related further increase in insulin output, despite the fact that the cytosolic Ca^{2+} concentration was significantly lower in the presence of D-glucose than in its absence but coinciding, at the high extracellular K^+ concentration, with a progressive increase of the ATP/ADP ratio in the islets exposed to increasing concentrations of D-glucose. From these findings, it was indeed concluded that D-glucose is able to affect insulin secretion by acting on a target distinct from the ATP-sensitive K^+ channels.

The present chapter deals mainly with such a second modality for the control of insulin secretion by glucose.

NBCe1 Na^+/HCO_3^- Cotransporters

In the search of a complementary mechanism for the stimulus-secretion coupling of glucose-induced insulin release, attention was first paid to the possible role of NBCe1 Na^+/HCO_3^- cotransporters in such a process.

The NBCe1 Na^+/HCO_3^- cotransporters represent a possible modality for the passage of bicarbonate ions across the cell membrane. Na^+/HCO_3^- cotransporter (NBC) isoform 1 is a member of the SLC4A4 gene family. NBCe1 has two protein variants, which mediate electrogenic Na^+/HCO_3^- cotransport, namely, NBCe1-A (formerly called kNBC1) and NBCe1-B (formerly called pNBC1). They are differentially expressed in a cell- and tissue-specific manner (Parker and Boron 2008). NBCe1-B is the most ubiquitous variant, being expressed, for instance, in the exocrine pancreas, brain, heart, prostate, small and large intestine, stomach, and epididymis. The NBCe1-A variant is more restricted, being most highly expressed in the kidney epithelia and eye. A third NBCe1 variant has also been described in the rat brain and has been named NBCe1-C.

The stoichiometry of the transporter can be altered from $1Na^+$/$2HCO_3^-$ to $1Na^+$/$3HCO_3^-$ by phosphorylation of a residue near the carboxyl terminus (Muller-Berger et al. 2001). Functional studies in exocrine pancreatic ducts established that NBCe1-B mediates the influx of one Na^+ with two HCO_3^-. This ion stoichiometry and electrochemical driving forces appear to result, in the pancreas and intestinal tract, in Na^+ and HCO_3^- entry into the cell likely to mediate HCO_3^- uptake across the basolateral membrane to support transepithelial anion secretion (Gawenis et al. 2007). In the kidney, however, the ion stoichiometry and electrochemical driving forces for NBCe1 result in Na^+ and HCO_3^- extrusion across the basolateral membrane, thus participating in HCO_3^- reabsorption in the proximal tubule.

Considering the possible role of changes in Na^+ and HCO_3^- fluxes associated with stimulation of insulin release, e.g., by nutrient secretagogues, attention was recently paid to the expression, variant identity, and function of NBCe1 in rat pancreatic islet cells.

Expression of NBCe1-A and NBCe1-B in Rat Pancreatic Islet Cells

In the first study, Wistar rats were sacrificed under CO_2 anesthesia. The pancreas and kidney were quickly excised and immediately frozen in liquid nitrogen or processed for either microscopy or islet isolation. The methods used for reverse transcription-polymerase chain reaction, Western blot analysis, tissue preparation for immunocytochemistry, immunohistochemistry following the standard ABC-DAB technique, immunofluorescence labeling using universal anti-NBC1 antibody, immunofluorescence labeling using variant-specific anti-NBCe1-A and NBCe1-B antibodies and functional studies including insulin release and D-glucose metabolism in isolated rat islets, intracellular pH measurements and electrophysiological experiments carried out with dispersed rat islet cells, and ^{22}Na net uptake by dispersed cells are all described in detail elsewhere (Soyfoo et al. 2009).

Amplicons corresponding to the expected pair of bases were observed for the NBCe1-A and NBCe1-B and the universal NBCe1 isoforms and β-actin in all specimen tissues, i.e., the kidney, pancreas, and pancreatic islets. Amplification seemed similar in all tissues in the case of both the universal NBCe1 and β-actin.

While pancreatic tissue offered apparently a similar level of amplification for both A and B variants, pancreatic islets yielded a stronger amplification signal for NBCe1-B compared to NBCe1-A.

Immunoblotting with antibodies specific for each variant indicated the expression of both NBCe1-A and NBCe1-B in rat pancreatic islets. The NBCe1-A band in the islets was less intense than that in the kidney despite the fact that the amount of protein used for pancreatic islets was 20 times higher. Using islet and pancreatic homogenates containing equivalent amounts of protein, the staining of the band corresponding to NBCE1-B was more pronounced in islets than in the pancreas. The findings on the expression of NBCe1-A and NBCe1-B in Western blots were superimposable to those obtained by RT-PCR.

In rat pancreatic sections, the antibody recognizing all three variants of the cotransporter stained much more intensely pancreatic islets than the surrounding exocrine tissue. Using the same antibody, NBC1 was localized in an isolated rat pancreatic islet. Both insulin- and glucagon-producing cells appeared to express NBCe1. In order to distinguish whether the labeling observed with this antibody could be attributed to either NBCe1-A or NBCe1-B, variant-specific antibodies were used. NBCe1-A immunolabeling of weak intensity was observed in pancreatic islets, whereas pancreatic acinar cells were completely devoid of NBCe1-A immunoreactivity. Immunoreactivity for NBCe1-B was found in both pancreatic acinar cells and islets, with a labeling intensity considerably stronger in islets than in exocrine pancreas, confirming immunoblotting data. Double labeling using anti-insulin and anti-glucagon antibodies showed partial co-localization of NBCe1-A with insulin, whereas in glucagon-expressing cells NBCe1-A immunoreactivity was absent. The NBCe1-B antibody clearly labeled insulin-producing cells located at the center of the islets, but apparently failed to do so in the glucagon-producing cells located at the periphery of the islets.

Tenidap (3–100 μM) caused a concentration-related inhibition of insulin release evoked over 90-min incubation by D-glucose (16.7 mM) in rat isolated islets, with a half-maximal inhibition close to 50 μM. At a concentration of 100 μM, tenidap failed to affect significantly the basal release of insulin recorded in the presence of 5.6 mM D-glucose, abolished the secretory response at 8.3 mM D-glucose, and severely decreased the insulinotropic action of 16.7 mM D-glucose.

Tenidap (100 μM) also decreased significantly the insulin secretory response to non-glucidic nutrient secretagogues such as 2-ketoisocaproate (10.0 mM) or L-leucine (20.0 mM).

Last, in islets exposed to 8.3 mM D-glucose, tenidap suppressed the enhancing action of non-nutrient secretagogues, including theophylline (1.4 mM), forskolin (5 μM), glibenclamide (5 μM), and cytochalasin B (0.2 mM) upon glucose-stimulated insulin secretion, otherwise obvious in the absence of tenidap.

As judged from the net uptake of $^{22}Na^+$, after 10-min incubation, by dispersed islet cells and the apparent distribution space of both L-[1-^{14}C] glucose, used as an extracellular marker, and ^3HOH, the net uptake of $^{22}Na^+$ corresponded to an estimated intracellular concentration of 35.8 ± 5.7 mM. Whether in the absence or presence of ouabain (1.0–2.0 mM), tenidap (0.1 mM) increased the mean value

for $^{22}Na^+$ net uptake, yielding an overall mean value of 132.5 ± 11.3 % ($n = 66$) as compared ($p < 0.001$) to a mean reference value of 100.0 ± 6.3 % ($n = 64$).

When isolated rat islets were incubated for 90 min in the presence of 16.7 mM D-glucose, tenidap, tested at a 30 μM concentration, inhibited both the utilization of D-[5-^{3}H]glucose and the oxidation of D-[U-^{14}C]glucose. The relative extent of such an inhibition did not differ significantly for the two metabolic variables under consideration, with an overall mean value of 61.0 ± 13.2 % (df = 33).

Tenidap (50–100 μM) provoked a rapid and slowly reversible cellular acidification as judged from the 440 to 480 nm fluorescence ratio in rat islet cells loaded with the pH-sensitive dye BCECF (2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein).

Tenidap was found to provoke within 1–2 min a pronounced hyperpolarization of the β-cell plasma membrane, whether in the presence of 4 or 16 mM D-glucose, this coinciding at the high concentration of the hexose with the suppression of spiking activity.

In the light of the findings so far described, attention should be drawn to the somewhat unexpected increase of $^{22}Na^+$ net uptake caused by tenidap. It could indeed be objected that the prevailing NBCe1-A variant expressed in islet cells is currently considered to work following an influx mode, e.g., in pancreatic duct cells (Parker and Boron 2008). Inter alia, however, the relatively high concentrations of HCO_3^- (alkaline pH) and Na^+ (about 36 mM) in islet cells exposed to 16.7 mM D-glucose could conceivably allow NBCe1-B to cotransport one Na^+ and two HCO_3^- ions from inside the islet cells into the extracellular fluid. This would generate a net inward depolarizing current, consistent with the hyperpolarization accompanying inhibition of the transporter by tenidap. An alternative or complementary modality for the latter hyperpolarization is discussed later in this chapter.

Expression of NBCe1 in Tumoral Insulin-Producing BRIN-BD11 Cells

The methods used for the culture of BRIN-BD11, INS-1, and MIN6 cells, for reverse transcription-polymerase chain reaction, Western blot analysis, immunofluorescence, insulin release, and sodium uptake in the investigations concerning the expression of NBCe1 in tumoral insulin-producing cells are described in detail elsewhere (Bulur et al. 2009).

The BRIN-BD11 cells expressed mainly the NBCe1-B variant, while in INS-1 cells both NBCe1-B and, to a lesser extent, NBCe1-A provided sizeable amplification signals. Such was also the case in the pancreas and kidney.

Western blotting analysis documented the presence, in both BRIN-BD11 cells and the kidney, of a predominant NBCe1 band with a molecular weight close to 130 kDa.

In the 50–100 μM range, tenidap decreased basal insulin output, as measured in the presence of 1.1 mM D-glucose, to 72.2 ± 5.0 % ($n = 5$) of paired control value (26.7 ± 7.7 μU per 30 min; $n = 5$). When the concentration of NaCl was decreased by 50 mM, the release of insulin averaged 215.3 ± 11.6 % ($n = 5$) of paired basal value. In such a hypotonic medium, tenidap lowered insulin secretion down to 85.0 ± 12.2 % ($n = 5$) of paired basal value (recorded in the isoosmotic medium in

the absence of tenidap). The latter percentage was not significantly different from that recorded, also in the presence of tenidap, in the isotonic medium. The relative magnitude of the inhibitory action of tenidap was thus higher in the hypotonic medium than in the isotonic one, resulting in the suppression of the secretory response to extracellular hypoosmolarity.

