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Regulation of Na^+/Ca^{2+} exchange in the rat pancreatic B cell

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Na^+/Ca^{2+} exchange in the B cell was recently characterized by measuring intracellular-Na^+-dependent $^{45}Ca^{2+}$ uptake in isolated rat pancreatic islet cells. The aim of the present study was to investigate the regulation of this process. Extracellular pH (pH_o) and intracellular pH (pH_i) markedly affected Na^+/Ca^{2+} exchange. A fall of 0.04 unit in pH_i decreased the exchange by 45%, whereas a rise of 0.13 unit increased the uptake by 70%. Mitochondrial poisons (oligomycin, antimycin A and 2,4-dinitrophenol) inhibited reverse Na^+/Ca^{2+} exchange by about 25–50%. The exchanger displayed a low Q_{10} (temperature coefficient), indicating that it is only indirectly dependent on metabolic energy. The phorbol ester phorbol 12-myristate 13-acetate did not affect Na^+/Ca^{2+} exchange. Likewise, lowering the extracellular K^+ concentration did not inhibit $^{45}Ca^{2+}$ uptake. In conclusion, the pH_i and the metabolic state of the cell may represent important modulatory signals by which insulin secretagogues such as glucose could regulate reverse Na^+/Ca^{2+} exchange in the B cell. The process does not appear to co-transport K^+ nor to be influenced by protein kinase C.

INTRODUCTION

Na^+/Ca^{2+} exchange represents an important modulator of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in several types of cells (Sheu & Blaustein, 1986; DiPolo & Beaugé, 1988; Reeves & Philipson, 1989). In excitable cells, the system may participate in both Ca^{2+} outflow (forward mode) or Ca^{2+} inflow (reverse mode), depending on the state of cell activity (Sheu & Blaustein, 1986).

For more than a decade, the existence of a process of Na^+/Ca^{2+} exchange that could participate in Ca^{2+} extrusion was postulated in the pancreatic B cell (Donatsch *et al.*, 1977; Herchuelz *et al.*, 1980; Hellman *et al.*, 1980). Recently the process working in its reverse mode was characterized in normal rat pancreatic islet cells (Plasman *et al.*, 1990). The exchanger was shown to display quite a large capacity and to be stimulated by both glucose and membrane depolarization (Plasman *et al.*, 1990). Direct evidence was also provided showing that Na^+/Ca^{2+} exchange participated in the regulation of $[Ca^{2+}]_i$ by favouring Ca^{2+} outflow from the cell (Herchuelz, 1991).

Because the B cell is electrically excitable, Na^+/Ca^{2+} exchange could also drive Ca^{2+} inflow, e.g. during the depolarizing phases that occur when the cell is stimulated by nutrient secretagogues such as glucose. Hence the regulation of reverse Na^+/Ca^{2+} exchange may be of great interest in the understanding of the stimulus–secretion coupling of glucose-induced insulin release from the pancreatic B cell.

The aim of the present study was to characterize further the regulation of Na^+/Ca^{2+} exchange in the pancreatic B cell.

MATERIALS AND METHODS

The methods used were described previously (Plasman *et al.*, 1990).

Media

The medium used to isolate and dissociate the islets was a Hepes-buffered Earle's solution (Gobbe & Herchuelz, 1989), supplemented with 0.2% (w/v) BSA (fraction V; Sigma Chemical Co., St. Louis, MO, U.S.A.) and equilibrated with O_2/CO_2 (19:1). The medium used to incubate the islet cells was a Krebs–Ringer solution buffered with Hepes/NaOH (10 mM, pH 7.4), containing 1 mM-$CaCl_2$ and equilibrated against O_2 (100%). In some experiments, NaCl was iso-osmotically replaced by sucrose (241 mM) and Hepes/NaOH was replaced by Hepes/KOH. All reagents were of analytical grade. Rotenone, phorbol 12-myristate 13-acetate (PMA), imidazole and monensin were from Sigma. Nifedipine was from Bayer, Brussels, Belgium. Sodium acetate, KCN and NH_4Cl were from Merck, Darmstadt, Germany. Lithium acetate was from Aldrich-Chemie, Steinheim, Germany. Oligomycin and antimycin A were from Boehringer, Mannheim, Germany. 2,4-Dinitrophenol (DNP) was from BDH, Poole, Dorset, U.K., and 4α-phorbol 12,13-didecanoate (4αPDD) was from Calbiochem, San Diego, CA, U.S.A. The tetra-acetoxymethyl ester of 2,7-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF) was from HSC Research Development Corp., Toronto, Ont., Canada. These drugs, except KCN, were dissolved in dimethyl sulphoxide, which was added to both control and test media at final concentrations not exceeding 0.1% (v/v). At this concentration, dimethyl sulphoxide fails to affect islet function (Levy *et al.*, 1976).

