Biochemical Pharmacology, Vol. 40, No. 3, pp. 411–416, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

COMMENTARY

CYTOSOLIC pH AND PANCREATIC β-CELL FUNCTION

ANGELA LYNCH and LEONARD BEST*
Department of Medicine, University of Manchester, Manchester M13 9PT, U.K.

The regulation of cytosolic pH (pHi) in mammalian cells and the importance of this process in cellular function have received a considerable amount of attention in recent years and, accordingly, have been reviewed extensively [1–5]. The purpose of this article is to examine the mechanisms by which pHi is regulated in pancreatic islet cells and the functional consequences of manipulating pHi, to assess the possible role of pHi as a coupling factor in nutrient-induced insulin release, and to speculate upon the possible consequences of pHi regulation.

It has become well established that pHi in mammalian cells is closely regulated, and does not fluctuate under varying cellular conditions. The observed values of pHi are also considerably higher than they would be if protons were distributed across the plasma membrane according to electrochemical gradients and membrane potential. Furthermore, there is evidence that changes in pHi can be elicited by certain hormones and mitogens such that pHi may play an important role in the control of the cell cycle [3–6]. Intracellular pH homeostasis is probably of critical importance for the activity of pH-dependent enzymes, ion channels and numerous cellular processes.

In considering the pancreatic islet, particularly the β-cell, secretory responses to nutrients such as glucose are thought to be linked closely to the high capacity of the cell to oxidise these nutrients. Thus, the possibility exists that changes in pHi as a result of the generation of acidic metabolites of these nutrients (such as lactic acid and CO_2) could play a role in the modulation of secretory activity. In addition, it is clear that regulatory mechanisms must exist to counteract the excessive acidification which may otherwise occur.

Effects of stimuli on islet cell pHi

Conflicting reports have appeared regarding the effects of glucose on islet cell pHi, and it is likely that these inconsistencies have resulted, as least in part, from differences in experimental design, particularly regarding the techniques used to assess cytosolic pH.

A number of studies have utilised a method based upon the distribution of a labelled weak acid, 5,5-dimethyl-[2-^{14}C]oxazolidine-2,4-dione ([^{14}C]-DMO), across the plasma membrane. Using such a technique, Lindstrom and Sehlin [7] reported that 20 mM glucose stimulates the equilibrium uptake of [^{14}C]-DMO in obese mouse islets corresponding to a rise in pHi of approximately 0.15 units, whilst Lebrun and colleagues [8] observed a similar increase in pHi in rat islets incubated in high concentrations of glucose. In contrast, Pace and Tarvin [9] subsequently reported that glucose reduces [^{14}C]-DMO equilibrium uptake in rat islets with a half-maximally effective concentration of 4 mM. A similar reduction in pHi in rat islets, assessed by [^{14}C]-DMO distribution, was observed in response to another nutrient, α-ketoisocaproate [10]. These authors also calculated that α-ketoisocaproate causes intracellular acidification in islet cells using an indirect method based upon extracellular pH measurement and buffering capacity of islet homogenates [11].

The use of fluorescent, pH-sensitive dyes has permitted the continuous monitoring of pHi, which, unlike isotope equilibrium experiments, readily enables kinetic studies to be made of responses to stimulation. In a series of studies employing fluorescein diacetate, Deleers and colleagues [12, 13] demonstrated that glucose induces a rapid, sustained rise in pHi. A similar increase in pHi was observed in response to α-ketoisocaproate [13], whereas non-nutrient stimuli such as glibenclamide or high concentrations of K^+ decrease intracellular pH.

Subsequent studies, using the fluorescent dye 2′7′-biscarboxyethyl-5′(6′)-carboxyfluorescein (BCECF) have shown that the rise in islet cell pHi following glucose stimulation is preceded by an immediate, transient intracellular acidification [14, 15]. Similar findings have been obtained subsequently, using this dye with a cultured insulinoma (H1T-T15) cell line [16]. In addition, the nutrients α-ketoisocaproate and glyceraldehyde, together with several non-nutrient stimuli including high K^+ and Ba^{2+}, were found to cause a pronounced acidification in these cells.