Incidentally, at higher concentrations (0.5 and 1.0 mM), tenidap provoked a concentration-related augmentation of insulin release in both cells incubated in the isoosmotic and hypotonic medium, this coinciding with the suppression of any significant difference in insulin output from BRIN-BD11 cells exposed to the isotonic or hypotonic medium. A comparable tenidap-induced and concentration-related increase in insulin output was observed in rat pancreatic islets. For instance, in the isotonic medium, tenidap (1.0 mM) increased insulin output from a control value of 42.7 ± 2.0 to 357.2 ± 29.7 μU per 30 min in BRIN-BD11 cells and from 33.5 ± 2.7 to 491.8 ± 24.3 μU/islet per 90 min in isolated rat pancreatic islets. Such dramatic increases in insulin output from either BRIN-BD11 cells or rat isolated pancreatic islets exposed to high concentrations of tenidap might well correspond to an unspecific damaging effect on insulin-producing cells.

At this point, it should be stressed that in a more recent report, tenidap (50–100 μM) was found not only to suppress the regulatory volume decrease otherwise observed in dispersed rat pancreatic islet cells exposed to a hypotonic medium, to hyperpolarize the β-cell membrane potential and suppress glucose-induced electrical activity and to cause a concentration-related inhibition of VRAC currents, whether VRAC activity was provoked by the use of a hypertonic pipette solution in order to induce cell swelling or exposure of the islet cells to 10 mM D-glucose. Indeed, in the same study, tenidap (100 μM) was found to also provoke the activation of K_{ATP} channels and, by doing so, to contribute to the tenidap-induced hyperpolarization (Best et al. 2010a).

Over 5–20 min incubation in the absence of tenidap, the time course for ^{22}Na net uptake by BRIN-BD11 cells was compatible with an apparent Na^{+} intracellular concentration of 34.3 ± 10.1 mM. As expected, ouabain (1.0 mM) significantly increased ^{22}Na net uptake. Pooling together results recorded in either the absence or presence of ouabain, tenidap increased ^{22}Na uptake to 143.1 ± 12.6 % ($n = 70$; $p < 0.003$) of the corresponding reference values recorded in the absence of tenidap (100.0 ± 5.9 %; $n = 70$).

Expression of SLC4A4 in Human Pancreatic Islets

In the third and most recent study, the expression of NBCe1 or SLC4A4 was explored in human pancreatic islets. The pancreases were obtained from human cadaveric donors without any primary or secondary quantifiable pathology, from the Transplant Services Foundation of the Hospital Clinic (Barcelona, Spain), after informed consent from their families and approval by the hospital's ethics committee. One part of the tissue was fixed in paraformaldehyde, embedded in paraffin, and sliced for further immunofluorescence studies. From another part of the pancreas,

islets were isolated as previously described (Casas et al. 2007). The last part of the pancreatic gland was utilized as a total pancreatic sample. The techniques used for total RNA isolation, real-time PCR, and immunofluorescent studies are detailed in this recent publication (Hanzu et al. 2012).

After total RNA isolation from isolated islets or total pancreatic sample, qRT-PCR yielded lower gene expression levels of SLC4A4 in isolated islets than in total pancreas, such levels being normalized to the housekeeping gene TBP (TATA box binding protein). The paired pancreas/islet ratio averaged 4.51.

At the protein level, immunostaining of SLC4A4 was as intense in insulin-producing cells as in exocrine pancreatic cells.

Further information concerning the possible participation of NBCe1 in the process of insulin secretion is provided later in this chapter (section "Soluble Adenylyl Cyclase").

The Volume-Regulated Anion Channel Hypothesis

As first proposed in 1997 (Best et al. 1997) and as recently reviewed (Malaisse et al. 2008; Best et al. 2010b), another complementary hypothesis for the stimulus-secretion coupling of glucose-induced insulin release postulates the participation of volume-regulated anion channels in such a process. It is proposed that the entry of D-glucose in insulin-producing cells as mediated by GLUT2, the phosphorylation of the hexose catalyzed mainly by glucokinase and the subsequent acceleration of glucose metabolism lead to the intracellular accumulation of metabolites generated by the catabolism of the hexose, such as lactate and bicarbonate anions. The resulting increase in intracellular osmolarity might then provoke, through increased water uptake, cell swelling and subsequent gating of volume-sensitive anion channels. In the insulin-producing β-cells, the gating of these channels may allow the exit of anions, such as Cl^-, and, hence, provoke a further depolarization of the plasma membrane, with subsequent gating of voltage-sensitive calcium channels. The β-cell volume-regulated anion channel (VRAC) shares several characteristics with that expressed in other tissues and only appears to be distinct from that in other cell types by its halide selectivity (Best et al. 1996).

The postulated activation of VRAC by D-glucose in the pancreatic islet β-cell is supported by a number of observations. This effect was indeed demonstrated at the whole-cell level (Best 1997, 2000) and in single channel recordings (Best 1999, 2002). The increase in β-cell Cl^- permeability provoked by D-glucose will be later discussed in this dissertation. Likewise, the identity of the volume-activated anion channels and the several anionic candidates possibly concerned by such a process are duly considered in the following sections of the present contribution. At this point, it should be stressed that a rise in D-glucose concentration indeed causes a concentration-related increase of β-cell volume and that during sustained exposure to D-glucose, such an increase in β-cell volume persists with often an oscillatory pattern (Miley et al. 1997). Raising the concentration of D-glucose to 20 mM caused

a similar degree of cell swelling in the presence of 2 mM Co^{2+}, a blocker of voltage-sensitive Ca^{2+} channels. This suggests that increase in cell volume in response to glucose is not merely a consequence of an enhanced rate of exocytosis. When 3-O-methyl-D-glucose, a non-metabolizable analog which is transported into β-cells in the same manner as D-glucose, was substituted for an equivalent concentration of mannitol, which is relatively impermeant, this only resulted in a modest and transient increase in cell volume probably attributable to the entry of 3-O-methyl-D-glucose in the β-cell, as supported by the finding that addition of 3-O-methyl-D-glucose with no substitution of mannitol caused no significant increase in β-cell volume. The findings that the glucokinase activator GKA50 causes an increase in cell volume and activation of volume-regulated anion channels in rat pancreatic β-cells (McGlasson et al. 2011) and that the effect of D-glucose to activate the volume-sensitive anion channel is reproduced by 2-ketoisocaproate (Best 1997) supported the view that the increase in β-cell volume evoked by these insulin secretagogues is linked to their capacity to act as nutrient in the β-cell. Last, the abovementioned effect of D-glucose to gate VRAC is suppressed by anion channel inhibitors such as 5-nitro-2(3-phenylpropylamino)benzoic acid.

The Experimental Model of Extracellular Hypotonicity

An acute reduction in the osmolality of the medium bathing isolated pancreatic islets has been recognized as early as in 1975 as a stimulus for insulin release and was found to reproduce the first phase of glucose-induced insulin release (Blackard et al. 1975). In several investigations concerning the volume-regulated anion channel hypothesis, the experimental model of extracellular hypotonicity was often used. In the first extensive study of the stimulus-secretion coupling of hypotonicity-induced insulin release conducted in BRIN-BD11 cells, the following information was gathered (Beauwens et al. 2006).

In the first series of experiments, the secretory response to hypotonicity, as provoked by a decrease in NaCl concentration by 50 mM, was examined in three lines of insulin-producing cells. The findings indicated that the BRIN-BD11 cells, as distinct form either MIN-6 or INS-1 cells, not only display a relatively greater secretory response to hypotonicity but also a positive modulation of such a response by the extracellular concentration of D-glucose. All further experiments were, therefore, conducted only in BRIN-BD11 cells.

The release of insulin recorded in the hypotonic medium averaged $230 \pm 17\%$ ($n = 37$) of the paired basal value measured in the presence of 1.1 mM D-glucose using a salt-balanced iso-osmolar medium. In the latter iso-osmolar medium, a rise in D-glucose concentration from 1.1 to 11.1 mM augmented insulin release by no more than $23 \pm 8\%$ ($n = 15$; $p < 0.01$). The time course for the secretory response to hypoosmolarity was characterized after the peak value recorded over the first 15 min of exposure to the hypotonic medium, by an exponential decrease during the subsequent incubation of 15 min each up to the 90th min of the experiment.

The inhibitor of volume-sensitive anion channels 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB, 0.1 mM) abolished the secretory response to hypotonicity. Such a secretory response represented a Ca^{2+}-dependent process, being inhibited either in the absence of extracellular Ca^{2+} and presence of EGTA (0.5 mM) or in the presence of the organic calcium antagonist verapamil (10.0 μM).

The possible role of ATP-sensitive K^+ channels in the process of hypotonicity-induced insulin release was examined in three series of experiments. First, diazoxide (0.1 mM) decreased to the same relative extent both basal- and hypotonicity-stimulated insulin output, suggesting that the gating of ATP-sensitive K^+ channels by diazoxide played a comparable modulatory role under these two experimental conditions. Second, the hypoglycemic sulfonylurea tolbutamide (10 μM) was found to increase modestly but significantly the release of insulin recorded in the hypotonic medium, possibly by minimizing the fall in insulin secretion otherwise characterizing the secretory response to hypoosmolarity. Last, a rise in extracellular K^+ concentration up to 30 mM while increasing, as expected, insulin release at normal osmolarity decreased the increment in insulin output otherwise attributable to hypoosmolarity. Thus, the rise in K^+ concentration apparently prevented hypoosmolarity to provoke a further depolarization of the plasma membrane. Taken as a whole, these findings suggest that a closing of ATP-sensitive K^+ channels is not involved in hypotonicity-induced insulin release.

The concentration dependency of the response to hypoosmolarity was also examined. For instance, the incorporation of increasing concentrations of sucrose (25–100 mM) to the hypotonic medium provoked in the 25–75 mM range of sucrose concentration a progressive decrease of the hypotonicity-induced increment in insulin output.

Last, two sets of experimental data were consistent with the view that a high intracellular concentration of Cl^- anions, as presumably achieved in β-cells at the intervention of the Na^+-K^+-2Cl^- cotransporter specifically expressed in rat islet β-cell, is required to allow the process of hypotonicity-induced insulin release. First, the Cl^- ionophore tributyltin (1.0 μM), which did not affect significantly basal insulin output, decreased the increment in insulin output attributable to hypoosmolarity. When the concentration of tributyltin was increased to 2.5 μM, the output of insulin was virtually identical at normal osmolarity and in the hypotonic medium. Second, the inhibitor of the Na^+-K^+-2Cl^- cotransporter furosemide (0.1 mM) again did not affect basal insulin output, but severely decreased the output of insulin recorded under hypoosmolar conditions.