Islet-cell preparation

Pancreatic islets (800–1200) were isolated by the collagenase technique from the pancreas of fed albino rats. The method used to isolate pancreatic islet cells has been described elsewhere (Gobbe & Herchuelz, 1989). In brief, after exposure to a Ca^{2+}-deprived medium, the islets were disrupted by using Dispase and were centrifuged through 4% BSA in Hanks solution to remove debris and dead cells. Cell viability as assessed by Trypan Blue exclusion was ~ 97%. The responsiveness of the cell preparation to glucose and other insulin secretagogues has been previously assessed (Gobbe & Herchuelz, 1989; Plasman *et al.*, 1990). After isolation, the cells were incubated at 37 °C for 1 h in 0.5 ml of RPMI 1640 culture medium (GIBCO Europe, Gent, Belgium) containing 0.5% BSA, 2.3 mM-glutamine and 11.1 mM-glucose under O_2/CO_2 (19:1).

$^{45}Ca^{2+}$ uptake

After centrifugation (500 *g*, 3 min) to remove the supernatant, the cells were preincubated at 37 °C in 1 ml of a non-radioactive

Abbreviations used: $[Ca^{2+}]_i$, intracellular Ca^{2+} concn.; pH_i, intracellular pH; pH_o, extracellular pH; BCECF, 2,7-biscarboxyethyl-5(6)-carboxyfluorescein; DNP, 2,4-dinitrophenol; PMA, phorbol 12-myristate 13-acetate; 4αPDD, 4α-phorbol 12,13-didecanoate.

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Krebs–Ringer solution containing 5 μM-nifedipine. After 30 min, the cells were again centrifuged and incubated at 37 °C for 1 or 5 min in 1 ml of the same medium also containing $^{45}Ca^{2+}$ (10 μCi/ml) and, when required, no Na^+. When required, oligomycin, antimycin A, DNP, rotenone, KCN, PMA or 4αPDD was added to both the preincubation and incubation media. Sodium acetate, lithium acetate, NH_4Cl and imidazole were added to the incubation medium only.

At the end of the incubation, the uptake of ^{45}Ca was stopped by addition of 5 ml of an ice-cold 'stop solution', consisting of a Krebs–Ringer solution containing $LaCl_3$ (2 mM) adjusted to pH 7.1 to avoid any precipitation of La^{3+}. La^{3+} and/or low temperature (1 °C) have been shown practically to abolish ^{45}Ca efflux and to impair $^{45}Ca^{2+}$ uptake severely in pancreatic islets (Hellman, 1978; Herchuelz & Malaisse, 1978). After incubation for 20 min in this medium, the cells were centrifuged (500 *g*, 3 min), the radioactive supernatant was discarded, and the cells were resuspended in 1 ml of the same ice-cold stop solution. Portions (0.1 ml) of this suspension were then placed in polyethylene micro-centrifuge tubes. A first centrifugation (30s; Beckman Microfuge) was performed to deposit the cells in the tip of the tube. Di-n-butyl phthalate (0.1 ml; BDH) was layered on top of the solution, and a second centrifugation (30s) was performed to separate the islet cells from the medium. The bottom of the tube was cut and transferred to a counting vial to which 5 ml of scintillation fluid was added (Lumagel; Lumac, Olen, Belgium). The uptake of ^{45}Ca was expressed as mol of $^{45}Ca^{2+}$ with the same specific radioactivity as that of the incubation medium.

For the effect of temperature on Na^+/Ca^{2+} exchange, data are presented as an Arrhenius plot by using the following equation (Segel, 1975):

$$\log k = \frac{E_a}{2.303R} \times \frac{1}{T} + \log A$$

where k is the reaction velocity constant, T is the absolute temperature, A is a constant, R is the universal gas constant and E_a is the Arrhenius activation energy. E_a was calculated from the slope of the straight line obtained, according to the equation:

$$E_a = -\text{slope} \times 2.303 \times R$$

The temperature coefficient Q_{10}, defined as the factor by which the reaction velocity is increased on raising the temperature by 10 °C ($T1$, $T2$) was calculated from the equation (Segel, 1975):

$$E_a = \frac{2.303R \times T1 \times T2 \times \log Q_{10}}{10}$$

Intracellular pH (pH_i)

For measurement of pH_i pancreatic islet cells were preincubated for 30 min in the presence of 1.0 μM of the tetraacetoxymethyl ester of BCECF, washed twice and placed in a quartz cuvette (1.5 ml) containing 0.75 ml of a Hepes/NaOH buffer (25 mM) equilibrated against ambient air and adjusted to pH 7.4.

