Effect of intracellular alkalinization on pancreatic islet calcium uptake and insulin secretion

Per LINDSTRÖM and Janove SEHLIN

Department of Histology and Cell Biology, University of Umeå, S-901 87 Umeå 6, Sweden

Microdissected β -cell-rich pancreatic islets of *ob/ob* mice were used in studies of the relationship between intracellular pH (pH_i) and $^{45}\text{Ca}^{2+}$ uptake and insulin release. Stepwise increases in extracellular pH (pH_o) from 6.80 to 8.00 resulted in a parallel, although less pronounced, elevation of pH_i from 7.24 to 7.69. Experimental conditions that alkalinize the islet cell interior, i.e. addition of 5 mM- NH_4^+ , sudden withdrawal of extracellular bicarbonate buffer or increase in pH_o , induced insulin secretion in the absence of other types of secretory stimulation (1 mM-D-glucose). Intracellular acidification by lowering pH_o below 7.40 or sudden addition of bicarbonate buffer did not induce insulin secretion. The removal of extracellular bicarbonate buffer, increase in pH_o from 7.40 to 8.00, or the addition of 5 mM-L-5-hydroxytryptophan or 5 mM- NH_4^+ , which all alkalinize the islet cells and induce insulin secretion, also increased the La^{3+} -non-displaceable $^{45}\text{Ca}^{2+}$ uptake in the presence of 1 mM-D-glucose. The results suggest that intracellular alkalinization in β -cells can trigger insulin secretion. Taken together with the fact that D-glucose increases pH_i in the islet cells, the results also point to the possibility that alkalinization may be a link in the stimulus–secretion coupling sequence in β -cells.

INTRODUCTION

The intracellular pH (pH_i) has an important regulatory role in many cell types (Roos & Boron, 1981; Nuccitelli & Deamer, 1982). It has been inferred from indirect evidence that intracellular acidification may be a link between glucose metabolism in the pancreatic β -cells and later steps in the stimulus–secretion coupling sequence (Malaisse *et al.*, 1980; Pace *et al.*, 1983). However, attempts to measure pH_i directly in β -cells stimulated by glucose have either failed to show any change (Hellman *et al.*, 1972; Malaisse *et al.*, 1979; Lebrun *et al.*, 1982) or indicated an intracellular alkalinization (Deleers *et al.*, 1983; Lindström & Sehlín, 1984). It has been argued that the alkalinization might be the result of a cellular overcompensation of a primary acidification (Deleers *et al.*, 1983). In attempts to elucidate further the role of β -cell pH_i in insulin secretion, we have measured the effects of modified pH_i on insulin release. There are several ways to change pH_i acutely in cells. Studies on model cells have shown that exposure to NH_4^+ , elevated extracellular pH or sudden removal of $\text{HCO}_3^-/\text{CO}_2$ buffer increase the pH_i , whereas acute addition of $\text{HCO}_3^-/\text{CO}_2$ buffer or extracellular acidification decrease the pH_i (Boron, 1983). Although each of these treatments may also affect islet-cell parameters other than pH_i , a study comparing the effects of several of these treatments might be conclusive in indicating whether intracellular alkalinization or acidification is associated with the induction of insulin secretion. The aim of the present study was thus to investigate how experimentally induced intracellular alkalinization or acidification in the exceptionally β -cell-rich pancreatic islets of *ob/ob* mice affects insulin secretion. To analyse whether change in pH_i can produce secretory signals through the traditional stimulus–secretion coupling sequence, the islet-cell $^{45}\text{Ca}^{2+}$ uptake was monitored.

MATERIALS AND METHODS

Chemicals

[^{14}C]DMO and $^{45}\text{CaCl}_2$ were from Amersham International, Amersham, Bucks., U.K. L-[1- ^3H (n)]Glucose was from NEN Research Products, Dreieich, Germany. Hepes and L-5-hydroxytryptophan were from Sigma Chemical Co., St. Louis, MO, U.S.A. Bovine serum albumin (fraction V) was from Miles Laboratories, Stoke Poges, Bucks., U.K. Other chemicals were commercially available reagents of analytical grade. Double-quartz-distilled water was used throughout.