In the same study, it was documented that exposure to the hypotonic medium indeed increased the volume of BRIN-BD11 cells, followed by a regulatory volume decrease, itself suppressed in the presence of NPPB. This inhibitor of VRAC also decreased in a rapid and reversible manner both the inward and outward currents provoked by ±100 mV voltage pulses in conventional whole-cell recording with hypertonic intracellular medium to induce BRIN-BD11 cell swelling. NPPB also opposed the effect of hypotonicity to provoke depolarization and induction of spiking activity in the BRIN-BD11 cells. Last, exposure of the BRIN-BD11 cells to a hypotonic medium provoked a rapid increase in the cytosolic Ca^{2+} concentration (Beauwens et al. 2006).

The Possible Role of NAD(P)H Oxidase-Derived H_2O_2 in the Activation of VRAC in β-Cells Exposed to a Hypotonic Medium

It was recently proposed that, in several cell lines, the activation of VRAC under hypotonic extracellular conditions and the ensuing volume regulatory decrease results from NAD(P)H oxidase (NOX)-derived H_2O_2. In a recent study, it was investigated whether a comparable situation prevails in insulin-producing cells, i.e., whether an increase in intracellular H_2O_2 is instrumental in the opening of VRAC in the process of hypotonicity-induced insulin release (Crutzen et al. 2012).

The following findings supported the latter view.

First, exogenous H_2O_2 stimulates insulin release from BRIN-BD11 cells, with a threshold value close to 40 μM and a maximal stimulation at about 100 μM. The secretory response to exogenous H_2O_2, like that evoked by extracellular hypotonicity, was suppressed by 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB; 100 μM).

Second, NAD(P)H oxidase inhibitors, such as diphenylene iodonium chloride (DPI, 10 μM) or plumbagin (30 μM), suppressed in the BRIN-BD11 cells the secretory response to hypotonicity. Such was also the case after preincubation of the BRIN-BD11 cells either with N-acetyl-L-cysteine for 24 h or with betulinic acid for 48 h, the latter agent causing a time-related decrease of NOX4 gene expression (as assessed by RT-PCR) in the BRIN-BD11 cells.

Third, exposure of the BRIN-BD11 cells to either exogenous H_2O_2 or extracellular hypotonicity increases their intracellular content in reactive oxygen species. In this respect, the response of the BRIN-BD11 cells to hypotonicity was rapid and sustained, it being abolished by DPI.

Fourth, exogenous H_2O_2 provoked membrane depolarization and electrical activity in the BRIN-BD11 cells, such an effect being opposed by NPPB. Likewise, exogenous H_2O_2 induced the activation of single chloride channels, an effect again opposed by NPPB.

Fifth, hypotonicity provoked cell swelling followed by a regulatory volume decrease in the BRIN-BD11 cells, the latter RVD being suppressed by NPPB, by the NAD(P)H oxidase inhibitors (DPI, plumbagin) and after preincubation of the BRIN-BD11 cells with either N-acetyl-L-cysteine or betulinic acid.

Sixth, in dispersed rat islet cells, as distinct from BRIN-BD11 cells, exogenous H_2O_2 again provoked a concentration-related depolarization of the plasma membrane, such an effect being suppressed by NPPB.

Last, in both dispersed rat islet cells and freshly isolated rat pancreatic islets, H_2O_2 (100–200 μM) again stimulated insulin release over 20-min incubation, an effect itself again opposed by NPPB.

Candidate Anions

The volume-regulation anion channel hypothesis here under consideration raises the question as to the identity of the concerned anions under physiological conditions.

Sehlin was the first to report that a rise in D-glucose concentration caused a concentration-related decrease of the $^{36}Cl^-$ content of prelabeled islets prepared from *ob/ob* mice, a current model of inherited obesity (Sehlin 1978). From these findings, it was inferred that the hexose stimulates the Cl^- efflux from islet cells and that such an increase in Cl^- permeability may partly mediate the glucose-induced depolarization of insulin-producing cells.

The possible extension of these findings to islets prepared from normal rats was more recently investigated by measuring the changes evoked by increasing concentrations of D-glucose in $^{36}Cl^-$ outflow from prelabeled islets (Malaisse et al. 2004). For such a purpose, after 60-min preincubation at 37 °C in the presence of 3.0 mM D-glucose and $^{36}Cl^-$, the rat islets were incubated for 8–10 min at 37 °C in the presence of increasing concentrations of D-glucose (3–20 mM). After preincubation the $^{36}Cl^-$ content of the islets corresponded to an estimated intracellular Cl^- concentration of 126 ± 13 mM, as compared to 128 mM in islets from *ob/ob* mice. The rise in D-glucose concentration during the final incubation period caused a concentration-related increase of $^{36}Cl^-$ efflux from the prelabeled rat islets, with a threshold value close to 5.0 mM D-glucose and a half-maximal response at a D-glucose concentration close to 10.0 mM. These two features are similar to those characterizing the effect of D-glucose upon insulin release from rat islets. The D-glucose concentration-response relationship found in this study was also virtually identical to that obtained by Best when measuring the effect of increasing concentrations of D-glucose upon the channel open probability of a 200 pS anion-selective channel in recordings of cell-attached rat pancreatic β-cells (Best 2000).

Thus, the salient finding in these three series of investigations (Best 2000; Sehlin 1978; Malaisse et al. 2004) consisted in the fact that the concentration-related effect of D-glucose to cause the gating of voltage-sensitive anion channels closely parallels that of the hexose as an insulinotropic agent. This is in sharp contrast to the concentration-related response for the effect of D-glucose to provoke the closing of ATP-sensitive K^+ channels (Carpinelli and Malaisse 1981). Thus, in the latter case, a maximal response is already recorded at a concentration of D-glucose close to 5.0 mM.

Already in 1974, Freinkel et al. reported that a rise in extracellular D-glucose concentration causes a transient increase in inorganic phosphate release from isolated pancreatic islets (Freinkel et al. 1974). Virtually all the radioactive material released from islets prelabeled with ^{32}P-orthophosphate also consists of ^{32}P-orthophosphate. Such a phosphate flush is provoked by nutrient secretagogues, such as D-glucose, D-mannose, D-glyceraldehyde, L-leucine, its non-metabolized analog b(-)2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH), and 2-ketoisocaproate (Freinkel et al. 1974, 1976; Freinkel 1979; Carpinelli and Malaisse 1980). It coincides with a sizeable decrease in the inorganic phosphate content of the islets (Bukowiecki et al. 1979).

It is only in 2007 that the glucose-induced phosphate flush in pancreatic islets was proposed to be attributable to the gating of volume-sensitive anion channels (Louchami et al. 2007). Thus, it was documented that an increase in D-glucose

concentration from 1.1 to 8.3 mM induces a typical phosphate flush and biphasic stimulation of insulin release. Extracellular hypoosmolarity, as provoked by reducing the NaCl concentration by 50 mM, caused a monophasic increase in both ^{32}P fractional outflow from the islets prelabeled with ^{32}P-orthophosphate and insulin output. The inhibitor of volume-sensitive anion channels 5-nitro-2(3-phenylpropylamino)benzoate, used at a 0.1 mM concentration, inhibited both stimulation of insulin release and phosphate flush induced by either the increase in D-glucose concentration or extracellular hypoosmolarity. It should be underlined that, in these as in previous experiments, the secretory response to D-glucose was biphasic, while that to extracellular hypoosmolarity was monophasic with a rapid exponential return of the secretory rate toward basal value.

The proposed role attributed to the gating of volume-regulated anion channels as a key determinant of the phosphate flush is also compatible with the concentration-response relationship for the stimulation by D-glucose of effluent radioactivity from prelabeled and perifused rat pancreatic islets (Carpinelli and Malaisse 1980). Thus, the threshold concentration of D-glucose for induction of a phosphate flush is close to 4.0 mM with a close-to-maximal response at 16.7 mM.

The findings just mentioned suggest that, in the process of glucose-induced insulin release, another anion or other anions than inorganic phosphate may participate in the second phase of the insulin secretory response, accounting for the oscillation in cell volume recorded during prolonged exposure of islet cells to D-glucose (Miley et al. 1997). For instance, it was proposed that, during the second and sustained phase of insulin secretion evoked by D-glucose or other nutrient secretagogues, the gating of volume-regulated anion channels could provide a route of bicarbonate efflux in insulin-producing cells (Louchami et al. 2007). This proposal takes into account the finding that, in glucose-stimulated islets, the generation of bicarbonate catalyzed by mitochondrial carbonic anhydrase accounts for the majority of CO_2 produced through the oxidative catabolism of the hexose (Sener et al. 2007).

Incidentally, in the study on the possible role of carbonic anhydrase in rat pancreatic islets, acetazolamide, which was used to inhibit the latter enzyme and indeed decreased, when used in the 3.0–10.0 mM range, the production of $H^{14}CO_3^-$ by islets exposed to 16.7 mM D-[U-^{14}C]glucose, was found to slightly decrease intracellular pH and to lower the cytosolic concentration of Ca^{2+}. Whether these ionic effects of acetozolamide could be attributed, in part at least, to an altered cotransport of HCO_3^- and Na^+ by NBC1 remains to be assessed. Nevertheless, it should not be ignored that, in the proximal colon, the NBC1 activity can be increased during carbonic anhydrase inhibition by acetazolamide to maintain maximal levels of HCO_3^- secretion (Gawenis et al. 2007).

In a manner comparable to that just considered in the case of bicarbonate anions, the exit of lactic acid generated by the catabolism of D-glucose may occur at the intervention of volume-regulated anion channels during sustained exposure of the islets to D-glucose. The output of lactic acid from rat islets exposed for 90 min to D-glucose progressively increases from a basal value measured in the absence of the hexose averaging 23 ± 1 pmol/islet per 90 min ($n = 11$) to a value as high as

218 ± 7 pmol/islet per 90 min ($n = 6$) in the presence of 27.8 mM D-glucose (Sener and Malaisse 1976). At 16.7 mM D-glucose, the intracellular lactate content of the islets reaches a steady-state value not exceeding about 20 pmol/islet (Sener and Malaisse 1976), while the amount of lactate accumulated in the extracellular medium amounts to 181 ± 6 pmol/islet per 90 min (n = 102). The activity and expression of the lactate (monocarboxylate) transporter MCT are low or absent in β-cells (Best et al. 1992; Zhao et al. 2001), potentially leading to intracellular lactate accumulation during glucose stimulation. Thus, such an accumulation may account for both the glucose-induced β-cell swelling and the efflux of lactate via the VRAC, in which β-cells indeed show significant permeability to lactate (Best et al. 2001). This proposal is supported by the finding that the accumulation of D-lactate formed from methylglyoxal leads to β-cells swelling and VRAC activation (Best et al. 1999).