BCECF fluorescence was monitored in a Perkin–Elmer spectrofluorimeter (LS 5), the cuvette being maintained at 37 °C and the cell suspension continuously stirred. Excitation and emission wavelengths were 500 and 530 nm respectively. After equilibration for 15–20 min, fluorescence measurements were started and test agents were added to the cuvette in small portions (2–10 μl) about 10 min later. Calibration of the BCECF florescence was carried out at the end of each experiment by adding digitonin (final concn. 50 μM) and 1.0 μl portions of 0.1 M-HCl, to allow measurement of fluorescence at decreasing pH, the latter being measured by a pH-sensitive electrode. The pH_i was then judged from the fluorescence recorded before addition of digitonin and by reference to the calibration curve established at the end of the same experiment. The limit of sensitivity of the method was estimated to be less than an 0.003 pH.

Results are expressed as means ± S.E.M. The statistical significance of differences between data was assessed by using a non-paired Student's t test for two comparisons and analysis of variance for multi-sample comparison.

RESULTS

Effect of pH

In the presence of extracellular Na^+ (139 mM), basal ^{45}Ca uptake averaged 496 ± 32 fmol/min per 1000 cells ($n = 47$) and was minimally affected by extracellular pH (pH_o) and pH_i. All data were corrected for this basal uptake observed in the presence of 139 mM-Na^+ at the various pH values investigated, so that only intracellular-Na^+-dependent Ca^{2+} movements are further reported. pH_o markedly affected reverse Na^+/Ca^{2+} exchange (Fig. 1*a*; $P < 0.001$). Indeed, at pH_o 6.0, Na^+/Ca^{2+} exchange was decreased by about 65 ± 5 % ($P < 0.01$), whereas at pH_o 9 it was increased by about 70 ± 13 % ($P < 0.001$).

pH_i affected reverse Na^+/Ca^{2+} exchange even more markedly than pH_o (Fig. 1*b*; $P < 0.001$). The pH_i was altered by using acetate (10 mM) and NH_4Cl (10 mM). Acetate was used either as its sodium salt (pH measurements) or as its lithium salt (Ca^{2+}-

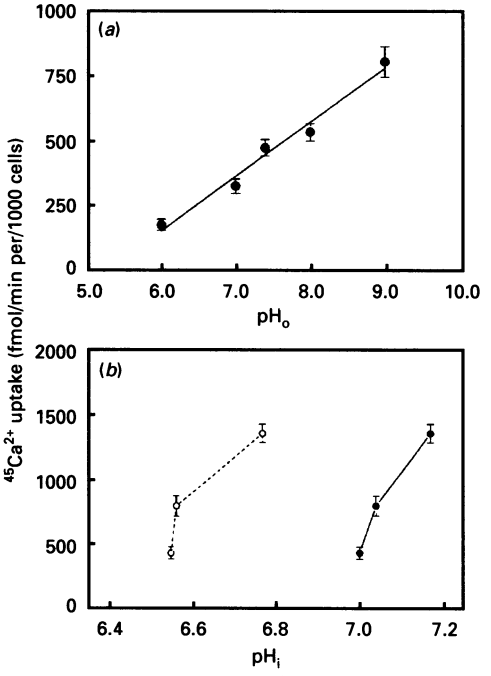

Fig. 1. Effect of pH_o (*a*) and pH_i (*b*) on reverse Na^+/Ca^{2+} exchange in isolated islet cells

$^{45}Ca^{2+}$ uptake was measured over 1 min periods in the absence of extracellular Na^+ (replaced by sucrose) and at different pH_o (*a*) and pH_i (*b*) values. pH_i was altered by using acetate (10 mM) and NH_4Cl (10 mM). Acetate was used either as its Na^+ salt (pH measurement) or as its Li^+ salt (Ca^{2+}-uptake experiments). The effects of acetate and NH_4Cl on pH_i were measured either in the absence (○) or in the presence of extracellular Na^+ (●). The data are corrected for basal uptake observed in the presence of 139 mM-Na^+ at the various pH values investigated. Mean values (± S.E.M.) are for 21–48 samples in each case.

