The interaction between manganese and calcium fluxes in pancreatic β -cells

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Electrothermal atomic-absorption spectroscopy was employed for measuring manganese in β -cell-rich pancreatic islets isolated from *ob/ob* mice. The efflux from preloaded islets was estimated from the amounts remaining after 30 min of subsequent test incubations in the absence of Mn^{2+} . An increase in the extracellular Mg^{2+} concentration promoted the Mn^{2+} efflux and removal of Na^+ from a Ca^{2+} -deficient medium had the opposite effect. Addition of 25 mM- K^+ failed to affect Mn^{2+} outflow as did 3-isobutyl-1-methylxanthine and dibutyl cyclic AMP. Whereas tolbutamide caused retention of manganese, the ionophore Br-X537A promoted an efflux. D-Glucose was equally potent in retaining the islet manganese when the external Ca^{2+} concentration ranged from 15 μM to 6.30 mM. Subcellular-fractionation experiments indicated a glucose-stimulated incorporation of manganese into all fractions except the microsomes. The effect was most pronounced in the mitochondrial fraction, being as high as 164%. The glucose-induced uptake of intracellular ^{45}Ca was abolished in the presence of 0.25 mM- Mn^{2+} . When added to medium containing 2.5 mM- Mn^{2+} , glucose even tended to decrease $^{45}Ca^{2+}$ uptake. The inhibitory effect of Mn^{2+} was apparent also from a diminished uptake of ^{45}Ca into all subcellular fractions. The efflux of $^{45}Ca^{2+}$ was markedly influenced by Mn^{2+} as manifested in a prominent stimulation followed by inhibition. In addition to demonstrating marked interactions between fluxes of Mn^{2+} and Ca^{2+} , the present studies support the view that the glucose inhibition of the efflux of bivalent cations from pancreatic β -cells is accounted for by their accumulation in the mitochondria.

It is well established that, even below the threshold concentration for insulin release, D-glucose induces a transient or permanent decrease of $^{45}Ca^{2+}$ efflux from pancreatic islets (Malaisse *et al.*, 1979; Abrahamsson *et al.*, 1981). However, it has proven difficult to elucidate the processes underlying this phenomenon. The glucose-induced inhibition of $^{45}Ca^{2+}$ efflux may either reflect increased retention in subcellular organelles (Hellman *et al.*, 1979) or result from direct interference with the outward transport across the plasma membrane (Herchuelz *et al.*, 1980). The high concentrations of calcium normally present in the pancreatic β -cells (Berggren *et al.*, 1978) represent a major difficulty when attempting to assess minute net changes of the calcium content. These difficulties can be circumvented by the introduction into the β -cells of ions similarly affected by glucose but normally not present in significant amounts. It was apparent from

a recent study (Rorsman *et al.*, 1982) that these criteria were fulfilled by Mn^{2+} .

Mn^{2+} has been used as a paramagnetic Ca^{2+} analogue in numerous e.s.r. and n.m.r. studies. Although the chemistry of Mn^{2+} resembles that of Mg^{2+} rather than Ca^{2+} (Williams, 1970), there is now considerable evidence that Mn^{2+} transport has some features in common with the translocation of Ca^{2+} in whole cells (Getz *et al.*, 1979; Impraïm *et al.*, 1979) and subcellular organelles such as the mitochondria (Fiskum & Lehninger, 1980). Two chemically related ions such as Mn^{2+} and Ca^{2+} might well share some properties in biological tissues, but not necessarily all. Whereas exposure to D-glucose results in an enhanced uptake and retention of manganese there is no concomitant stimulation of insulin release (Rorsman *et al.*, 1982).

In the present study further attention has been paid to the unique possibilities offered by Mn^{2+} for elucidating the effects of glucose on the β -cell handling of Ca^{2+} . Evidence is presented both for interactions between Mn^{2+} and Ca^{2+} fluxes and a

Abbreviation used: IBMX, 3-isobutyl-1-methylxanthine.

significant role of the mitochondria in the process of glucose-induced retention of manganese.