One potentially important consideration in the measurement of cytosolic pH is the ionic composition of the medium, particularly the bicarbonate content, since at least one pHi regulatory mechanism depends upon the presence of bicarbonate (see below). Indeed, Deleers *et al.* [13] found that glucose induces a rise or fall in rat islet cell pHi in the presence and absence of bicarbonate respectively. However, the glucose-induced rise in pHi in mouse islets observed by Lindstrom and Sehlin [7] persisted in the absence of bicarbonate. Similarly, omission of this anion does not appear to affect stimulus-induced changes in pHi in H1T-T15 cells (Trebilcock R, Lynch A and Best L, unpublished observations). Thus, the bicarbonate content of the medium could influence pHi responses in islet cells from certain species.

* Correspondence: Dr Leonard Best, Department of Medicine, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

Regulation of pHi in insulin-secreting cells

There are clearly a number of mechanisms by which a stimulus could influence islet cytosolic pH. As mentioned earlier, it would be predicted that metabolism of nutrients such as glucose, with the resultant production of acidic metabolites, would cause a fall in pHi. Whilst such an acidification, albeit transient, has been detected in certain studies, the majority of reports suggest that the long-term response to glucose and possibly α-ketoisocaproate is an intracellular alkalinisation. Thus, it appears that the β-cell possesses at least one mechanism by which the acidification which would be expected to result from nutrient oxidation can be masked and overcome. The most probable candidates for such a regulation of cytosolic pH are Na^+/H^+ exchange and Na^+-dependent Cl^-/HCO_3^- exchange [2–5].

Several studies have provided evidence that regulation of pHi in rat pancreatic islet cells [13–15] and in HIT cells [16] is highly sensitive to amiloride, an inhibitor of Na^+/H^+ exchange. Furthermore, when islets or HIT cells were incubated in the presence of amiloride, a progressive intracellular acidification, rather than alkalinisation, was observed [13, 16, 17], suggesting that glucose may, in some way, activate Na^+/H^+ exchange. The mechanism by which glucose might activate this process is unknown, although a number of possibilities exist. It has been proposed that activity of the antiporter is enhanced by the binding of protons to an allosteric "modifier" site on its cytoplasmic face [18, 19]. Such a mechanism could also explain the "overshoot" rise in pHi which has been observed in HIT cells following intracellular acidification [16]. An alternative possibility is that Na^+/H^+ exchange can be stimulated by diacylglycerol via an activation of protein kinase C [19]. It is well established that glucose, in common with several other nutrients, induces inositol lipid hydrolysis in islets [20, 21], and it therefore seems likely that diacylglycerol levels could be elevated in nutrient-stimulated islet cells.

A possible involvement of Cl^-/HCO_3^- exchange in islet cell pHi homeostasis was suggested by the observation that glucose also provoked an intracellular acidification in islets incubated in the absence of bicarbonate [13]. In addition, an increase in $^{14}CO_3H^-$ uptake has been observed in glucose-stimulated islets [12], suggesting that uptake of bicarbonate, perhaps via the anion antiporter, could at least contribute toward the neutralisation of acid equivalents derived from nutrient oxidation. Such an exchanger would presumably be the Na^+-dependent system which acts as a cell alkalinising mechanism [5]. In contrast, the Na^+-independent Cl^-/HCO_3^- exchange system acts as a cell-acidifying mechanism, promoting recovery from an alkaline load [5]. In recent experiments using BCECF-loaded HIT-T15 cells incubated in the absence of chloride or bicarbonate, we have failed to observe any modification of pHi responses to nutrients, weak acids or bases, thereby questioning the existence of either type of Cl^-/HCO_3^- exchange system in these cells (Trebilcock R, Lynch A and Best L, unpublished observations).

Whether Na^+/H^+ or Cl^-/HCO_3^- exchange is predominantly responsible for the dissipation of protons from the cytosol, the net effect of nutrient oxidation in islet cells would be the extrusion of H^+ from the cell into the extracellular medium. Malaisse and colleagues [22] have indeed demonstrated that glucose causes a concentration-related increase in output of H^+ from pancreatic islets, whilst no change in pHi was detected, thus providing evidence for one or more regulatory mechanisms in islet cells responsible for pHi homeostasis.