Animals

Pancreatic islets were microdissected (Hellerström, 1964) from adult non-inbred *ob/ob* mice (Umeå *ob/ob*). Islets from these animals are large and contain an unusually high proportion of β cells (> 90%; Hellman, 1965). Although the mice from this stock are metabolically abnormal, with a mild hyperglycaemia, which may be due to a peripheral insulin resistance and hyperphagia (Stauffer *et al.*, 1967; Stauffer & Renold, 1969), their islets show a normal regulation of insulin secretion *in vitro* (Hahn *et al.*, 1974; Hellman *et al.*, 1974; Idahl *et al.*, 1976).

Incubation media

The experiments were performed with either of two different basal media. Krebs–Ringer bicarbonate–Hepes medium (KRBH) was gassed with 5% CO_2 in air and had the following composition: 115 mM-NaCl, 4.7 mM-KCl, 2.56 mM- CaCl_2 , 1.2 mM- KH_2PO_4 , 1.2 mM- MgSO_4 , 25 mM- NaHCO_3 and 20 mM-Hepes, adjusted with NaOH to pH 7.40. From the other Krebs–Ringer Hepes medium (KRH), the bicarbonate was omitted, only Hepes was used as buffer, and the gas phase was ambient

Abbreviations used: pH_i , intracellular pH; pH_o , extracellular pH; [^{14}C]DMO, 5,5-dimethyl[2- ^{14}C]oxazolidine-2,4-dione.

Fig. 1. Effects of changes in extracellular pH on the intracellular pH of islet cells

Isolated pancreatic islets were initially exposed to a preliminary incubation for 30 min in KRH medium containing 1 mM-D-glucose and buffered to 7.40. Then they were incubated for 7 min in the same type of medium containing 1 mM-D-glucose, 89 μ M-[14 C]DMO (2 TBq/mol), and 0.9 μ M-L-[3 H]glucose (400 TBq/mol), and with the pH adjusted to different values. The data on the uptake of [14 C]DMO in excess of the extracellular (L-glucose) space have been calculated in terms of pH_i by using the equation of Waddell & Butler (1959). Results are expressed as mean $\pm$ S.E.M. (bars) for 5–12 experiments.

air. The composition of this medium was: 130 mM-NaCl, 4.7 mM-KCl, 2.56 mM- $CaCl_2$, 1.2 mM- KH_2PO_4 , 1.2 mM- $MgSO_4$ and 20 mM-Hepes; pH was adjusted with NaOH to 7.40. Both basal media were supplemented with 1 mM-D-glucose and 1 mg of bovine serum albumin/ml.

Experimental procedures

Insulin secretion was measured either in static incubations (Lindström & Sehlin, 1984) or in a perfusion system (Asplund *et al.*, 1979; Lindström & Sehlin, 1982). Briefly, batches of 3 (static incubations) or

about 30 islets (perifusions) were preincubated for 30 min at 37 °C in 1 ml of basal medium. In static incubations, they were then incubated for 60 min in 300 μ l of incubation medium as specified in the legend to Fig. 2. In perfusions, the islets were placed in a perfusion chamber and medium was pumped at a rate of 1 ml/min. The time required for a new medium to reach the chamber was 50 s. Correction for this lag period has been made in Fig. 3.

The intracellular pH was determined by using [14 C]DMO as a probe and L-[3 H]glucose as an extracellular-space marker, by a previously described procedure (Lindström & Sehlin, 1984). After preincubation as above, batches of five islets were transferred to new vials and incubated for 7 min at 37 °C in 200 μ l of medium. Control experiments have shown that [14 C]DMO equilibrates in the islet cells within 7 min (Lindström & Sehlin, 1984). The short incubation time was chosen because changes in pH_i in model cells have been shown to be transient (see Boron, 1983). To be able to compare the effects on pH_i with effects on insulin release over the first few minutes of treatment, the dynamics of insulin release were studied in perfusion (see above). The islet-cell uptake of [14 C]DMO in excess of the extracellular space was calculated in relation to the islet dry weight, as assessed by freeze-drying and weighing on a quartz-fibre balance. The pH_i was calculated from the [14 C]DMO-uptake values corrected for [14 C]DMO in the L-glucose (extracellular) space by calculating the intracellular [14 C]DMO concentration. This was done by assuming an intracellular water volume of 1.2 litres/kg dry wt. (Hellman *et al.*, 1971a).