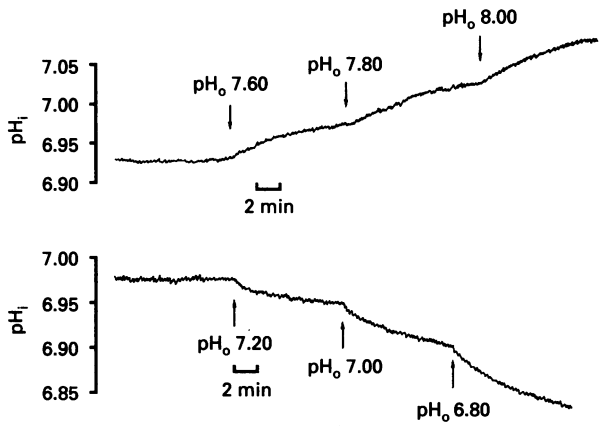

Fig. 2. Effect of changes in pH_o on pH_i in isolated islet cells

pH_i was measured by using BCECF. Basal pH_o (7.4) was changed by successive addition of NaOH (upper curve) or HCl (lower curve). Changes in pH_o were measured with a pH electrode. The traces are representative of three experiments in each case.

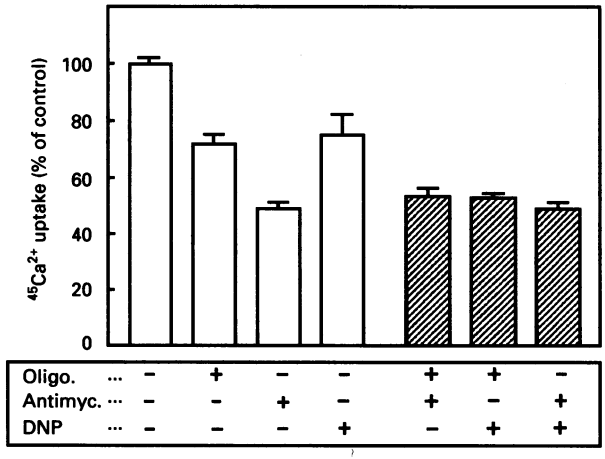

Fig. 3. Effect of metabolic poisons on reverse Na^+/Ca^{2+} exchange in isolated islet cells

$^{45}Ca^{2+}$ uptake was measured over 5 min periods in the absence of extracellular Na^+ (replaced by sucrose) and in the absence or the presence of oligomycin (Oligo.; 4 μM), antimycin A (Antimyc.; 2 μM) and DNP (0.2 mM) alone or in combination. The data are corrected for basal uptake observed in the presence of 139 mM-Na^+ and the metabolic inhibitors. Mean values (±S.E.M.) are for 26–138 samples in each case.

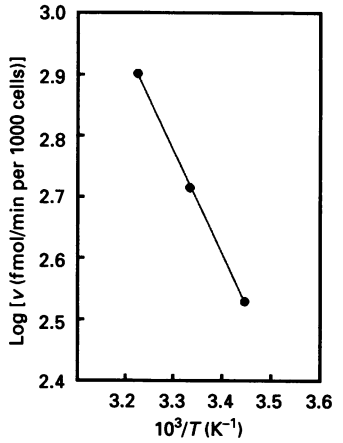

Fig. 4. Arrhenius plot of Na^+/Ca^{2+} exchange activity in isolated islet cells

$^{45}Ca^{2+}$ uptake induced by the absence of extracellular Na^+ (replaced by sucrose) was measured at 17 °C, 27 °C and 37 °C. The data are corrected for basal uptake observed in the presence of 139 mM-Na^+ at the various temperatures investigated. Mean (±S.E.M.) values are for 21 samples in each case.

uptake experiments). The latter salt was preferred for Ca^{2+}-uptake experiments, since it did not affect reverse Na^+/Ca^{2+} exchange while producing a decrease in pH_i that was comparable with that with sodium acetate (results not shown). Basal pH_i at pH_o 7.4 and in the presence of extracellular Na^+ averaged 7.04±0.02 ($n = 11$). Over 1 min, acetate decreased pH_i by 0.04±0.003 unit ($n = 19$), whereas NH_4Cl increased pH_i by 0.13±0.03 unit ($n = 4$). In the absence of extracellular Na^+, basal pH averaged 6.56±0.2 ($n = 3$). Acetate decreased pH_i by 0.014±0.003 unit ($n = 3$), whereas NH_4Cl increased it by 0.214±0.006 unit ($n = 3$). Fig. 1(*b*) shows that the decrease in pH_i owing to acetate decreased reverse Na^+/Ca^{2+} exchange by about 40±6%, whereas the increase by NH_4Cl enhanced Na^+/Ca^{2+} exchange by about 70±9% ($P < 0.001$). The weak base imidazole (10 mM) increased reverse Na^+/Ca^{2+} exchange by about 40±8% (results not shown; $P < 0.01$). All these experiments were carried out over a 1 min period, the islet cells being exposed to the test condition for only 1 min. Indeed, changes in pH_i as induced by acetate, imidazole and NH_4Cl may not persist over periods exceeding 1 min, owing to the presence in islet cells of pH_i-regulatory mechanisms (for a review, see Lynch & Best, 1990).