It should be emphasised that processes other than nutrient oxidation could influence islet cell pHi. For example, the H^+-coupled transport of nutrients or metabolites across the plasma membrane would be predicted to cause an intracellular acidification. Such a process could at least contribute towards the fall in pHi observed upon stimulating islets or HIT cells with α-ketoisocaproate [10, 11, 16], pyruvate and lactate [16]. In particular, the pHi response of HIT cells to pyruvate and lactate strongly resembles the effects of addition of weak acids, suggesting that these metabolites could be H^+-cotransported, or otherwise enter the islet cell in the undissociated form.

The acidification of islet and HIT cells following exposure to non-nutrient secretagogues such as glibenclamide [13, 23], tolbutamide [23], high K^+ [13, 16, 23], calcium ionophores [23] or Ba^{2+} [16] is unlikely to be the result of H^+ transport into the cell, but rather a consequence of increased cytosolic $[Ca^{2+}]$, a response common to the above treatments. It is well established that changes in $[Ca^{2+}]$ can influence pHi [24, 25], presumably owing to protons and calcium ions competing for common intracellular binding sites [25, 26].

Thus, the overall effect of a given agonist on islet cell pHi is likely to be determined by a number of factors including proton co-transport, oxidative metabolism, changes in cytosolic calcium concentration and activation of Na^+/H^+ and possibly Cl^-/HCO_3^- exchange. Such a multifactorial regulation could account for the diversity of and time-dependence of cytosolic pH responses to different stimuli.

Manipulation of pHi; consequences for islet cell activation

In attempting to investigate the role of cytosolic pH in the regulation of islet cell function, several studies have examined the consequences of pharmacological manipulation of pHi. Such manipulations can be achieved in a number of ways (Fig. 1). The inhibition of Na^+/H^+ exchange by amiloride results in an intracellular acidification [9, 13–17] and prevents recovery from an acid load [16], although it should also be noted that amiloride has been shown to inhibit other Na^+-dependent processes including Na^+/Ca^{2+} exchange [27], (Na^+, K^+)-ATPase activity and Na^+-coupled amino acid transport [28].

In all except one study [29], amiloride treatment of insulin-secreting cells resulted in an enhanced basal rate of insulin release [14–17, 30, 31] although, paradoxically, secretion evoked by a high concentration of glucose (16.7 mM) was suppressed [31]. In an attempt to rationalise these conflicting findings, we have shown recently that whilst glucose did indeed potentiate insulin release in the presence of

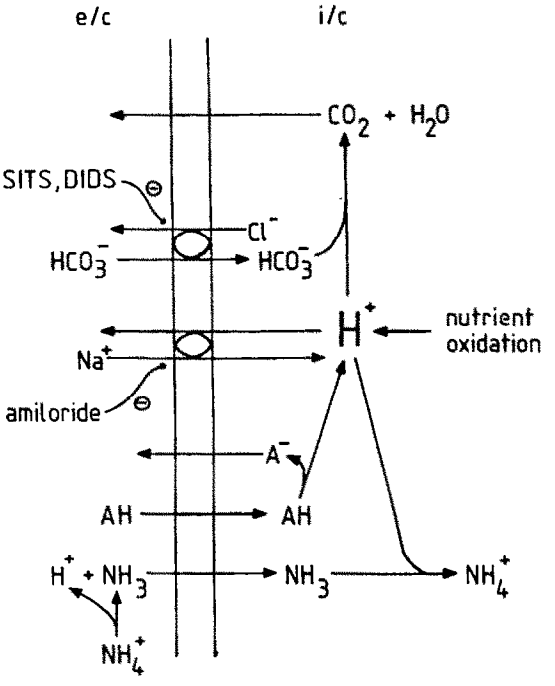

Fig. 1. Regulation and manipulation of islet cell pHi: possible roles of nutrient oxidation, Na^+/H^+ exchange and HCO_3^-/Cl^- exchange. Abbreviations: SITS, 4-acetamido-4'-isothiocyanatostilbene 2,2'-disulphonic acid; DIDS, 4.4'-diisothiocyano-2,2'-stilbene disulphonic acid; AH, weak acid; e/c, extracellular; and i/c, intracellular.

a submaximal glucose concentration, longer exposure to the drug inhibited secretion at high glucose concentrations (Best L and Yates AP, unpublished observations). Furthermore, amiloride was also found to impair glucose metabolism. It is likely that both of these effects are the result of intracellular acidification. The inhibition of glucose metabolism may explain the inhibitory effect of amiloride on insulin secretion, particularly at high glucose concentrations and in longer incubations.