Measurements of $^{45}Ca^{2+}$ uptake with the La^{3+} -wash technique were performed essentially as described by Hellman *et al.* (1976), except that the temperature during washing was 0 °C (Hellman, 1978). Incubated pancreatic islets were freeze-dried overnight (0.1 Pa, -40 °C), weighed on a quartz-fibre balance, and their radioactive contents were analysed by liquid-scintillation spectrometry.

RESULTS

Intracellular pH

Fig. 1 shows how changes in extracellular pH affected the pH_i . If the curve is approximated to a linear function

Table 1. Effect of 4 mM-L-5-hydroxytryptophan or the omission of bicarbonate buffer on islet intracellular pH in the presence of 1 mM-D-glucose

Isolated mouse pancreatic islets were initially preincubated for 30 min (period 1) in basal medium containing 1 mM-D-glucose. Then they were incubated for 7 min (period 2) in media with the same glucose concentration and modifications as described in the Figure and supplemented with 89 μ M-[14 C]DMO (2 TBq/mol) and 0.9 μ M-L-[3 H]glucose (400 TBq/mol). Results are expressed as mean $\pm$ S.E.M. for the numbers of experiments in parentheses: * P < 0.02 and ** P < 0.005 for difference from respective control, by using Student's *t* test for paired data.

Incubation medium		[14 C]DMO uptake (μ mol/kg dry wt. of islets)	Calculated pH_i
Period 1 (30 min)	Period 2 (7 min)		
KRBH	KRBH (control)	68.2 $\pm$ 5.4 (10)	7.18 $\pm$ 0.04 (10)**
KRBH	KRH (lacking HCO_3^-/CO_2)	92.8 $\pm$ 8.2 (10)	7.32 $\pm$ 0.04 (10)**
KRH	KRH (control)	119.2 $\pm$ 12.0 (8)	7.43 $\pm$ 0.05 (8)
KRH	KRH + 4 mM-L-5-hydroxytryptophan	157.7 $\pm$ 21.9 (8)	7.55 $\pm$ 0.06 (8)*

Fig. 2. Effects of changes in extracellular pH on insulin release

Isolated pancreatic islets were preincubated as in Fig. 1 and then incubated for 60 min in KRH medium containing 1 mM-D-glucose and with the pH adjusted to different values. The data are presented as means $\pm$ S.E.M. (bars) for 9–10 experiments: * $P < 0.05$ for difference from the mean insulin release at pH 6.8–7.6 in each experiment by using the Student's t test for paired data. For comparison, in parallel experimental groups, the insulin secretion in the presence of 20 mM-D-glucose at pH 7.40 was 2.27 ± 0.32 ($n = 10$).

the slope becomes 0.36, supporting the view that the islet cells have a considerable buffering capacity (Hutton *et al.*, 1980). Nevertheless, change in pH_o from 6.80 to 8.00 resulted in a parallel shift in apparent pH_i from 7.24 to 7.69. The data also indicate that the transmembrane pH gradient is zero at a pH_o of just below 7.40. At lower pH_o there seems to be an inwardly directed chemical gradient for protons, and at higher pH_o this gradient is reversed.

Previous work has shown that sudden addition of bicarbonate buffer to *ob/ob*-mouse islets results in intracellular acidification (Lindström & Sehlin, 1984). The data in Table 1 indicate that this is a reversible phenomenon, i.e. a sudden change to a medium deficient in bicarbonate buffer resulted in intracellular alkalization of the islet cells. We have previously demonstrated that addition of 4 mM-L-5-hydroxytryptophan increases the pH_i in islet cells in the presence of 20 mM-D-glucose (Lindström & Sehlin, 1984) and potentiates glucose-induced insulin secretion (Lindström & Sehlin, 1983), as well as stimulating basal release at 3 mM-D-glucose (P. Lindström & J. Sehlin, unpublished work). Table 1 shows that L-5-hydroxytryptophan at 4 mM increased the pH_i in the presence of only 1 mM-D-glucose.