Expression and Function of Anoctamin

The TMEM16 transmembrane protein family consists of 10 different proteins with numerous splice variants that contain 8–9 transmembrane domains. TMEM16A (also called anoctamin1 or ANO1) has been identified as a subunit of activated Cl^- channels that are expressed in epithelial and non-epithelial tissues. All vertebrate cells regulate their volume by activating chloride channels. TMEM16A together with other TMEM16 proteins are activated by cell swelling, leading to a regulatory volume decrease (RVD). As a rule, it is considered that intracellular Ca^{2+} plays a role as a mediator for activation of volume-regulated chloride currents.

Activation of volume-regulated chloride channels is reduced in the colonic epithelium and in salivary acinar cells from mice lacking expression of TMEM16A. Hence, TMEM16 proteins appear to be a crucial component of epithelial volume-regulated Cl^- channels (Almaca et al. 2009). Studies on expression and function of the TMEM16A calcium-activated chloride channel conducted by Huang et al. have contributed to their subcellular location and function in a number of organs, including the epithelial cells, exocrine glands, and trachea, as well as airway and reproductive tract smooth muscle cells (Huang et al. 2009). Anoctamin 6 (or TMEM16F) was proposed as an essential component of the outwardly rectifying chloride channel in airway epithelial cells (Martins et al. 2011).

The expression of all ten members (ANO1–ANO10) in a broad range of murine tissues was also analyzed, each tissue expressing a set of anoctamin that forms cell- and tissue-specific Ca^{2+}-dependent Cl^- channels (Schreiber et al. 2010). In the perspective of the present chapter, two findings merit to be underlined. First, ANO1 produces large and rapidly activating Ca^{2+}-dependent Cl^- current, requiring 10 μM of cytosolic Ca^{2+} for full activation, while being inhibited at higher Ca^{2+} concentrations. Second, among some 26 organs examined for such a purpose, the pancreatic gland was found to express large amounts of ANO1 (Schreiber et al. 2010).

Two recent studies were devoted to the possible role of TMEM16A, also called anoctamin 1, as a volume-regulated anion channel in insulin-producing cells.

Expression and Role of Anoctamins in Rodent Insulin-Producing Cells

The methods used in the first of these two studies for reverse transcription-polymerase chain reaction, immunohistochemistry, measurement of BRIN-BD11 cell volume, insulin release and D-glucose metabolism in rat isolated pancreatic islets, and the monitoring of mouse β-cell membrane potential are detailed elsewhere (Malaisse et al. 2012, 2013).

Anoctamin Expression

Screening of anoctamin mRNA expression by RT-PCR documented its presence in both rat and human pancreas, isolated rat pancreatic islets, and kidney. In rat islets, anoctamin 1 and anoctamin 6 were predominant, with a lower level of anoctamin 10, while in BRIN-BD11 cells, anoctamin 6 predominated with much lower levels of either anoctamin 1 or anoctamin 10. The expression of anoctamin 1 was documented by immunohistochemistry, in mouse and rat and pancreas, with a more intense staining of pancreatic islets, as compared to exocrine pancreas.

BRIN-BD11 Cell Volume

Tannic acid (100 μM) suppressed the regulatory volume decrease otherwise occurring in BRIN-BD11 cells exposed to a hypotonic extracellular medium in the presence of 5.0 mM D-glucose.

Insulin Release

Tannic acid (100 μM) abolished the secretory response to extracellular hypoosmolarity in rat pancreatic islets incubated for 30 min in the presence of 2.8 mM D-glucose. As judged from the effects of increasing concentrations of tannic acid upon insulin output evoked by 16.7 mM D-glucose in rat islets incubated for 90 min and taking into account the basal value for insulin release, as measured in the presence of 2.8 mM D-glucose, the regression line concerning the release of insulin at increasing concentrations of tannic acid (logarithmic scale) suggested a threshold concentration for the inhibitory action of tannic acid close to 3.1 μM and an ED_{50} close to 65.6 μM. Two further series of experiments provided the following results. It was first observed that tannic acid (100 μM) indeed inhibits the secretory response to 16.7 mM, while failing to affect significantly insulin output at 8.3 mM D-glucose. The latter finding was confirmed, no significant difference in insulin output being observed when the islets were incubated in the presence of 8.3 mM D-glucose and increasing concentrations of tannic acid. Pooling together all available data, the output of insulin recorded in the presence of 8.3 mM D-glucose and 100 μM tannic acid averaged 92.7 ± 5.7 % ($n = 35$; $p > 0.35$) of the mean corresponding control values recorded within the same experiment in the absence of tannic acid (100.0 ± 5.4 %; $n = 36$). In the presence of 16.7 mM D-glucose and 100 μM tannic acid, however, the output of insulin represented no more than 57.2 ± 3.5 % ($n = 44$; $p < 0.001$) of the mean corresponding control values recorded in the absence of tannic acid. The output

of insulin remained significantly higher ($p < 0.001$) in the presence of 16.7 mM D-glucose and 100 µM tannic acid than in the sole presence of 8.3 mM D-glucose.

D-glucose Metabolism

The results of the experiments aiming at assessing the effects of tannic acid upon D-glucose metabolism in rat islets provided the following information. Relative to the mean value for D-[U-^{14}C]glucose oxidation recorded within each of four experiments in islets exposed to 16.7 mM in the absence of tannic acid (100.0 ± 8.7 %; $n = 27$), the measurements made at 2.8 mM D-glucose also in the absence of tannic acid averaged 14.6 ± 1.3 % ($n = 26$; $p < 0.001$), while those found in the concomitant presence of 16.7 mM D-glucose and 100 µM tannic acid amounted to 140.3 ± 11.8 % ($n = 28$; $p < 0.009$). Inversely, the generation of ^{3}HOH from D-[5-^{3}H]glucose by islets exposed to 16.7 mM D-glucose in the presence of tannic acid represented no more than 55.3 ± 7.0 % ($n = 21$; $p < 0.003$) of the mean corresponding values recorded within the same experiments at the same hexose concentration but in the absence of tannic acid. As a result of these opposite metabolic effects of tannic acid, the mean absolute value for the paired ratio between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization, which, in the absence of tannic acid, was much lower at 2.8 mM D-glucose than at 16.7 mM D-glucose, was, at the high concentration of the hexose, significantly higher in the presence of tannic acid than in its absence.

As judged from these findings and assuming that the difference between D-[5-^{3}H]glucose conversion to ^{3}HOH and that of D-[U-^{14}C]glucose to ^{14}CO$_2$ corresponds to the generation of lactic acid from D-glucose, the ATP generation rate attributable to the catabolism of the hexose, which did not exceed 171.8 ± 18.4 pmol/islet per 90 min at 2.8 mM D-glucose, amounted to 995.4 ± 98.4 and 998.4 ± 77.1 pmol/islet per 90 min at 16.7 mM D-glucose, respectively, in the absence and presence of tannic acid. The latter two values being virtually identical, they indicate that, in the presence of tannic acid, the increased oxidation of D-glucose compensated, in terms of energy yield, for the decreased rate of glycolysis.

Bioelectrical Activity

Tannic acid (100 µM) was found to impair the bioelectrical activity induced by D-glucose (16.7 mM) in mouse β-cells. From a detailed analysis of the changes induced by tannic acid in the bioelectrical response to 16.7 mM D-glucose, it was calculated that, over the same period of time, the influx of Ca^{2+} ions only represented in the presence of tannic acid about 41.4 % of that taking place in its absence.

Concluding Remarks

A salient finding in this study consists in the fact that at a D-glucose concentration of 8.3 mM as distinct from 16.7 mM, tannic acid failed over 90-min incubation to cause any sizeable decrease in insulin output. Hence, it would appear that inhibition by tannic acid of anoctamin 1 preferentially impairs the increase in insulin output provoked by a rise in D-glucose concentration from 8.3 to 16.7 mM, i.e., in the range of hexose

concentrations in which the gating of volume-regulated anion channels may play its major role in the stimulus-secretion coupling of glucose-induced insulin secretion.

Expression of TMEM16A in Human Pancreatic Islets

In the same report at that mentioned in section "Expression of SLC4A4 in Human Pancreatic Islets" of the present chapter, the expression of TMEM16A was also assessed in human pancreatic islets (Hanzu et al. 2012).

In mirror image to that found for SLC4A4, qRT-PCR yielded higher gene expression of TMEM16A in isolated islets than in the total pancreas with a mean paired pancreas/islet ratio of 0.50.

At the protein level, immunohistochemistry for TMEM16A documented its presence in both insulin-producing cells and exocrine cells. The immunostaining of TMEM16A appeared somewhat less pronounced in insulin-producing cells than in the exocrine cells.

Soluble Adenylyl Cyclase

Insulin-producing β-cells have long been known to be equipped with a family of G protein-responsive transmembrane adenylyl cyclases. Incretins released by the intestine in response to food intake, such as glucagon-like peptide 1 (GLP-1), increase adenosine $3'$-$5'$-cyclic monophosphate (cAMP) in β-cells, at the intervention of specific G protein-coupled receptors, e.g., GLP-1 receptor, by activating transmembrane adenylyl cyclase.

A rise in extracellular D-glucose concentration also provokes a rapid and sustained increase in the cAMP content of rat pancreatic islets. Other nutrient secretagogues, such as L-leucine, also increase cAMP generation. In both cases, the latter increase is suppressed when the islets are incubated in the absence of extracellular Ca^{2+} (Valverde et al. 1983). As a matter of fact, the accumulation of cAMP evoked by D-glucose in islet cells was proposed to be attributable to a calcium-dependent stimulation of adenylate cyclase by endogenous calmodulin indeed present in pancreatic islets (Valverde et al. 1979).

More recently, a soluble adenylyl cyclase was identified in insulin-producing INS-1E cells by RT-PCR, Western blot, and immunocytochemistry. The activity of this soluble adenylyl cyclase can be modulated by Ca^{2+}, bicarbonate, and ATP. It was proposed that this soluble adenylyl cyclase is the predominant source of glucose-induced cAMP, at least in INS-1E cells (Ramos et al. 2008).

This information led to further experimental work conducted within the framework of the issues discussed in the present chapter. Thus recent investigations aimed at exploring the interaction between adenosine $3'5'$-cyclic monophosphate (cAMP), volume-regulated anion channels (VRAC), and the Na^+-HCO_3^--cotransporter NBCe1 in the regulation of nutrient- and hypotonicity-induced insulin

release from both rat pancreatic islets and tumoral insulin-producing BRIN-BD11 cells (Bulur et al. 2013). The major findings collected in this last series of investigations may be summarized as follows.