The potentiation of secretion by amiloride appeared to require a threshold concentration of glucose, suggesting that intracellular acidification could not mimick the effect of glucose on the islet cell. The potentiation of insulin secretion by amiloride in the presence of threshold glucose concentrations is accompanied by depolarisation and enhanced electrical activity of β-cells [30–33] together with an extensive modification of ionic fluxes, namely inhibition of the rates of efflux of $^{45}Ca^{2+}$ [14, 15, 17] and $^{86}Rb^+$[15, 17] from pre-loaded islets. Essentially similar results were obtained by inhibition of Na^+/H^+ exchange by omitting Na^+ from the incubation medium [32, 34]. Conversely, glucose-induced insulin secretion [31, 35] and electrical activity [31, 36] are inhibited by monensin, an Na^+/H^+ exchanger.

Fewer data are available concerning the effects of manipulating Cl^-/HCO_3^- exchange in islet cells. Pace *et al.* [30–32] have shown that 4,4'diisothiocyano-2,2'-stilbene disulphonic acid (DIDS), an inhibitor of Cl^-/HCO_3^- exchange, potentiates glucose-induced electrical activity. Inhibition of this exchanger by omission of Cl^- or HCO_3^- or by 4-acetamido-4'-isothiocyanatostilbene 2,2'-disulphonic acid (SITS; [33]) or probenicid [31–33] had similar results. However, whilst the potentiation of electrical activity by DIDS was accompanied by enhanced insulin release under those conditions [31], it is well established that, in the absence of bicarbonate, the secretory response to glucose, in contrast to electrical activity, is suppressed [8, 37]. In an attempt to resolve this discrepancy, it may be worthwhile considering the possible consequences of bicarbonate omission from the incubation medium. On the one hand, one would predict that inhibition of Na^+-dependent HCO_3^-/Cl^- exchange by bicarbonate omission would render the cell less able to recover from intracellular acidification. However, the effect of rapid bicarbonate withdrawal (with a simultaneous reduction in pCO_2) is an intracellular alkalinisation [8], presumably as a result of the exit of CO_2 from the cell. Such a rise in pHi would be consistent with impaired secretory function and may well mask any additional effects caused by inhibiting HCO_3^-/Cl^- exchange, particularly in short-term experiments.

Alternative approaches for manipulating pHi include the use of weak acids and bases ([2], Fig. 1). The permeant weak acid sulfamerazine has been shown to cause islet cell depolarisation, to increase spike activity and to potentiate insulin release [38, 39]. In addition, the weak acids acetate and propionate, which cause an immediate, transient intracellular acidification of islet and HIT cells [9, 14–16], induce a transient potentiation of glucose-induced insulin release [15], an effect associated with reduced rates of efflux of $^{45}Ca^{2+}$ [14, 15] and $^{86}Rb^+$ [15]. A reduction in islet cell pHi by increasing pCO_2 is also accompanied by restricted outflow of these isotopes [40, 41].

In most cases, the use of a weak base to raise intracellular pH has been shown to impair islet cell function. Thus, treatment of islet and HIT cells with NH_4Cl raises pHi [9, 14–16, 42], inhibits glucose-induced insulin secretion [15, 31, 37], stimulates $^{45}Ca^{2+}$ and $^{86}Rb^+$ efflux [15], causes β-cell hyperpolarisation and suppresses electrical responses to nutrient secretagogues [31, 37, 41] whilst the weak base imidazole causes islet cell hyperpolarisation, inhibition of electrical activity and impaired insulin release [31, 36, 39]. The withdrawal of HCO_3^- from the incubation medium also results in intracellular alkalinisation, facilitation of $^{45}Ca^{2+}$ outflow and impaired glucose-induced insulin release [8]. In contrast to the above studies, most of which employed rat islets, Lindstrom and Sehlin [7, 43] have reported that raising pHi in mouse islets by treatment with NH_4Cl results in a potentiation of insulin secretion. Whether these discrepancies are solely a reflection of species differences remains to be established.