To examine to what extent changes in pH_o could affect Na^+/Ca^{2+} exchange indirectly by modifying pH_i, the effect of pH_o on pH_i was investigated. Changes in pH_o induced parallel changes in pH_i (Fig. 2), though the changes in pH_i were smaller than those in pH_o. Thus, over the pH_o range 6.8–8.0, a 0.2-pH_o-unit change induced a 0.07±0.0042 ($n = 18$) unit change in pH_i.

Effects of mitochondrial poisons

Three different types of mitochondrial poisons were used to study the influence of metabolic energy on Na^+/Ca^{2+} exchange: oligomycin, an ATP synthase inhibitor, antimycin A, rotenone and KCN, three electron-transfer inhibitors, and DNP, an uncoupler of oxidative phosphorylation. Oligomycin, antimycin A and KCN have been shown to decrease the ATP concentration markedly in rat pancreatic islets (Malaisse *et al.*, 1979*a*): in the absence of glucose they decreased ATP concentration by 56%, 62% and 56% respectively.

The drugs minimally affected basal $^{45}Ca^{2+}$ uptake measured in the presence of extracellular Na^+ (139 mM). The values observed under the latter condition were subtracted from those recorded in the absence of Na^+. Fig. 3 shows the effects of oligomycin (4 μM), antimycin A (2 μM) and DNP (0.2 mM) alone or in combination on Na^+/Ca^{2+} exchange. They inhibited $^{45}Ca^{2+}$ uptake by 28±3%, 48±2% and 25±7% respectively ($P < 0.001$). A larger effect was observed when oligomycin and DNP were used in combination than when either drug was used alone (Fig. 3). KCN (2 mM) and rotenone (10 μM) decreased $^{45}Ca^{2+}$ uptake by 42% and 35% respectively (results not shown; $P < 0.001$). The presence of glucose (16.7 mM) in the incubation did not reverse the effect of the metabolic inhibitors (results not shown).

Effect of temperature

Cooling from 37 °C to 27 °C and 17 °C produced a temperature-dependent decrease in Na^+/Ca^{2+} exchange activity. Fig. 4 shows an Arrhenius plot of $^{45}Ca^{2+}$ uptake as a function of the temperature. An E_a of 16 kJ (3822 cal)/mol was calculated,

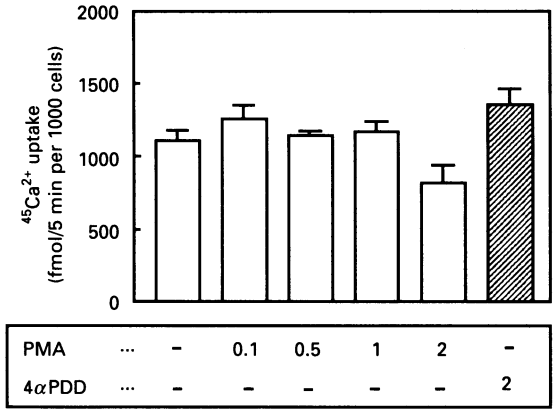

Fig. 5. Effect of PMA and 4αPDD on reverse Na^+/Ca^{2+} exchange in isolated islet cells

$^{45}Ca^{2+}$ uptake was measured over 5 min periods in the absence of extracellular Na^+ (replaced by sucrose) and in the absence or the presence of PMA (0.1–2 μM) or 4αPDD (2 μM). The data are corrected for basal uptake observed in the presence of 139 mM-Na^+ and the phorbol esters. Mean values (±S.E.M.) are for 11–42 samples in each case.

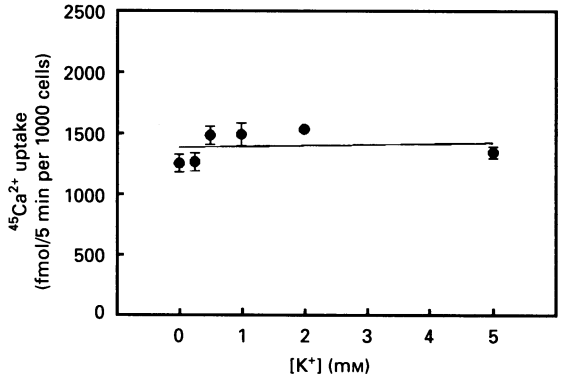

Fig. 6. Effect of extracellular K^+ on reverse Na^+/Ca^{2+} exchange in isolated islet cells

$^{45}Ca^{2+}$ uptake was measured over 5 min periods in the absence of extracellular Na^+ (replaced by sucrose) and at various extracellular K^+ concentrations. The data are corrected for basal uptake observed in the presence of 139 mM-Na^+ and the different K^+ concentrations. Mean values (±S.E.M.) are for 20–36 samples in each case.

corresponding to a Q_{10} of 1.23 between 27 and 37 °C. These experiments were carried out over 1 min to approximate the initial fast component of the uptake (Plasman *et al.*, 1990).