Experiments in Rat Pancreatic Islets

Tenidap (50 μM) and the inhibitor of VRAC 5-nitro-2-(3-phenylpropylamino)benzo-ate (NPPB, 0.1 mM) inhibited the secretory response evoked by D-glucose (8.3 mM) in rat pancreatic islets. Either 8-bromoadenosine-3′-5′-cyclic monophosphate (8-Br-cAMP, 1.0 mM) or dibutyryladenosine-3′-5′-cyclic monophosphate (db-cAMP, 1.0 mM) increased glucose-stimulated insulin release. The relative mag-nitude of such an increase was not significantly different with each of these two cAMP analogs with an overall mean enhancing action of $41.1 \pm 11.4\,\%$, when the measure-ments of insulin output were corrected for basal value. In the presence of tenidap, the two cAMP analogs augmented the mean value for insulin release. The overall mean relative magnitude of such an increase was virtually identical to that recorded in the absence of any potential inhibitor of insulin release. In the islets exposed to NPPB, however, the enhancing action of the cAMP analogs failed to achieve statistical significance, suggesting that, under the present experimental conditions, NPPB suppressed an essential component of the secretory response to D-glucose. It is indeed well established that agents increasing the cAMP content of non-tumoral insulin-producing cells fail to augment insulin output from islets incubated at low D-glucose concentrations (Malaisse et al. 1967). In contrast, the maintenance of a significant positive response to the cAMP analogs in the presence of tenidap suggests that the participation of NBCe1 in ionic fluxes does not represent an essential permissive process for the expression of D-glucose insulinotropic action.

Experiments in BRIN-BD11 Cells

Reference Data

The basal insulin release from BRIN-BD11 cells incubated in the isotonic medium containing 1.1 mM D-glucose averaged 61.5 ± 4.1 μU/ml per 30 min ($n = 39$). It was increased by 70.0 ± 5.8 μU/ml per 30 min (paired comparison; $n = 33$) in a hypotonic medium and by 30.5 ± 2.8 μU/ml per 30 min (paired comparison; $n = 8$) in the presence of 2-ketoisocaproate (KIC; 10 mM).

Effects of cAMP Analogs and Phosphodiesterase Inhibitors

When BRIN-BD11 cells were incubated in an isotonic medium, the association of adenosine-3′,5′-cyclic monophosphate acetoxymethyl ester (cAMP-AM; 0.1–0.2 mM) and 3-isobutyl-1-methylxanthine (IBMX; 0.5 mM) and even the sole presence of IBMX approximately doubled insulin output. The association of cAMP and IBXM also augmented insulin output when the BRIN-BD11 cells were incubated in a hypotonic medium. In this case, however, the relative magnitude for the

increase in insulin output evoked by the association of cAMP and IBMX was much lower than in the isotonic medium, this difference coinciding with the fact that the control values found in the absence of cAMP and IBXM averaged, in the hypotonic medium, close to 250 % of that recorded in the isotonic medium.

Effects of NPPB

The inhibitor of VRAC, NPPB (0.1 mM), abolished the secretory response to KIC, the cAMP analogs failing to fully restore the insulinotropic action of KIC.

When BRIN-BD11 cells are incubated in an isotonic medium, NPPB (0.1 mM) slightly enhances basal insulin output to 115.9 ± 7.6 % ($n = 8$; $p < 0.005$) of paired control values (Beauwens et al. 2006). However, at the same concentration NPPB abolished the secretory response to hypotonicity. Once again, the cAMP analogs failed to restore the secretory response evoked by the exposure of the BRIN-BD11 cells to the hypotonic medium. Nevertheless, the well-known enhancing action of the phosphodiesterase inhibitor IBMX upon insulin secretion remained operative in the cells exposed to both NPPB and a cAMP analog.

Effects of Tenidap

In the 50–100 μM range, tenidap decreases insulin output from BRIN-BD11 cells incubated in an isotonic medium containing 1.1 mM D-glucose (Bulur et al. 2009). Even in the concomitant presence of IBMX (0.5 mM) and cAMP-AM (0.1 mM), tenidap (50 μM) decreased significantly insulin output from BRIN-BD11 cells incubated in the isotonic medium below the mean control value recorded in the absence of tenidap.

Tenidap (50 μM) also inhibited KIC-stimulated insulin release. The cAMP analogs 8-Br-cAMP (1.0 mM) or db-cAMP (0.1 mM) failed to augment significantly insulin release recorded in the presence of both KIC and tenidap. Only dioctanoyl adenosine-3′,5′-cyclic monophosphate (dioctanoyl-cAMP; 1.0 mM) and 2′-O-monosuccinyladenosine 3′,5′-tyrosyl methyl ester (0.1 mM) augmented significantly insulin release evoked by KIC in the presence of tenidap.

When BRIN-BD11 cells were incubated in a hypotonic medium in the presence of tenidap, the release of insulin was significantly lower ($p < 0.01$) than the paired value recorded in an isotonic medium in the absence of tenidap. Among various cAMP analogs examined for such a purpose, and whether in the concomitant presence of IBMX (0.5 mM) or not, only dioctanoyl-cAMP (1.0 mM) increased, when tested in the absence of IBMX, insulin output to a sizeable extent from BRIN-BD11 cells exposed to the hypotonic medium in the presence of tenidap.

Effects of MAP-Kinase Inhibitors

The MAP-kinase inhibitors U0126 (1,4-diamino-2,3-dicyano-1,4bis(O-aminophenylmercapto)butadiene ethanolate; 10 μM) and PD98,059 (2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; 50 μM) decreased modestly, and to the

same relative extent, the release of insulin from BRIN-BD11 cells incubated in the isotonic medium, whether in the presence or absence of KIC (10 mM) or in the hypotonic medium. These results are compatible with the participation of cAMP-responsive MAP-kinase in the secretory activity of BRIN-BD11 cells.

Effects of 2-Hydroxyestriol

The inhibitor of soluble adenylate cyclase 2-hydroxyesteriol, when tested at a 50 µM concentration, failed to affect significantly insulin release, whether from BRIN-BD11 cells incubated in an isotonic medium or exposed to the hypotonic medium. Even at a 100 µM concentration, 2-hydroxyesteriol only decreased insulin output to 84.2 ± 3.1 % ($n = 8$; $p < 0.002$) of the paired control value found under the same experimental conditions (isotonic or hypotonic medium) in the absence of 2-hydroxyestriol. Within the same experiments the release of insulin from BRIN-BD11 cells exposed to the hypotonic medium was decreased to a comparable extent by either 2-hydroxyestriol (100 µM) or the membrane permeant, metabolically stable inhibitor of cAMP-dependent protein kinase 8-bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp-8-Br-cAMPS; also 100 µM) with an overall mean value of 82.8 ± 5.6 % ($n = 8$; $p < 0.025$) of the paired measurement made, also in the hypotonic medium, in the absence of the latter two agents. These findings could suggest a limited participation of soluble adenylate cyclase in the secretory activity of BRIN-BD11 cells. It should be underlined, however, that in this case, like in the experiments conducted with the MAP-kinase inhibitors, basal and stimulated insulin output from the BRIN-BD11 cells were affected to a comparable relative extent by the tested inhibitors.

Effects of HCO_3^- and/or Cl^- Omission

The omission of $NaHCO_3$ severely decreased the secretory response to either KIC (10 mM) or extracellular hypotonicity. In the absence of $NaHCO_3$, a modest further decrease in insulin output was noticed when tenidap (50 µM) was present in the incubation medium. Under the latter experimental conditions, neither 8-Br-cAMP (1.0 mM) nor db-cAMP (also 1.0 mM) affected significantly insulin output. Dioctanoyl-cAMP (1.0 mM), however, still dramatically increased insulin release from the BRIN-BD11 cells exposed, in the absence of $NaHCO_3$ and presence of tenidap, to either KIC or a hypotonic medium. The inhibition of insulin release from BRIN-BD11 cells attributable to the absence of $NaHCO_3$ is reminiscent of comparable results recorded in rat pancreatic islets (Malaisse et al. 1979b; Sener and Malaisse 2012).

In the absence of Cl^- or both Cl^- and HCO_3^-, the paired ratio between insulin output in the hypotonic/isotonic medium was also abnormally low.

Effects of Na^+ Omission

The release of insulin from BRIN-BD11 cells incubated in an isotonic medium deprived of Na^+, as achieved by the substitution of NaCl (115 mM) by an equimolar mixture of 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS), N-methyl-D-glucosamine, and sucrose and that of $NaHCO_3$ (24 mM) by an equimolar amount of

choline bicarbonate, was two to three times higher than that found, within the same experiment, in the usual isotonic medium. The hypotonic/isotonic ratio for insulin output, which was as expected above 200 % under the usual experimental conditions, did not exceed 103.6 ± 3.3 % in the Na^+-free hypotonic medium. When the BRIN-BD11 cells were exposed to the Na^+-free hypotonic medium, a sizeable increase in insulin output was provoked by either 8-Br-cAMP (1.0 mM) or both IBMX (0.5 mM) and cAMP-AM (0.1 mM). These findings may suggest a favorable effect of Na^+ omission, on basal insulin output at least. This could, conceivably, involve a lesser consumption of ATP by the Na^+, K^+-ATPase.

Facts and Hypotheses

In a physiologically relevant perspective and, hence, in the experiments conducted in rat pancreatic islets, a salient finding was that, under experimental conditions in which tenidap and NPPB inhibited to a comparable extent the insulinotropic action of 8.3 mM D-glucose, the enhancing action of cAMP analogs was suppressed in the islets exposed to NPPB, but not so in the islets exposed to tenidap. This finding is compatible with the view that NPPB, by opposing the gating of VRAC, suppressed an essential component of the secretory response to D-glucose. In contrast, the maintenance of a significant positive response to cAMP analogs in the presence of tenidap suggests that the participation of NBCe1 in ionic fluxes does not represent an essential permissive process for the expression of D-glucose insulinotropic action. A different situation prevailed in tumoral insulin-producing cells which, however, otherwise display a relatively poor secretory response to D-glucose and apparently express a lower level of TMEM16A mRNA than that found in rat pancreatic islets. The experiments conducted in these tumoral cells also failed to ascribe to activation or inactivation of soluble adenylyl cyclase a key role in their response to either a nutrient secretagogue or extracellular hypoosmolarity.

Possible Roles of Aquaporins

Several insulinotropic agents were recently reported to cause β-cell swelling. The possible participation of aquaporins to water transport in pancreatic islet cells was investigated, therefore, in several recent reports. Aquaporins are channel-forming membrane proteins which allow water movement through the plasma membrane (Agre 2004). Aquaglyceroproteins represent a subfamily of aquaporins permeable not only to water but also to small solutes like glycerol and urea (Agre 2004; Rojek et al. 2008). Aquaglyceroporin 7 (AQP7) is expressed in rat and mouse pancreatic islet β-cells and tumoral insulin-producing BRIN-BD11 cells (Best et al. 2009; Delporte et al. 2009; Matsumura et al. 2007). Five recent publications deal with the possible role of AQP7 and other aquaporins in β-cell function.