Insulin secretion

Fig. 2 shows insulin secretion in static incubations during 60 min in the presence of 1 mM-D-glucose and with the pH_o adjusted to different values. The hormone

release remained the same as the pH_o was raised step-wise from 6.80 to 7.60. However, further elevation of pH_o to 7.80 and 8.00 induced an increase in the insulin output by 75% and 188% respectively.

To test further whether intracellular alkalization of the β -cells can induce insulin secretion, we measured the dynamics of secretion in a perfusion system, while modifying the pH_i in several different ways. Fig. 3(a) shows for comparison the biphasic insulin release induced by 20 mM-D-glucose in *ob/ob*-mouse islets. To facilitate comparisons of the different test situations, all results have been expressed in relation to the basal insulin secretion in each perfusion. Fig. 3(a) thus shows that 20 mM-D-glucose induced a 15–20-fold increment in insulin release. A sudden increase in extracellular pH from 7.40 to 8.00 (Fig. 3b) induced insulin release ($P < 0.02$ for increase above basal during 0–15 min), although the effect was smaller and the development of it was slower as compared with glucose-induced secretion. When the pH_o was changed back to 7.40, the stimulated insulin release rapidly dropped towards the low basal value.

The addition of 5 mM-NH₄⁺ (Fig. 3c) induced a prompt, although rather modest, increment above the basal insulin release at 1 mM-D-glucose ($P < 0.005$ for increase above basal during 0–15 min). The effect was completely reversible on removal of NH₄⁺.

Intracellular alkalization by a sudden change from a Hepes/bicarbonate-buffered medium to one lacking bicarbonate buffer (Fig. 3d) induced a rapidly developing and transient insulin release ($P < 0.05$ for increase above basal during 0–15 min). After 15 min the secretory rate was not different from the basal release. Re-addition of bicarbonate buffer, which leads to intracellular acidification of islet cells (Lindström & Sehlin, 1984), did not induce insulin secretion.

Ca²⁺ uptake

Insulin secretion induced by most stimuli is associated with an increased islet uptake of ⁴⁵Ca²⁺ (Wollheim & Sharp, 1981). To see whether increased pH_i in the β -cells can induce secretion by signalling through the ordinary stimulus-secretion coupling sequence, we tested how intracellular alkalization affects the islet cell ⁴⁵Ca²⁺ uptake. As shown in Fig. 4, step-wise increases in the extracellular pH from 6.80 to 8.00 were accompanied by increases in ⁴⁵Ca²⁺ uptake similar to the effect on pH_i.

Table 2 shows how three other procedures that increase pH_i affected the ⁴⁵Ca²⁺ uptake. The addition of 5 mM-NH₄⁺, the addition of 5 mM-5-hydroxytryptophan, and the sudden change to a medium deficient in HCO₃⁻/CO₂ buffer all increased the La³⁺-non-displaceable ⁴⁵Ca²⁺ uptake. In contrast, the sudden addition of HCO₃⁻/CO₂ buffer, which decreases pH_i, decreased the ⁴⁵Ca²⁺ uptake in the presence of 1 mM-glucose.

DISCUSSION

Previous experiments on β -cell-rich *ob/ob*-mouse islets revealed that acute stimulation of the pancreatic β -cells by D-glucose is accompanied by an intracellular alkalization (Lindström & Sehlin, 1984). The effect was half-maximal at about 4 mM and the maximum, reached at about 10 mM-D-glucose, corresponded to 0.15 pH unit. The glucose-induced alkalization was of approximately the same magnitude regardless of the presence or

Fig. 3. Effects of 20 mM-glucose, increased extracellular pH (8.00), 5 mM- NH_4Cl , or omission of bicarbonate buffer on the insulin release

In panels (a)–(c), pancreatic islets were preincubated as in Fig. 1 and then pre-perfused for 45 min in KRH medium containing 1 mM-D-glucose and at pH 7.40. After 45 min (t_0), the perfusion medium was changed as indicated in the Figure and, after another 30 min of perfusion, the basal medium was re-introduced. In panel (d), the pre-perfusion medium was gassed KRBH (see the Materials and