Effect of the protein kinase C activator PMA

The phorbol ester PMA and its inactive analogue 4αPDD did not affect basal $^{45}Ca^{2+}$ uptake recorded in the presence of extracellular Na^+ (139 mM). PMA up to 1 μM also failed to affect reverse Na^+/Ca^{2+} exchange (after subtraction of basal value) (Fig. 5). At a higher concentration (2 μM), PMA decreased Na_i-dependent Ca^{2+} uptake by 36 % ($P < 0.001$), whereas at the same concentration its negative control, 4αPDD, was ineffective ($P > 0.5$). Since phorbol esters act on B-cell Ca^{2+} fluxes within the nanomolar range (Berggren *et al.*, 1989), the effect of PMA at 2 μM can be considered as non-specific.

Effect of extracellular K^+

In an attempt to examine whether the B-cell Na^+/Ca^{2+} exchanger can co-transport K^+, the effect of extracellular K^+ on Na^+/Ca^{2+} exchange was examined. Lowering the extracellular K^+ concentration provoked a dose-related decrease in both basal $^{45}Ca^{2+}$ uptake and the uptake observed in the absence of extracellular Na^+ (results not shown). When the former was subtracted from the latter, no effect of extracellular K^+ below 5 mM was evident (Fig. 6).

Effect of monensin

The Na^+ ionophore monensin was used to increase intracellular [Na^+] and hence to dissipate the Na^+ gradient. At 10 μM, monensin decreased reverse Na^+/Ca^{2+} exchange by about 60 % (results not shown; $P < 0.001$). This observation further confirms the view that the uptake of ^{45}Ca produced by the absence of extracellular Na^+ results from the activation of reverse Na^+/Ca^{2+} exchange (Plasman *et al.*, 1990).

DISCUSSION

The aim of the present study was to examine the effects of potential intracellular signals on Na^+/Ca^{2+} exchange, in order to understand better the regulation of this process within the pancreatic B cell.

Effect of pH

Changes in pH_i have been proposed as coupling or modulatory factors in the process of glucose-induced insulin release (Malaisse *et al.*, 1980*a*; Pace, 1984; Lynch & Best, 1990). Indeed, intracellular acidification or alkalinization respectively decreases or increases the efflux of both Ca^{2+} and K^+ from the B cell (Carpinelli & Malaisse, 1980; Carpinelli *et al.*, 1980; Lebrun *et al.*, 1982; Best *et al.*, 1988). For Ca^{2+}, the effect of pH is largely dependent on the presence of Na^+ in the medium, suggesting that intracellular protons inhibit forward Na^+/Ca^{2+} exchange (Lebrun *et al.*, 1982; Best *et al.*, 1988).

The present study shows that reverse Na^+/Ca^{2+} exchange is exquisitely sensitive to changes in pH_i and that this sensitivity occurred within the physiological range. Since the absence of extracellular Na^+ may modify the pH_i, the actual pH_i-dependence of reverse Na^+/Ca^{2+} exchange should be intermediate between the dependences determined in the presence and the absence of extracellular Na^+ (Fig. 1). The effects of pH_o on Na^+/Ca^{2+} exchange were parallel to those of pH_i, but less marked. Since changes in pH_o were attended by parallel changes in pH_i also of decreased magnitude (one-third), it is not inconceivable that pH_o acted indirectly to inhibit Na^+/Ca^{2+} exchange, or in other words, that pH_o had no direct or proper effect on Na^+/Ca^{2+} exchange. Indeed, in squid axon, in which good control of the pH_i can be exerted by internal dialysis, reverse Na^+/Ca^{2+} exchange is clearly unaffected by changes in pH_o within the range 7.3–8.8 (DiPolo & Beaugé, 1982). Since glucose appears to induce a progressive alkalinization of the B cell (for a review see Lynch & Best, 1990), our data suggest reverse Na^+/Ca^{2+} exchange as a potential target for this alkalinization. Indeed, the effect of glucose to increase pH_i and to stimulate reverse Na^+/Ca^{2+} exchange has a low K_m value for both (≤ 4 mM; Lindström & Sehlin, 1984; Deleers *et al.*, 1985; Plasman *et al.*, 1990). By this action, glucose could favour Ca^{2+} entry into the B cell, provided that during stimulation by glucose Ca^{2+} indeed enters the cell by reverse Na^+/Ca^{2+} exchange.