   Matsumura et al. (2007) first found expression of AQP7, but not that of AQP3 or AQP9, in mouse pancreatic islets at both the mRNA and protein levels.

Immunohistochemistry revealed a complete overlap between insulin and AQP7 immunostaining in the pancreatic islet. Intraislet glycerol and triglyceride content was increased in AQP7$^{-/-}$ mice. Despite reduced pancreatic β-cell mass and islet insulin content, islets isolated from AQP7$^{-/-}$ mice secreted insulin at a higher rate both under basal low-glucose conditions and on exposure to a high concentration of D-glucose (25.0 mM). Incidentally and quite surprisingly, assuming an islet protein content close to 1.0 μg/islet, the secretion of insulin by islets from AQP7$^{+/+}$ mice recorded in the presence of 25.0 mM D-glucose was about two orders of magnitude lower (ca. 23.8 $\pm$ 1.5 pg/μg protein per hour; $n = 3$) in the study by Matsumura et al. (2007) than that found by Li et al. (2009) in islets from wild-type mice incubated in the presence of 20.0 mM D-glucose (about 2.0 $\pm$ 0.1 ng/μg protein per hour; $n = 9$–23) or by Bulur et al. (2010) in islets from NRMI mice incubated at 16.7 mM D-glucose (3.8 $\pm$ 0.5 ng/μg protein per hour). An even more pronounced difference (about 400-fold) prevails when comparing the insulin content from wild-type mice in the report by Matsumura et al. (113.4 $\pm$ 7.2 pg/μg protein; $n = 8$) and either Li et al. (47 $\pm$ 3 ng/μg protein; $n = 6$–8) or Bulur et al. (46 $\pm$ 1 ng/μg protein; $n = 88$).

Louchami et al. then documented by RT-PCR the expression, in addition to AQP7, of AQP5 and AQP8 mRNA in mice pancreatic islets, as well as the presence of AQP5 and AQP8 in insulin-producing β-cells by immunostaining (Louchami et al. 2012). In the same study, the secretion of insulin evoked by the omission of 50 mM NaCl, the substitution of 50 mM NaCl by 100 mM glycerol, or a rise in D-glucose concentration from 2.8 to 8.3 and 16.7 mM was severely impaired in the islets from AQP7$^{-/-}$ mice. Yet, exposure of β-cells to either the hypotonic medium or a rise in D-glucose concentration caused a similar degree of cell swelling and comparable pattern of electrical activity in cells from AQP7$^{+/+}$ and AQP7$^{-/-}$ mice. Both the cell swelling and change in membrane potential were only impaired in AQP7$^{-/-}$ cells when exposed to 50 mM glycerol. These findings are consistent with the previous suggestion that AQP7 mediates both the influx (Delporte et al. 2009) and efflux (Matsumura et al. 2007) of glycerol from insulin-producing cells. Second, they apparently imply the existence of at least one water transport pathway in mouse β-cells other than AQP7. Last, the impaired insulin secretory activity found in the islets from AQP7$^{-/-}$ mice, despite normal volume and electrical responses, to insulinotropic stimuli other than glycerol suggests that the glyceroaquaporin AQP7 could play a role at a distal site of the exocytotic pathway. For example, it might imply the perturbed participation of some cytosolic protein otherwise tightly coupled in functional terms to AQP7. Alternatively, the impaired secretory activity of AQP7$^{-/-}$ β-cells could be related to a secondary consequence of AQP7 absence, such as the accumulation of triglyceride previously reported in these cells (Matsumura et al. 2007).

In another study, the functional role of AQP7 expression in the tumoral pancreatic β-cell line BRIN-BD11 was investigated (Delporte et al. 2009). The BRIN-BD11 cell line is an insulin-secreting cell line established by electrofusion of normal rat pancreatic β-cell from New England Deaconess Hospital with immortalized RINm5F cells (McClenaghan et al. 1996). AQP7 mRNA and protein were

detected by RT-PCR and Western blot analysis, respectively, in these BRIN-BD11 cells. In an isoosmolar medium, the net uptake of [2-^{3}H]glycerol displayed an exponential time course reaching an equilibrium plateau value close to its extracellular concentration. Within 2 min of incubation in a hypotonic medium (caused by a 50 mM decrease in NaCl concentration), the [2-^{3}H]glycerol uptake averaged 143.2 $\pm$ 3.8 % ($n = 24$; $p < 0.001$) of its control value in isotonic medium, declining thereafter consistently with previously demonstrated volume regulatory decrease. When isoosmolarity was restored by the addition of 100 mM urea to the hypotonic medium, [2-^{3}H]glycerol uptake remained higher (112.1 $\pm$ 2.8 %, $n = 24$; $p < 0.001$) than its matched control under isotonic conditions, indicating rapid entry of urea and water. Insulin release by BRIN-BD11 cells was three times higher in hypotonic than in isotonic medium. When glycerol (100 mM) or urea (100 mM) was incorporated in the hypotonic medium, the insulin release remained significantly higher than that found in the control isotonic medium, averaging, respectively, 120.2 $\pm$ 4.2 and 107.0 $\pm$ 3.8 % of the paired value recorded in the hypotonic medium. These findings document the rapid entry of glycerol and urea in BRIN-BD11 cells, likely mediated by AQP7.

In the fourth report, rat pancreatic β-cells were investigated (Best et al. 2009). AQP7 mRNA was detected by RT-PCR in both rat pancreas and rat isolated pancreatic islets. The AQP7 protein was identified in rat pancreatic islets by Western blot analysis. Double fluorescent immunolabeling documented that AQP7 labeling overlaps with that of either insulin or somatostatin, but not with that of glucagon in rat pancreatic islets. The major functional results may be summarized as follows. The standard incubation medium used for islet cell preparation and incubation consisted of 130 mM NaCl, 5 mM KCl, 1 mM MgSO$_4$, 1 mM NaH$_2$PO$_4$, 1.2 mM CaCl$_2$, 25 mM Hepes-NaOH (pH 7.4), and 5 mM D-glucose. For isoosmotic substitution experiments, the basal medium contained 50 mM mannitol substituted for 25 mM NaCl. The addition of urea, glycerol, and 1,3 propanediol to the medium was then substituted for an equivalent amount of mannitol. The isoosmotic addition of urea (50 mM) increased relative cell volume in rat pancreatic β-cells. Such a cell swelling was followed by a gradual regulatory volume decrease (RVD). A similar degree of cell swelling was provoked by the isoosmotic addition of 50 mM glycerol. However, in this case no subsequent RVD was observed, possibly due to the intracellular accumulation of glycerol metabolites. Consistent with this suggestion, the isoosmotic addition of non-metabolizable 1,3 propanediol caused cell swelling followed by RVD. The isoosmotic addition of urea caused, as a rule, membrane depolarization and electrical activity in isolated rat β-cells. This effect of urea was transient, possibly reflecting the process of RVD. In contrast, the isoosmotic addition of glycerol (50 mM) caused a marked and sustained depolarization with a brief period of electrical activity. Last, 1,3 propanediol (50 mM) caused a modest and transient depolarization with resulting electrical activity in some cells. The volume-regulated anion channel (VRAC) inhibitor 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB, 50 μM) reversibly inhibited the depolarizing action of glycerol. The isoosmotic addition of urea, glycerol, or 1,3 propanediol evoked a noisy inward current at the whole-cell level

using the perforated patch configuration. The characteristics of this current resembled those of the VRAC current and were inhibited in the presence of NPPB. These findings are consistent with the uptake of urea, glycerol, or 1,3 propanediol, possibly via aquaporin, accompanied by water uptake leading to cell swelling, VRAC activation, depolarization, and electrical activity.

Last, in the most recent publications, the expression of several aquaporin isoforms was investigated in pancreatic islets from both wild-type and AQP7$^{-/-}$ knockout mice (Virreira et al. 2012). In the wild-type mice, RT-PCR detection revealed the presence of the mRNA of AQP1, AQP4, AQP5, AQP6, AQP8, AQP11, and AQP12, while that of AQP2, AQP3, and AQP9 was close to or below the limit of detection. With the exception of AQP7, comparable results were recorded in the AQP7$^{-/-}$ mice with, on occasion, an apparently somewhat more pronounced mRNA expression, e.g., in the case of AQP1, AQP4, and AQP11. This recent study thus draws attention to the high number of distinct aquaporin isoforms indeed expressed in mouse pancreatic cells. It was acknowledged, however, that further work is obviously required both to assess the possible physiological significance of these various aquaporin isoforms in mouse pancreatic islets and to conduct comparable investigations both in other species including humans and in distinct populations of endocrine cells (e.g., insulin- versus glucagon-producing cells).

Volume-Regulated Anion Channels and Glucagon Release

A possible role for volume-regulated anion channels in the process of glucose-induced inhibition of glucagon release was recently considered. Insulin-producing β-cells express a Na$^+$-K$^+$-2Cl$^-$ cotransporter, which maintains a high chloride electrochemical potential gradient (Best 2005). Such is not the case in rat glucagon-producing cells (Majid et al. 2001). The latter cells, however, express K$^+$-Cl$^-$ cotransporters (KCC) of the KCC1 and KCC4 isoforms, which are not present in either β-cells or δ-cells (Davies et al. 2004). Exposure of α-cells to hypotonic solutions caused cell swelling followed by a regulatory volume decrease (RVD). An inhibitor of KCC blocked such an RVD in α-cells, while having no effect on the RVD in β-cells. Inversely, an activator of KCC significantly decreased α-cell volume, but had no effect on β-cell volume (Davies et al. 2004). Under physiological conditions, the K$^+$-Cl$^-$ cotransporter extrudes Cl$^-$ from the cell interior and would therefore be expected to maintain the chloride electrochemical potential gradient at a low value. In such a case, the activation of volume-regulated anion channels, e.g., by D-glucose, would result in Cl$^-$ entry into the cell, thus generating an outward, hyperpolarizing current, cell membrane hyperpolarization and, hence, inhibition of glucagon release. This proposed sequence of events was also considered in the light of a study documenting the expression and localization of the cystic fibrosis transmembrane conductance regulatory (CFTR) protein at much higher level in glucagon-secreting α-cells then in insulin-producing β-cells in the rat endocrine pancreas (Boom et al. 2007).

Concluding Remarks

The present chapter deals mainly with the expression and role of anionic transporters and channels in insulin-producing cells.