Fig. 4. Effects of changes in extracellular pH on the $^{45}Ca^{2+}$ uptake

Islets were preincubated as described in Fig. 1 and then incubated for 7 min in KRH medium containing 1 mM-D-glucose and labelled with 2.56 mM- $^{45}Ca^{2+}$ (0.3 TBq/mol) and with the pH adjusted to different values. Results are expressed as mean $\pm$ S.E.M. (bars) for 7–8 experiments.

absence of bicarbonate buffer (Lindström & Sehlin, 1984). This is in contrast with published data on rat islets indicating that D-glucose increases pH_i in the presence of bicarbonate buffer, but decreases islet pH_i in the absence of bicarbonate (Deleers *et al.*, 1985). We have no ready explanation for this discrepancy. However, it would seem that interpretation of the fluorimetric measurements on whole rat islets in terms of β -cell function may be complicated by the fact that such islets contain as much as 30–40% endocrine non- β -cells (Hellman, 1959), which have a preferentially peripheral location in the islets (cf. Orci, 1982) and therefore may quench or otherwise interfere with any fluorescent signal emanating from the centrally located β -cells.

The precise mechanism underlying the glucose-induced alkalinization is unclear, but can obviously comprise both perturbations in islet intermediary metabolism (Gevers, 1977) and transmembrane redistribution of protons (Boron, 1983). The available data raise the question whether alkalinization of the β -cells has a direct role in the regulation of secretion, or whether it is a

methods section), which was changed to KRH medium at t_0 and then back to KRBH after another 30 min. The results (large filled circles) are expressed as percentages of the mean basal insulin release during the last 5 min (–5 min to t_0) of pre-perfusion in each experiment. These mean rates of basal release were (ng/min per μ g islet dry wt.): (a) 1.4 ± 0.2 , (b) 3.0 ± 0.8 , (c) 3.2 ± 0.8 and (d) 1.9 ± 0.3 . The small filled circles in the lower part of panels (b)–(d) represent control experiments with islets pre-perfused and perfused with KRH medium at pH 7.40 containing 1 mM-D-glucose throughout (mean basal insulin release from –5 min to t_0 : 1.6 ± 0.2 ng/min per μ g islet dry wt.). Results are expressed as mean $\pm$ S.E.M. (bars) for the numbers of experiments (n) indicated in the Figure.

Table 2. Effect of 5 mM-NH₄Cl, 5 mM-L-5-hydroxytryptophan, or the addition or omission of bicarbonate buffer on the islet ⁴⁵Ca²⁺ uptake in the presence of 1 mM-D-glucose

Pancreatic islets were preincubated for 30 min (period 1) in basal medium containing 1 mM-D-glucose. Then they were incubated for 7 min (period 2) in media with the same glucose concentration and modifications as indicated in the Table and containing 2.56 mM-⁴⁵Ca²⁺ (0.3 TBq/mol). Results are expressed as mean $\pm$ S.E.M. for the numbers of experiments in parentheses: **P* < 0.05 and ***P* < 0.01 for difference from control; †*P* < 0.025 for difference from 2.07 $\pm$ 0.12. The statistical analyses were made by using Student's *t* test for paired data.

Incubation medium		
Period 1 (30 min)	Period 2 (7 min)	⁴⁵ Ca ²⁺ uptake (mmol/kg dry wt. of islets)
KRH	KRH (control)	2.99 $\pm$ 0.17 (24)
KRH	KRH + 5 mM-NH ₄ Cl	3.43 $\pm$ 0.25 (17)*
KRH	KRH + 5 mM-L-5-hydroxytryptophan	3.37 $\pm$ 0.20 (17)**
KRH	KRBH (containing HCO ₃ ⁻ /CO ₂)	2.07 $\pm$ 0.17 (7)*
KRBH	KRBH	2.07 $\pm$ 0.12 (8)**
KRBH	KRH (lacking HCO ₃ ⁻ /CO ₂)	3.59 $\pm$ 0.34 (25)*†