There are a number of mechanisms by which changes in pHi could alter islet cell electrical and secretory activity. The observations that intracellular acidification and alkalinisation are associated with reduced and increased rates, respectively, of efflux of $^{45}Ca^{2+}$ and $^{86}Rb^+$ suggest that protons can regulate the permeability of the plasma membrane to calcium and potassium. The modulation of $^{45}Ca^{2+}$ outflow by

pHi is largely dependent upon the presence of Na^+ in the medium [8, 15, 40], suggesting that protons inhibit the activity of the Na^+/Ca^{2+} exchange system [44]. Such an action would be expected to result in an increased cytosolic calcium concentration, which would be consistent with the potentiation of insulin secretion caused by lowering pHi. The reduction of $^{86}Rb^+$ efflux by intracellular acidification is likely to be the result of inhibition of the calcium-activated K^+ channel by protons [42, 45], which would again explain the depolarisation and potentiation of electrical and secretory activity accompanying intracellular acidification. The possibility that protons may exert additional actions upon islet cells cannot, of course, be ruled out.

Thus, there is little doubt that manipulations of pHi can influence ionic fluxes and hence electrical and secretory activity in insulin-secreting cells. However, the marked dependence of these effects upon the presence of glucose (or another nutrient) must question whether pHi acts as a coupling factor *per se* in nutrient-stimulated insulin release. The lack of a clear correlation between observed changes in pHi and secretory activity casts further doubt on this possibility.

Conclusions and perspectives

In common with a wide variety of mammalian cell types, pancreatic islet cells have a number of mechanisms for the regulation of pHi. It is likely that an ability to maintain intracellular pH homeostasis is of particular importance in the case of the β-cell, where nutrient-stimulated insulin release is intimately associated with the high capacity of the cell to oxidise those nutrients, a process resulting in the generation of acidic metabolites.

The operation of such regulatory mechanisms, which is apparent from measurements of pHi in nutrient-stimulated islet cells, makes it unlikely that intracellular acidification plays a major role in coupling nutrient oxidation to insulin release except, perhaps, for the initial phase of secretion. This is not to deny the possibility that other, non-nutrient secretagogues and pharmacological agents may exert their influence on islet cell activity, at least in part, by promoting intracellular acidification.

In the case of nutrients such as glucose, the predominant response of islet cell pHi to nutrient stimuli appears to be a gradual alkalinisation, presumably as a result of activation of a regulatory mechanism(s). Whether this rise in pHi plays any active or permissive role in the stimulus–secretion coupling process remains to be established. One possibility is that a rise in pHi produces an enhanced rate of nutrient oxidation, which might be conducive to greater secretory activity. A rise in pHi could also be linked to the process of insulin biosynthesis, DNA replication or islet cell growth, all of which are regulated by nutrients [46–48].

One aspect of pHi regulation which could be of considerable importance arises from the fact that the two principal systems responsible for intracellular pH homeostasis, namely Na^+/H^+ and HCO_3^-/Cl^- exchange (both Na^+-dependent and -independent), are electroneutral. Since the metabolism of glucose or another nutrient will result in the production of

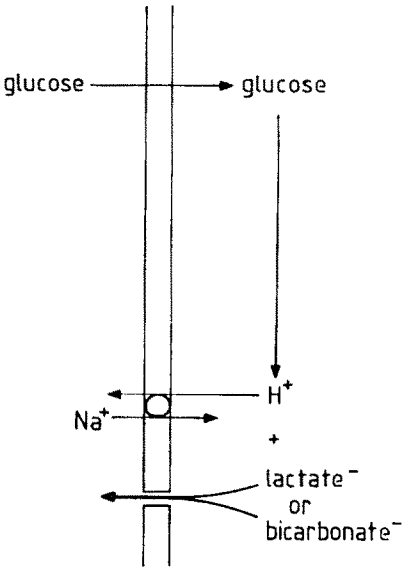

Fig. 2. A possible mechanism for islet cell depolarisation by the electrogenic efflux of lactate and/or bicarbonate, concomitant with electroneutral Na^+/H^+ (or (HCO_3^-/Cl^-) exchange.