In this respect, the first issue concerns the cotransporter(s) Na^+-HCO_3^- of the NBCe1 family. The expression of both the mRNA and protein of distinct NBCe1 isoforms was documented in rat islets. The expression of NBCe1 mRNA and protein was also assessed in tumoral insulin-producing cells of the BRIN-BD11 line and human pancreatic islets. In this first set of experiments, tenidap was used as a potential inhibitor of NBCe1. Attention is drawn however on an apparent lack of specificity of tenidap toward the NBCe1 cotransporter, this drug also causing the gating of ATP-sensitive K^+ channels, as documented in a subsequent study. In addition to this reservation, a major so far unsolved question concerns the precise subcellular location of the NBCe1 cotransporters and the anionic flux mediated by these cotransporters. For instance, the question comes inevitably in mind whether such cotransporters mediate either the influx or efflux of Na^+ and HCO_3^- across the β-cell plasma membrane. In turn, changes in the Na^+ and HCO_3^- cytosolic concentration may participate in the stimulus-secretion coupling of insulin release. To cite only one example, soluble adenylyl cyclase may be activated by HCO_3^-. In considering the just-mentioned question, it could be argued that the insulin-producing β-cell acts mainly as a fuel-sensor cell in which the CO_2 generated by nutrient secretagogues, such as D-glucose, escapes from the cell mainly as HCO_3^- generated in a reaction catalyzed by a mitochondrial carbonic anhydrase (Sener et al. 2007). Hence, in situations of sustained stimulation of insulin release by D-glucose or other nutrient secretagogues, the efflux of the bicarbonate anion may appear as a more relevant movement across the plasma membrane rather than the opposite influx of the same anion. Admittedly, however, the efflux of HCO_3^- may occur mainly at the intervention of volume-regulated anion channels.

The latter remark leads to the second major issue considered in the present chapter. Based on a number of prior findings, the present work was indeed conceived in the framework of the so-called VRAC hypothesis. This hypothesis postulates that the gating of volume-regulated anion channels represents, in addition to the closing of ATP-sensitive K^+ channels, the second essential component of stimulus-secretion coupling in the process of glucose-stimulated insulin secretion and prevails in the range of concentration of the hexose well above the threshold value for the insulinotropic action of this nutrient. In the present study, evidence is provided to support the view that anoctamin 1 represents, to say the least, one of the volume-regulated anion channels in insulin-producing cells. The expression of the mRNA for anoctamin 1 and the presence of anoctamin 1 protein, as assessed by immunohistochemistry, were indeed documented in mouse, rat, and human pancreatic islet cells. Moreover, tannic acid, an inhibitor of anoctamin 1, impaired the secretory and bioelectrical response to a high concentration of D-glucose (16.7 mM) in rat pancreatic islets and mouse β-cells, respectively. Somewhat unexpectedly, tannic acid also affected both D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation, the decrease in glycolytic flux provoked by tannic acid being compensated by an

increased oxidation of the hexose, with no change in the total energy yield resulting from the catabolism of the sugar in rat pancreatic islets. The underlying determinants of these metabolic effects of tannic acid remain to be identified. Likewise, the possible role of anoctamin 6 and anoctamin 10 in islet cells remains to be explored. It may also be safe to extend the present work by use of other inhibitors of TMEM16A such as the aminophenylthiazole (T16A(inh)AO1) (Namkung et al. 2011). Last, TMEM16A being referred to as a calcium-activated chloride channel, further work is obviously desirable to assess the effects of its potential inhibitors on cytosolic Ca^{2+} concentration in glucose-stimulated insulin-producing cells. In such a respect, it seems worthwhile to remind that, already in 1977, it was observed that the Ca^{2+} antagonist verapamil, which inhibits both ^{45}Ca uptake and glucose-stimulated insulin release by isolated islets and which does not affect the total production of lactate by islets exposed up to 90 min to 16.7 mM D-glucose, somewhat unexpectedly decreases the output of lactate into the incubation medium, this resulting in an increase of the lactate content of the islets (Malaisse et al. 1977). This finding may indeed be relevant to the participation of the Ca^{2+}-activated volume-regulated anion channel anoctamin 1 in the process of lactate anion efflux from pancreatic islets.

With these concluding remarks in mind, there is little risk to propose that the participation of anionic transporters and channels in pancreatic insulin-producing islet cells remains a field widely open to further investigations.

References

Agre P (2004) Aquaporin water channels. Angew Chem Int Ed Engl 43:4278–4290

Almaca J, Tian Y, Aldehni F, Ousingsawat J, Kongsuphol P, Rock JR, Harfe BD, Schreiber R, Kunzelmann K (2009) TMEM16A proteins produce volume regulated chloride currents that are reduced in mice lacking TMEM16A. J Biol Chem 284:28571–28578

Beauwens R, Best L, Markadieu N, Crutzen R, Louchami K, Brown P, Yates AP, Malaisse WJ, Sener A (2006) Stimulus-secretion coupling of hypotonicity-induced insulin release in BRIN-BD11 cells. Endocrine 30:353–363

Best L (1997) Glucose and a-ketoisocaproate induce transient inward currents in rat pancreatic β cells. Diabetologia 40:1–6

Best L (1999) Cell-attached recordings of the volume-sensitive anion channel in rat pancreatic B cells. Biochim Biophys Acta 1419:248–256

Best L (2000) Glucose-sensitive conductances in rat pancreatic β-cells: contribution to electrical activity. Biochim Biophys Acta 1468:311–319

Best L (2002) Study of a glucose-activated anion-selective channel in rat pancreatic β-cells. Pflugers Arch 445:97–104

Best L (2005) Glucose-induced electrical activity in rat pancreatic β-cells: dependence in intracellular chloride concentration. J Physiol (Lond) 568:137–144

Best L, Trebilcock R, Tomlinson S (1992) Lactate transport in insulin-secreting β-cells: contrast between rat islets and HIT-T15 insulinoma cells. Mol Cell Endocrinol 86:49–56

Best L, Sheader EA, Brown PD (1996) A volume-activated anion conductance in insulin-secreting cells. Pflugers Arch 431:363–370

Best L, Brown PD, Tomlinson S (1997) Anion fluxes, volume regulation and electrical activity in the mammalian β cell. Exp Physiol 82:957–966

Best L, Miley HE, Brown PD, Cook LJ (1999) Methylglyoxal causes swelling and activation of a volume-sensitive anion conductance in rat pancreatic β-cells. Membr Biol 167:65–71

Best L, Speake T, Brown PD (2001) Characterization of the volume-sensitive anion channel in rat pancreatic β-cells. Exp Physiol 86:145–150

Best L, Brown PD, Yates AP, Perret J, Virreira M, Beauwens R, Malaisse WJ, Sener A, Delporte C (2009) Contrasting effects of glycerol and urea transport on rat pancreatic β-cell function. Cell Physiol Biochem 23:255–264

Best L, Brown PD, Sener A, Malaisse WJ (2010a) Opposing effects of tenidap on the volume-regulated anion channel and K_{ATP} channel activity in rat pancreatic β-cells. Eur J Pharmacol 629:159–163

Best L, Brown PD, Sener A, Malaisse WJ (2010b) Electrical activity in pancreatic islet cells: the VRAC hypothesis. Islets 2:59–64

Blackard WG, Likuchi M, Rabinovitch A, Renold AE (1975) An effect of hypoosmolarity on insulin release in vitro. Am J Physiol 228:706–713

Boom A, Lybaert P, Pollet J-F, Jacobs P, Jijakli H, Golstein PE, Sener A, Malaisse WJ, Beauwens R (2007) Expression and localization of cystic fibrosis transmembrane conductance regulator in the endocrine pancreas. Endocrine 32:197–205

Bukowiecki L, Trus M, Matschinsky FM, Freinkel N (1979) Alteration in pancreatic islet phosphate content during secretory stimulation with glucose. Biochim Biophys Acta 583:370–377

Bulur N, Virreira M, Soyfoo MS, Louchami K, Delporte C, Perret J, Beauwens R, Malaisse WJ, Sener A (2009) Expression of the electrogenic Na^+ -HCO_3^- cotransporter NBCe1 in tumoral insulin-producing BRIN-BD11 cells. Cell Physiol Biochem 24:187–192

Bulur N, Zhang Y, Malaisse WJ, Sener A (2010) Insulin release from isolated pancreatic islets, dispersed islet cells and tumoral insulin producing cells: a re-examination. Metab Funct Res Diab 3:20–24

Bulur N, Crutzen R, Malaisse WJ, Sener A, Beauwens R, Golstein P (2013) Interaction between 3′,5′-cyclic monophosphate, volume-regulated anion channels and the Na^+-HCO_3^- cotransporter NBCe1 in the regulation of nutrient- and hypotonicity-induced insulin release from rat pancreatic islets and tumoral insulin-producing BRIN-BD11 cells. Mol Med Rep 7:1666–1672

Carpinelli AR, Malaisse WJ (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLIV. A possible link between glucose metabolism and phosphate flush. Diabetologia 19:458–464

Carpinelli AR, Malaisse WJ (1981) Regulation of ^{86}Rb outflow from pancreatic islets. V. The dual effect of nutrient secretagogues. J Physiol (Lond) 315:143–156

Casas S, Gomis R, Gribble FM, Altirriba J, Knuutila S, Novials A (2007) Impairment of the ubiquitin-proteasome pathway is a downstream endoplasmic reticulum stress response induced by extracellular human islet amyloid polypeptide and contributes to pancreatic β-cell apoptosis. Diabetes 56:2284–2294

Cook DL, Hales CN (1984) Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. Nature 311:271–273

Crutzen R, Shlyonsky V, Louchami K, Virreira M, Hupkens E, Boom A, Sener A, Malaisse WJ, Beauwens R (2012) Does NAD(P)H oxidase-derived H_2O_2 participate in hypotonicity-induced insulin release by activating VRAC in β-cells? Eur J Physiol 463:377–390

Davies SL, Roussa E, Le Rouzic P, Thevenod F, Alper SL, Best L, Brown PD (2004) Expression of K/Cl cotransporters in the a-cells of rat endocrine pancreas. Biochim Biophys Acta 1667:7–14

Delporte C, Virreira M, Crutzen R, Louchami K, Sener A, Malaisse WJ, Beauwens R (2009) Functional role of aquaglyceroporin 7 expression in the pancreatic β-cell line BRIN-BD11. J Cell Physiol 221:424–429

Freinkel N (1979) Phosphate translocation during secretory stimulation of pancreatic islets. In: Camerini-Davalos A, Hanover B (eds) Treatment of early diabetes. Plenum Press, New York, pp 71–77