phenomenon more loosely associated with β -cell activity. In attempts to resolve this, we have studied whether experimentally induced changes in islet-cell pH_i can trigger insulin release in the absence of other types of secretory stimulation. Several ways to manipulate the pH_i in model cells have been developed (for review, see Boron, 1983). We have characterized the effects of NH₄⁺ (Lindström & Sehlin, 1984), extracellular HCO₃⁻/CO₂ buffer (Lindström & Sehlin, 1984; the present work), pH_o (the present work), and L-5-hydroxytryptophan (Lindström & Sehlin, 1984; the present work). These studies show that the addition of 5 mM-NH₄⁺ or 5 mM-L-5-hydroxytryptophan or the sudden removal of HCO₃⁻/CO₂ buffer or the increase in pH_o all result in alkalinization of the islet cells, whereas the addition of HCO₃⁻/CO₂ buffer or the decrease in pH_o leads to acidification. Naturally, we cannot assume that these treatments act specifically on pH_i. We should rather assume that they may affect a variety of systems in the β -cells and thus produce a complex picture. Nevertheless, since all treatments used to alkalinize the islet cells also induce insulin release, whereas acidification of the cells does not, our results point to a possible causal relationship between β -cell alkalinization and insulin release. The fact that there seemed to be no clear-cut proportionality between the degree of alkalinization and the rate of insulin secretion does not rule out a causal relationship. Part of the discrepancy may be due to non-specific effects of the treatments on islet functions. Another aspect that should be kept in mind is that several different cellular mechanisms may be important for a full secretory response to glucose stimulation. Increased pH_i may represent only one part of the whole triggering system, thus giving less than maximum secretory response.

Several previous studies concerning the effects of modified pH_i on glucose-induced insulin release have been reported (for review, see Malaisse *et al.*, 1980; Smith & Pace, 1983). However, only few attempts have been made to see whether a change in intracellular pH can initiate insulin secretion from the basal value. Smith & Pace (1983) reported that neither extracellular acidification (pH_o 7.0) or alkalinization (pH_o 7.8) nor the addition of 5 mM-sulfamerazine or 10 mM-imidazole had any effect on basal insulin release in isolated rat islets. Hutton *et al.* (1980) did not find any significant change in basal insulin release in rat islets at either low (6.4–7.0) or high (7.8–8.0) pH_o. On the other hand, Henquin (1981) observed that the basal insulin release in mouse islets in the absence of glucose was decreased at a low pH_o (6.5) and increased at high pH_o (7.8) when compared with a pH_o of 7.40. This stimulatory effect is consistent with our present results on *ob/ob*-mouse islets. However, it cannot be decided from these studies whether there is a real species difference between rats and mice or whether other experimental differences explain the contradictory results.

It is well established that stimulation of insulin secretion by D-glucose is associated with β -cell uptake of Ca²⁺ ions (Hellman *et al.*, 1971b; Wollheim & Sharp, 1981). This is considered to be a distal step in the stimulus–secretion coupling sequence. Our present data show that intracellular alkalinization of the islet cells produced by four different techniques, i.e. increased pH_o, addition of 5 mM-NH₄⁺ or 5 mM-L-5-hydroxytryptophan, or the sudden omission of extracellular HCO₃⁻/CO₂ buffer, all were paralleled by an increased ⁴⁵Ca²⁺ uptake. In similar experiments, Henquin (1981) found that high pH_o increases ⁴⁵Ca²⁺ in the absence of D-glucose. We do not know whether increased pH_i stimulates the ⁴⁵Ca²⁺ uptake directly or whether it activates more proximal steps in the stimulus–secretion coupling sequence, eventually leading to increased Ca²⁺ uptake. However, taken together the present results strengthen the idea that intracellular alkalinization of the β -cell induces insulin secretion by activating the stimulus–secretion coupling. Since stimulation of insulin release by D-glucose in the islet preparation used here is paralleled by an acute alkalinization of the islet cells (Lindström & Sehlin, 1984), it is tempting to conclude that increased β -cell pH_i is indeed a natural link in the stimulus–secretion coupling sequence.

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