H^+ together with a corresponding anion (lactate or bicarbonate), it seems likely that the electroneutral exchange of H^+ for Na^+ will be associated with the *electrogenic* exit of that anion from the cell (see Fig. 2). Although there is, as yet, no direct evidence for electrogenic pathways of anion efflux in islet cells, it has been pointed out that channels which conduct chloride could also theoretically conduct a number of other anionic species [49].

In addition, the equilibrium distribution of lactate in a number of tissues, including liver and muscle [50], is not uniform, i.e. the intracellular concentration of lactate is considerably lower than would be predicted if lactate distribution were determined solely by the pH gradient across the cell membrane. The simplest explanation of this finding is that a channel exists which conducts lactate (and possibly other anions) out of the cell under the influence of the cell membrane potential.

The loss of lactate or bicarbonate from the cell by such an electrogenic process would represent the net loss of negative charge from the inside of the cell. Such a mechanism could result in depolarisation of the cell membrane and cell activation. Indeed, certain cell types [51], including insulin-secreting HIT-T15 cells [52], are depolarised by lactate-containing medium, an effect which could involve the electrogenic efflux of lactate anions from the cell, with concomitant Na^+/H^- exchange [52, 53]. Whether such a mechanism does play a role in coupling glucose metabolism to islet cell depolarisation and insulin release remains to be established.

REFERENCES

1. Roos A and Boron WF, Intracellular pH. *Physiol Rev* **61**: 296–434, 1981.
2. Boron WF, Transport of H^+ and of ionic weak acids and bases. *J Membr Biol* **72**: 1–16, 1983.