Effect of energy deprivation

It is generally accepted that the ability of glucose to stimulate insulin release is dependent on its metabolism within the cell (Malaisse *et al.*, 1979*b*). On the other hand, in a large variety of cells, Na^+/Ca^{2+} exchange, although not dependent on high-energy substrates (e.g. ATP), is activated by ATP. The present

finding of a profound but not total inhibition of reverse Na^+/Ca^{2+} exchange by mitochondrial poisons suggests that in the B cell Na^+/Ca^{2+} exchange is modulated by the metabolic state of the cell. Indeed, five different metabolic inhibitors decreased reverse Na^+/Ca^{2+} exchange without (or minimally) affecting basal $^{45}Ca^{2+}$ uptake measured in the presence of extracellular Na^+. This almost excludes any mechanisms, other than depletion of the cells of high-energy substrates, in mediating the inhibition of Na^+/Ca^{2+} exchange. For instance, the inhibition of Na^+/Ca^{2+} exchange by metabolic poisons was not mediated by a decrease in pH_i. Indeed, antimycin A (2 μM) did not decrease pH_i in isolated islet cells (results not shown). Our data are in agreement with a previous study showing that in unpoisoned cells glucose stimulated reverse Na^+/Ca^{2+} exchange (Plasman *et al.*, 1990). The effect was seen at low glucose concentrations, namely concentrations that may significantly elevate ATP levels (Malaisse & Sener, 1987). In that previous study, it was suggested that glucose could activate Na^+/Ca^{2+} exchange, as in other cells, by phosphorylating the carrier (Caroni & Carafoli, 1983; DiPolo & Beaugé, 1987). Therefore, our data suggest reverse Na^+/Ca^{2+} exchange as a potential target for the high-energy substrates (ATP) generated by glucose. In other words, through its metabolism glucose could activate reverse Na^+/Ca^{2+} exchange and, by doing so, favour Ca^{2+} entry into the B cell by another route than voltage-sensitive Ca^{2+} channels.

The finding of a relatively low Q_{10} value for Na^+/Ca^{2+} exchange activity is in agreement with previous findings in other tissues (Reuter & Seitz, 1968; Debetto *et al.*, 1990). It indicates that the process is indirectly dependent on metabolic energy, or that it is activated by, but not strictly dependent on, high-energy substrates (Debetto *et al.*, 1990). Indeed, the activity of the Na^+/Ca^{2+} exchanger is less affected by temperature than are membrane active-transport systems such as the Na^+–K^+-ATPase, for which a $Q_{10} > 2$ is observed (Eisner & Lederer, 1980).

Effect of protein kinase C activation

The involvement of protein kinase C in insulin secretion stimulated by glucose remains to be established (for a review see Wollheim & Regazzi, 1990). However, protein kinase C activation by phorbol esters was suggested to favour Ca^{2+} outflow from the B cell (Malaisse *et al.*, 1980*b*) and hence to assist the cell in recovery from raised $[Ca^{2+}]_i$ (Berggren *et al.*, 1989). The present study showing no effect of phorbol esters on Na^+/Ca^{2+} exchange suggests that the exchanger does not appear to be a target for protein kinase C, at least when working in its reverse mode.

Co-transport of K^+

In rod outer segment, Cervetto *et al.* (1989) and Schnetkamp *et al.* (1989) reported that K^+ was co-transported with Ca^{2+} by the exchanger at physiological K^+ concentrations (5 mM outside and 150 mM inside). It was suggested that K^+ co-transport would enable the exchange to decrease $[Ca^{2+}]_i$ to much lower values than previously supposed, and may be a general phenomenon (Cervetto *et al.*, 1989). Our data suggest that in the B cell Na^+/Ca^{2+} exchange does not depend on the presence of external K^+ and, presumably, does not co-transport K^+ with Ca^{2+} under physiological conditions. A similar conclusion was recently reached in cardiac cells (Yasui & Kimura, 1990).

In conclusion, the pH_i and the metabolic state of the cell may represent important modulatory signals by which insulin secretagogues such as glucose could regulate reverse Na^+/Ca^{2+} exchange in the B cell. The process does not appear to co-transport K^+, nor to be influenced by protein kinase C.