Materials and methods

Chemicals

Chemicals of analytical grade and deionized water were used. $^{45}\text{CaCl}_2$ was purchased from New England Nuclear Corp., Frankfurt/Main, Germany. Packard Instruments, Warrenville, IL, U.S.A., was the source of scintillator 299 and Hyamine. Sigma Chemical Co., St. Louis, MO, U.S.A., supplied EGTA, dibutyl cyclic AMP (N^6O^2 -dibutyladenosine 3':5'-cyclic monophosphate), Hepes [4-(2-hydroxyethyl)-piperazine-ethanesulphonic acid], bovine serum albumin (fraction V) and Triton X-100. Collagenase and tolbutamide were products of Boehringer Mannheim G.m.b.H., Mannheim, Germany, and Hoechst A.G., Frankfurt/Main, Germany, respectively. Fluorescamine (Fluram) and Br-X537A were both obtained from Hoffman-LaRoche & Co., Basel, Switzerland, and IBMX was supplied by EGA Chemie, Albuch/Steinheim, Germany.

Animals and isolation of islets

Adult obese-hyperglycaemic mice (*ob/ob*) of both sexes were taken from a local non-inbred colony (Hellman, 1965). The mice were starved overnight and the pancreases removed immediately after killing. Islets were isolated by microdissection for the study of intact islets or by collagenase digestion for analysis of subcellular fractions. The islets of the *ob/ob* mice contain more than 90% β -cells known to respond normally to glucose and other stimulators of insulin release (Hellman, 1970; Hahn *et al.*, 1974).

Preparation of media and general aspects of incubations

All incubations were performed at 37°C. The basal medium was a Hepes buffer, pH 7.4, physiologically balanced in cations with chloride as the sole anion (Hellman *et al.*, 1976). It should be noted that the media lacked phosphate, sulphate and carbonate, to avoid precipitation of Mn^{2+} . For the same reason pH was adjusted with NaOH before the addition of the Mn^{2+} salt. The concentration of Ca^{2+} was checked with an Orion microprocessor ion-analyzer (Orion Research, Cambridge, MA, U.S.A.) equipped with F2210 Ca^{2+} and K4040 calomel electrodes (Radiometer A/S, Copenhagen, Denmark). In the $^{45}\text{Ca}^{2+}$ uptake experiments the islets were allowed to equilibrate during a pre-incubation period of 30 min. When not taken for subcellular fractionation, the islets were freeze-dried overnight followed by weighing on a quartz-fibre balance.

Manganese mobilization from intact islets

Groups of five to six islets were incubated for 60 min with 0.25 mM- Mn^{2+} in the presence of 1.28 mM- Ca^{2+} and 3 mM-D-glucose followed by exposure to 5 ml of Mn^{2+} -free efflux medium for 30 min. Modifications of the latter medium are described in the legends to Figures and Tables. It was checked in separate experiments with choline $^{+}$ that the increased osmotic pressure after addition of Ca^{2+} , Mg^{2+} and K^{+} did not influence the mobilization of manganese. When lowering the Na^{+} concentration to 12 mM, this ion was replaced with equimolar amounts of choline $^{+}$.

$^{45}\text{Ca}^{2+}$ fluxes in intact cells

In the uptake experiments, groups of two islets were incubated for 60 min with 200 μl of medium containing 1.28 mM- $^{45}\text{Ca}^{2+}$ (sp. radioactivity 15.6 Ci/mol). After incubation, the islets were rinsed for 60 min in a cold La^{3+} solution to remove extracellular radioactivity (Hellman, 1978).

The dynamics of $^{45}\text{Ca}^{2+}$ efflux were studied during perfusion of the islets as previously described (Gylfe & Hellman, 1978). Briefly, groups of ten islets were loaded with $^{45}\text{Ca}^{2+}$ (sp. radioactivity 390 Ci/mol). The islets were then transferred to 10 μl chambers and perfused at a constant rate of approx. 40 $\mu\text{l}/\text{min}$. The use of two reservoirs made it possible to alter rapidly the composition of the medium supplied to the islets. The perfusates were collected over successive periods of 2 or 5 min.

In both the uptake and efflux experiments the islets were freeze-dried and weighed before being dissolved in 100 μl of Hyamine.

Subcellular fractionation experiments

Subcellular fractions were prepared as described by Kohnert *et al.* (1979). Groups of 100 islets were loaded with $^{45}\text{Ca}^{2+}$ (15.6 Ci/mol) or 0.25 mM- Mn^{2+} in 2 ml of oxygenated medium containing 1.28 mM- Ca^{2+} . After loading, the islets were washed for approx. 10 min at 2°C in the same medium as used for subsequent homogenization and differential centrifugation (0.25 M-sucrose, 1 mM-EGTA and 5 mM-Hepes, pH 6.0). The pelleted fractions were resuspended in either 100 μl of 1% Triton X-100 (^{45}Ca) or 50 μl of deionized water (Mn). The data for the ^{45}Ca and manganese were related to the content of protein measured with the fluorescamine method (Udenfriend *et al.*, 1972; Kohnert *et al.*, 1979). The recoveries of protein in the subcellular fractions compared with the homogenates were $100.4 \pm 5.3\%$. No differences were seen for the subcellular distribution of proteins between test and control incubations.