3. Busa WB, Mechanisms and consequences of pH-mediated cell regulation. *Annu Rev Physiol* **48**: 389–402, 1986.
4. Madshus IH, Regulation of intracellular pH in eukaryotic cells. *Biochem J* **250**: 1–8, 1988.
5. Frelin C, Vigne P, Ladoux A and Lazdunski M, The regulation of the intracellular pH in cells from vertebrates. *Eur J Biochem* **174**: 3–14, 1988.
6. Rosengurt E, Early signals in the microgenic response. *Science* **234**: 161–166, 1986.
7. Lindstrom P and Sehlin J, Effect of glucose on the intracellular pH of pancreatic islet cells. *Biochem J* **218**: 887–892, 1984.
8. Lebrun P, Malaisse WJ and Herchuelz A, Effect of the absence of bicarbonate upon intracellular pH and calcium fluxes in pancreatic islet cells. *Biochim Biophys Acta* **721**: 357–365, 1982.
9. Pace CS and Tarvin JT, Amiloride-sensitive regulation of intracellular pH in β-cells: Activation by glucose. *Metabolism* **35**: 176–181, 1986.
10. Hutton JC, Sener A and Malaisse WJ, The metabolism of 4-methyl-2-oxopentanoate in rat pancreatic islets. *Biochem J* **184**: 291–301, 1979.
11. Hutton JC, Sener A, Herchuelz A, Valverde I, Boschero AC and Malaisse WJ, The stimulus-secretion coupling of glucose-induced insulin release. XLII. Effects of extracellular pH on insulin release: Their dependency on nutrient concentration. *Horm Metab Res* **12**: 294–299, 1980.
12. Deleers M, Lebrun P and Malaisse WJ, Increase in HCO_3^- influx and cellular pH in glucose-stimulated pancreatic islets. *FEBS Lett* **154**: 97–100, 1983.
13. Deleers M, Lebrun P and Malaisse WJ, Nutrient-induced changes in the pH of pancreatic islet cells. *Horm Metab Res* **17**: 391–395, 1985.
14. Best L, Bone EA, Meats JE and Tomlinson S, Is intracellular pH a coupling factor in nutrient-stimulated pancreatic islets? *J Mol Endocr* **1**: 33–38, 1988.
15. Best L, Yates AP, Gordon C and Tomlinson S, Modulation by cytosolic pH of calcium and rubidium fluxes in rat pancreatic islets. *Biochem Pharmacol* **37**: 4611–4615, 1988.
16. Lynch AM, Meats JE, Best L and Tomlinson S, Effects of nutrient and non-nutrient stimuli on cytosolic pH in cultured insulinoma (HIT-T15) cells. *Biochim Biophys Acta* **1012**: 166–170, 1989.
17. Lebrun P, van Ganse E, Juvent M, Deleers M and Herchuelz A, Na^+-H^+ exchange in the process of glucose-induced insulin release from the pancreatic β-cell. Effects of amiloride on $^{86}Rb^+$, $^{45}Ca^{++}$ fluxes and insulin release. *Biochim Biophys Acta* **886**: 448–456, 1986.
18. Aaronson PS, Nee J and Suhm MA, Modifier role of internal H^+ in activating the Na^+/H^+ exchanger in renal microvillus membranes. *Nature* **299**: 161–163, 1982.
19. Grinstein S and Rothstein A, Mechanisms of regulation of the Na^+/H^+ exchanger. *J Membr Biol* **90**: 1–12, 1986.
20. Best L and Malaisse WJ, Phospholipids and islet function. *Diabetologia* **25**: 299–305, 1983.
21. Prentki M and Marschinski FM, Ca^{++}, cAMP and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol Rev* **67**: 1185–1248, 1987.
22. Malaisse WJ, Hutton JC, Kawazu S, Herschuelz A, Valverde I and Sener A, The stimulus–secretion coupling of glucose-induced insulin release. XXXV. Links between metabolic and cationic events. *Diabetologia* **16**: 331–341, 1979.
23. Deleers M, Lebrun P and Malaisse WJ, Effects of cations, ionophores and hypoglycemic sulfonylureas on the fluorescence of fluorescein-labelled pancreatic islets. *Res Commun Chem Pathol Pharmacol* **44**: 83–92, 1984.
24. Meech RW and Thomas RC, The effect of calcium injection on the intracellular sodium and pH of snail neurones. *J Physiol (Lond)* **265**: 867–879, 1977.
25. Ives HE and Daniel TO, Interrelationship between growth factor-induced pH changes and intracellular Ca^{++}. *Proc Natl Acad Sci USA* **84**: 1950–1954, 1987.
26. Hellman B, The significance of calcium for glucose stimulation of insulin release. *Endocrinology* **97**: 392–398, 1975.
27. Siegl PKS, Cragoe EJ, Trumble MJ and Kaczorowski GJ, Inhibition of Na^+/Ca^{++} exchange in membrane vesicle and papillary muscle preparations from guinea pig heart by analogues of amiloride. *Proc Natl Acad Sci USA* **81**: 3238–3242, 1984.
28. Renner EL, Lake JR, Cragoe EJ Jr and Scharschmidt BF, Amiloride and amiloride analogues inhibit Na^+/K^+-transporting ATPase and Na^+-coupled alanine transport in rat hepatocytes. *Biochim Biophys Acta* **938**: 386–394, 1988.
29. Bidem TJ, Janjic D and Wollheim CB, Sodium requirement for insulin release: Putative role in the regulation of intracellular pH. *Am J Physiol* **250**: C207–C213, 1986.
30. Pace CS, Role of pH as a transduction device in triggering electrical and secretory responses in islet B cells. *Fedn Proc* **43**: 2379–2384, 1984.
31. Pace CS, Tarvin JT and Smith JS, Stimulus–secretion coupling in β-cells: Modulation by pH. *Am J Physiol* **244**: E3–E18, 1983.
32. Pace CS and Tarvin JT, pH modulation of glucose-induced electrical activity in B-cells: Involvement of Na/H and HCO_3/Cl antiporters. *J Membr Biol* **73**: 39–49, 1983.
33. Eddlestone GT and Beigelman PM, Pancreatic β-cell electrical activity: The role of anions and the control of pH. *Am J Physiol* **244**: C188–C197, 1983.
34. Lebrun P, Plasman PO and Herchuelz A, Effect of extracellular sodium removal upon ^{86}Rb outflow from pancreatic islet cells. *Biochim Biophys Acta* **1011**: 6–11, 1989.
35. Kanatsuka A, Makino H, Sakurada M, Hashimoto N, Yamaguchi T and Yoshida S, Biphasic insulin response to high glucose and a role for protons and calcium. *Endocrinology* **120**: 77–82, 1987.
36. Tarvin JT, Sachs G and Pace CS, Glucose-induced electrical activity in the pancreatic β-cell: Modulation by pH. *Am J Physiol* **241**: C264–C268, 1981.
37. Henquin JC and Lambert AE, Extracellular bicarbonate ions and insulin secretion. *Biochim Biophys Acta* **381**: 437–442, 1975.
38. Pace CS and Goldsmith KT, Effect of substitution of a permeant weak acid for the permissive role of glucose in amino acid-induced electrical activity in β-cells. *Endocrinology* **119**: 2433–2438, 1986.
39. Smith JS and Pace CS, Modification of glucose-induced insulin release by alteration of pH. *Diabetes* **32**: 61–66, 1983.
40. Carpinelli AR, Sener A, Herchuelz A and Malaisse WJ, The stimulus–secretion coupling of glucose-induced insulin release. XLI. Effect of intracellular acidification upon calcium efflux from islet cells. *Metabolism* **29**: 540–545, 1980.
41. Carpinelli AR and Malaisse WJ, Regulation of $^{86}Rb^+$ outflow from pancreatic islets. II. Effect of changes in extracellular and intracellular pH. *Diabete Metab* **6**: 193–198, 1980.
42. Rosario LM and Rojas E, Modulation of K^+ conductance by intracellular pH in pancreatic β-cells. *FEBS Lett* **200**: 203–209, 1986.
43. Lindstrom P and Sehlin J, Effect of intracellular alkalinisation on pancreatic islet calcium uptake and insulin secretion. *Biochem J* **239**: 199–204, 1986.
44. Malaisse WJ, Herchuelz A and Sener A, The possible significance of intracellular pH in insulin release. *Life Sci* **26**: 1367–1371, 1980.