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REFERENCES

Berggren, P.-O., Arkhammar, P. & Nilsson, T. (1989) Biochem. Biophys. Res. Commun. **165**, 416–421
Best, L., Yates, A. P., Gordon, C. & Tomlinson, S. (1988) Biochem. Pharmacol. **37**, 4611–4615
Caroni, P. & Carafoli, E. (1983) Eur. J. Biochem. **132**, 451–460
Carpinelli, A. R. & Malaisse, W. J. (1980) Diabete Metab. **6**, 193–198
Carpinelli, A. R., Sener, A., Herchuelz, A. & Malaisse, W. J. (1980) Metab. Clin. Exp. **29**, 540–545
Cervetto, L., Lagnado, L., Perry, R. J., Robinson, D. W. & McNaughton, P. A. (1989) Nature (London) **337**, 740–743
Debetto, P., Cusinato, F. & Luciani, S. (1990) Arch. Biochem. Biophys. **278**, 205–210
Deleers, M., Lebrun, P. & Malaisse, W. J. (1985) Horm. Metab. Res. **17**, 391–395
DiPolo, R. & Beaugé, L. (1982) Biochim. Biophys. Acta **688**, 237–245
DiPolo, R. & Beaugé, L. (1987) Biochim. Biophys. Acta **897**, 347–354
DiPolo, R. & Beaugé, L. (1988) Biochim. Biophys. Acta **947**, 549–569
Donatsch, P., Lowe, D. A., Richardson, B. P. & Taylor, P. (1977) J. Physiol. (London) **267**, 357–376
Eisner, D. A. & Lederer, W. J. (1980) J. Physiol. (London) **303**, 441–474
Gobbe, P. & Herchuelz, A. (1989) Res. Commun. Chem. Pathol. Pharmacol. **63**, 231–247
Hellman, B. (1978) Biochim. Biophys. Acta **540**, 534–542
Hellman, B., Andersson, T., Berggren, P.-O & Rorsman, P. (1980) Biochem. Med. **24**, 143–152
Herchuelz, A. (1991) Diabetologia **34**, Suppl. 2, A73 (abstr.)
Herchuelz, A. & Malaisse, W. J. (1978) J. Physiol. (London) **238**, 409–424
Herchuelz, A., Sener, A. & Malaisse, W. J. (1980) J. Membr. Biol. **57**, 1–12
Lebrun, P., Malaisse, W. J. & Herchuelz, A. (1982) Biochim. Biophys. Acta **721**, 357–365
Levy, J., Herchuelz, A., Sener, A., Malaisse-Lagae, F. & Malaisse, W. J. (1976) Endocrinology (Baltimore) **98**, 429–437
Lindström, P. & Sehlin, J. (1984) Biochem. J. **218**, 887–892
Lynch, A. & Best, L. (1990) Biochem. Pharmacol. **40**, 411–416
Malaisse, W. J. & Sener, A. (1987) Biochim. Biophys. Acta **972**, 190–195
Malaisse, W. J., Hutton, J. C., Kawazu, S., Herchuelz, A., Valverde, I. & Sener, A. (1979*a*) Diabetologia **16**, 331–341
Malaisse, W. J., Sener, A., Herchuelz, A. & Hutton, J. C. (1979*b*) Metab. Clin. Exp. **28**, 373–386
Malaisse, W. J., Herchuelz, A. & Sener, A. (1980*a*) Life Sci. **26**, 1367–1371
Malaisse, W. J., Sener, A., Herchuelz, A., Carpinelli, A. R., Poloczek, P., Winand, J. & Castagna, M. (1980*b*) Cancer Res. **40**, 3827–3831
Pace, C. S. (1984) Am. J. Physiol. **244**, E3–E18
Plasman, P.-O., Lebrun, P. & Herchuelz, A. (1990) Am. J. Physiol. **259**, E844–E850
Reeves, J. P. & Philipson, K. D. (1989) in Sodium–Calcium Exchange (Allen, T. J. A.., Noble, D. & Reuter, H., eds.), pp. 27–53, Oxford Science Publications, Oxford
Reuter, H. & Seitz (1968) J. Physiol. (London) **195**, 451–470
Schnetkamp, P. P. M., Debesh, K. B. & Szerencsei, R. T. (1989) Am. J. Physiol. **257**, C153–C157
Segel, I. H. (1975) Enzyme Kinetics: Behaviour and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems, pp. 884–942, Wiley, New York
Sheu, S.-S. & Blaustein, M. P. (1986) in The Heart and the Cardiovascular System (Fozzard, H. A., ed.), pp. 509–535, Raven, New York
Wollheim, C. B. & Regazzi, R. (1990) FEBS Lett. **268**, 376–380
Yasui, K. & Kimura, J. (1990) Pflügers Arch. **415**, 513–515

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