Freinkel N, El Younsi C, Bonnar J, Dawson MC (1974) Rapid transient efflux of phosphate ions from pancreatic islets as an early action of insulin secretagogues. J Clin Invest 54:1179–1189

Freinkel N, El Younsi C, Dawson RMC (1976) Insulin release and phosphate ion efflux from rat pancreatic islets induced by L-leucine and its nonmetabolizable analogue, 2-amino-bicyclo [2-2-1]heptane-2-carboxylic acid. Proc Natl Acad Sci USA 73:3403–3407

Gawenis LR, Bradford EM, Prasad V, Lorenz JN, Simpson JE, Charke LL, Woo AL, Grisham C, Sanford LP, Doetschman T, Miller ML, Shull GE (2007) Colonic anion secretory defects and metabolic acidosis in mice lacking the NBC1 Na^+/HCO_3^- cotransporter. J Biol Chem 282:9042–9052

Hanzu FA, Gasa R, Bulur N, Lybaert P, Gomis R, Malaisse WJ, Beauwens R, Sener A (2012) Expression of TMEM16A and SLC4A4 in human pancreatic islets. Cell Physiol Biochem 29:61–64

Henquin JC, Detimary P, Gembal M, Jonas JC, Shepherd RM, Warnotte C, Gilon P (1994) Aspects biophysiques du contrôle de la sécrétion d'insuline. Journées de Diabétologie de l'Hôtel-Dieu:21–32

Huang F, Rock JR, Harfe BD, Cheng T, Huang X, Jan YN, Jan LY (2009) Studies on expression and function of the TMEM16A calcium-activated chloride channel. Proc Natl Acad Sci USA 106:21413–21418

Li D-Q, Jing X, Salehi A, Collins SC, Hoppa MB, Rosengren AH, Zhang E, Lundquist I, Oloffson CS, Mörgerlin M, Eliasson L, Rorsman P, Renström E (2009) Suppression of sulfonylurea- and glucose-induced insulin secretion in vitro and in vivo in mice lacking the chloride transport protein ClC-3. Cell Metab 10:309–315

Louchami K, Zhang Y, Beauwens R, Malaisse WJ, Sener A (2007) Is the glucose-induced phosphate flush in pancreatic islets attributable to gating of volume-sensitive anion channels? Endocrine 31:1–4

Louchami K, Best L, Brown P, Virreira M, Hupkens E, Perret J, Devuyst O, Uchida S, Delporte C, Malaisse WJ, Beauwens R, Sener A (2012) A new role for aquaporin 7 in insulin secretion. Cell Physiol Biochem 29:65–74

Majid A, Speake T, Best L, Brown PD (2001) Expression of the Na-K-2Cl cotransporter in and β cells isolated from the rat pancreas. Pflugers Arch 442:570–576

Malaisse WJ, Orci L (1979) The role of the cytoskeleton in pancreatic β-cell function. In: Gabbiani G (ed) Methods of achievements in experimental pathology, vol 9. S. Karger, Basel, pp 112–136

Malaisse WJ, Sener A, Koser M, Herchuelz A (1976) The stimulus-secretion coupling of glucose-induced insulin release. XXIV. The metabolism of α- and β-D-glucose in isolated islets. J Biol Chem 251:5936–5943

Malaisse WJ, Herchuelz A, Levy J, Sener A (1977) Calcium-antagonists and islet function. III. The possible site of action of verapamil. Biochem Pharmacol 26:735–740

Malaisse WJ, Sener A, Herchuelz A, Hutton JC (1979a) Insulin release: the fuel hypothesis. Metabolism 28:373–386

Malaisse WJ, Hutton JC, Kawazu S, Herchuelz A, Valverde I, Sener A (1979b) The stimulus-secretion coupling of glucose-induced insulin release. XXXV. The links between metabolic and cationic events. Diabetologia 16:331–341

Malaisse WJ, Malaisse-Lagae F, Mayhew D (1967) A possible role for the adenylcyclase system in insulin secretion. J Clin Invest 46:1724–1734

Malaisse WJ, Sener A, Welsh M, Malaisse-Lagae F, Hellerström C, Christophe J (1983) Mechanism of 3-phenylpyruvate-induced insulin release. Metabolic aspects. Biochem J 210:921–927

Malaisse WJ, Zhang Y, Louchami K, Jijakli H (2004) Stimulation by D-glucose of $^{36}Cl^-$ efflux from prelabeled rat pancreatic islets. Endocrine 25:23–25

Malaisse WJ, Best L, Beauwens R, Sener A (2008) Ionic determinants of the insulinotropic action of glucose: the anion channel hypothesis. Metab Funct Res Diab 1:2–6

Malaisse WJ, Virreira M, Zhang Y, Crutzen R, Bulur N, Lybaert P, Golstein PE, Sener A, Beauwens R (2012) Role of anoctamin 1 (TMEM16A) as a volume regulated anion channel in insulin-producing cells. Diabetologia 55(Suppl 1):S204

Malaisse WJ, Crutzen R, Bulur N, Virreira M, Rzajeva A, Golstein PE, Sener A, Beauwens R (2013) Effects of the inhibitor of anoctamin 1, tannic acid, on insulin-producing cells. Diabetologia 56(Suppl 1):S196

Martins JR, Faria D, Kongsuphol P, Schreiber R, Kunzelmann K (2011) Anoctamin 6 is an essential component of the outwardly rectifying chloride channel. Proc Natl Acad Sci USA 108:18168–18172

Matsumura K, Chang BH, Fujimiya M, Chen W, Kulkarni RN, Eguchi Y, Kimura H, Kojima H, Chan L (2007) Aquaporin 7 is a β-cell protein and regulator of intraislet glycerol content and glycerol kinase activity, β-cell mass, and insulin production and secretion. Mol Cell Biol 27:6026–6037

McClenaghan NH, Barnett CR, O'Harte FP, Flatt PR (1996) Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic β-cell line. J Endocrinol 151:349–357

McGlasson L, Best L, Brown PD (2011) The glucokinase activator GKA50 causes an increase in cell volume and activation of volume-regulated anion channels in rat pancreatic β-cells. Mol Cell Endocrinol 342:48–53

Miley HE, Sheader EA, Brown PD, Best L (1997) Glucose-induced swelling in rat pancreatic β-cells. J Physiol (Lond) 504:191–198

Muller-Berger S, Ducoudret O, Diakov A, Frömter E (2001) The renal Na-HCO$_3^-$ cotransporter expressed in *Xenopus laevis* oocytes: change in stoichiometry in response to elevation of cytosolic Ca^2 concentrations. Pflugers Arch 442:718–728

Namkung W, Phuan PW, Verkman AS (2011) TMEM16A inhibitors reveal TMEM16A as a minor component of calcium-activated chloride channel conductance in airway and intestinal epithelial cells. J Biol Chem 286:2365–2374

Orci L, Malaisse WJ (1980) Hypothesis: single and chain release of insulin secretory granules is related to anionic transport at exocytotic sites. Diabetes 29:943–944

Parker MD, Boron WF (2008) Sodium-coupled bicarbonate transporters. In: Alpern RJ, Hebert SC (eds) Seldin and Giebisch's. The kidney physiology and pathophysiology. Elsevier Academic Press, Amsterdam, pp 1481–1497

Ramos LS, Zippin JH, Kamenetsky M, Buck J, Levin LR (2008) Glucose and GLP-1 stimulate camp production via distinct adenylyl cyclases in INS-1^E insulinoma cells. J Gen Physiol 132:329–338

Rojek A, Praetorius J, Froklaer J, Nielsen S, Fenton RA (2008) A current view of the mammalian aquaglyceroporins. Annu Rev Physiol 70:301–327

Schreiber R, Uliyakina I, Kongsuphol P, Warth R, Mirza M, Martins JR, Kunzelmann K (2010) Expression and function of epithelial anoctamins. J Biol Chem 285:7838–7845

Sehlin J (1978) Interrelationship between chloride fluxes in pancreatic islets and insulin release. Am J Physiol 235:E501–E508

Sener A, Malaisse WJ (1976) Measurement of lactic acid in nanomolar amounts. Reliability of such a method as an index of glycolysis in pancreatic islets. Biochem Med 15:34–41

Sener A, Malaisse WJ (2012) Secretory, ionic and metabolic events in rat pancreatic islets deprived of extracellular NaHCO$_3$. Metab Funct Res Diab 5:1–3

Sener A, Malaisse-Lagae F, Malaisse WJ (1981) Stimulation of islet metabolism and insulin release by a nonmetabolizable amino acid. Proc Natl Acad Sci U S A 78:5460–5464

Sener A, Welsh M, Lebrun P, Garcia-Morales P, Saceda M, Malaisse-Lagae F, Herchuelz A, Valverde I, Hellerström C, Malaisse WJ (1983) Mechanism of 3-phenylpyruvate-induced insulin release. Secretory, ionic and oxidative aspects. Biochem J 210:913–919

Sener A, Jijakli H, Zahedi Asl S, Courtois P, Yates AP, Meuris S, Best LC, Malaisse WJ (2007) Possible role of carbonic anhydrase in rat pancreatic islets: enzymatic, secretory, metabolic, ionic, and electrical aspects. Am J Physiol 292:E1624–E1630

Somers G, Blondel B, Orci L, Malaisse WJ (1979) Motile events in pancreatic endocrine cells. Endocrinology 104:255–264

Somers G, Sener A, Devis G, Malaisse WJ (1980) The stimulus-secretion coupling of glucose-induced insulin release. XLV. The anion-osmotic hypothesis for exocytosis. Pflugers Arch 388:249–253

Soyfoo MS, Bulur N, Virreira M, Louchami K, Lybaert P, Crutzen R, Perret J, Delporte C, Roussa E, Thevenod F, Best L, Yates AP, Malaisse WJ, Sener A, Beauwens R (2009) Expression of the electrogenic Na^+-HCO_3^- cotransporters NBCe1-A and NBCe1-B in rat pancreatic islet cells. Endocrine 35:449–458

Valverde I, Vandermeers A, Anjaneyulu R, Malaisse WJ (1979) Calmodulin activation of adenylate cyclase in pancreatic islets. Science 206:225–227

Valverde I, Garcia-Morales P, Ghiglione M, Malaisse WJ (1983) The stimulus-secretion coupling of glucose-induced insulin release. LIII. Calcium-dependency of the cyclic AMP response to nutrient secretagogues. Horm Metab Res 15:62–68

Virreira M, Malaisse WJ, Sener A, Beauwens R (2012) Expression of aquaporin isoforms in mouse pancreatic islets. Metab Funct Res Diab 5:27–28

Zhao C, Wilson MC, Schuit F, Halestrap AP, Rutter GA (2001) Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50:361–366