45. Cook DL, Musatoshi I and Fujimoto WY, Lowering of pHi inhibits Ca^{++}-activated channels in pancreatic β-cells. *Nature* **311**: 269–271, 1984.
46. Lin BJ and Haist RE, Insulin biosynthesis: Effects of carbohydrates and related compounds. *Can J Physiol Pharmacol* **47**: 791–801, 1969.
47. Nielsen JH, Growth and function of the pancreatic β-cell *in vitro*: Effects of glucose, hormones and serum factors on mouse, rat and human pancreatic islets in organ culture. *Acta Endocrol (Copenh)* **108** (Suppl 266): 1–39, 1985.
48. Swenne I, Glucose-stimulated DNA replication of the pancreatic islets during the development of the rat foetus: Effects of nutrients, growth hormone and T3. *Diabetes* **34**: 803–809, 1985.
49. Wright EM and Diamond JM, Anion selectivity in biological systems. *Physiol Rev* **57**: 109–156, 1977.
50. Edlund GL and Halestrap AP, The kinetics of transport of lactate and pyruvate into rat hepatocytes. *Biochem J* **249**: 117–126, 1988.
51. Bashford CL and Pasternak CA, Plasma membrane potential of some animal cells is generated by ion pumping, not by ion gradients. *Trends Biochem Sci* **11**: 113–116, 1986.
52. Meats JE, Tuersley MD, Best L, Lynch AM and Tomlinson S, Lactate alters plasma membrane potential, increases the concentration of cytosolic Ca^{++} and stimulates the secretion of insulin by the hamster B-cell line HIT-T15. *J Mol Endocr* **3**: 121–128, 1989.
53. Best L, Yates AP, Meats JE and Tomlinson S, Effects of lactate on pancreatic islets: Lactate efflux as a possible determinant of islet cell depolarisation by glucose. *Biochem J* **259**: 507–511, 1989.