Measurements of manganese and ^{45}Ca

Manganese was determined by electrothermal atomic-absorption spectroscopy at 279.5 or

403.1nm (Rorsman *et al.*, 1982). The subcellular fractions were dissolved in an equal volume of conc. HNO_3 during 60 min of moderate shaking at 60°C. It has been demonstrated that HNO_3 does not interfere with the measurements of manganese (Rorsman & Berggren, 1982).

The radioactivities of the perifusates, dissolved islets and subcellular fractions were analysed by liquid-scintillation spectrometry after addition of 5 ml of scintillator 299. Samples of radioactive incubation media were used as external standards. In the uptake experiments, the radioactivity was expressed as mmol of ^{45}Ca /kg dry wt. or kg of protein assuming the same specific radioactivity as in the loading medium. Since isotopic equilibrium did not exist, it was impossible to estimate absolute changes in different calcium pools. The recoveries of ^{45}Ca and manganese in the subcellular fractions were $90.1 \pm 6.6\%$ and $77.0 \pm 4.2\%$ respectively, no differences being seen between test and control incubations.

Statistical evaluation of results

The data were expressed per kg dry wt. (intact islets) or per kg of protein (subcellular fractionation experiments). Statistical significances were estimated by Student's *t* test of the differences between paired test and control incubations over a series of repeated experiments.

Results

Ionic modifications of Mn^{2+} efflux

Fig. 1 shows the amounts of manganese remaining in preloaded islets after 30 min of efflux incubation in the presence of different concentrations of Mg^{2+} and Ca^{2+} . Whereas a rise of the Mg^{2+} concentration to 12 mM reduced the manganese content to 1.61 ± 0.16 mmol/kg dry wt., increasing concentrations of Ca^{2+} did not significantly influence

the manganese content. The inclusion of 0.5 mM-EGTA in a Ca^{2+} -deficient medium diminished the manganese content from 2.12 ± 0.09 to 1.63 ± 0.18 mmol/kg dry wt. ($P < 0.025$; $n = 9$).

The effects of various concentrations of the univalent cations on the Mn^{2+} efflux are shown in Table 1. Addition of 25 mM- K^+ (final concentration 30.9 mM) had no effect on the Mn^{2+} efflux. After equimolar substitution of Na^+ with choline $^+$ there

Fig. 1. Effects of different concentrations of Mg^{2+} and Ca^{2+} on the islet content of manganese

The islets were incubated for 60 min in a medium containing 1.28 mM- Ca^{2+} , 3 mM-D-glucose and 0.25 mM- Mn^{2+} and subsequently transferred for 30 min of efflux incubation to a similar Mn^{2+} -free medium containing different concentrations of Mg^{2+} (●) or Ca^{2+} (○). Mean values $\pm$ s.e.m. of nine (Mg^{2+}) or six (Ca^{2+}) experiments are shown. Differences with regard to the lowest concentrations of respective ion were evaluated by Student's *t* test of paired data. * $P < 0.05$; ** $P < 0.025$.

Table 1. Effects of ionic modifications on the islet content of manganese

The islets were incubated for 60 min in a medium containing 1.28 mM- Ca^{2+} , 3 mM-D-glucose and 0.25 mM- Mn^{2+} and subsequently transferred for 30 min of efflux incubation to a similar Mn^{2+} -free medium modified as indicated. The final concentrations after addition of K^+ and removal of Na^+ were 30.9 mM and 12 mM respectively. The Ca^{2+} concentration of <1 nM was obtained by adding 0.5 mM-EGTA to a medium already low in Ca^{2+} . Mean values $\pm$ s.e.m. of five to 13 experiments are shown. Differences with regard to the ionic modifications were evaluated by Student's *t* test. * $P < 0.05$.

		Islet content of manganese (mmol/kg dry wt.)		
Ionic modifications	[Ca^{2+}]	Control (A)	Test (B)	(B) - (A)
+25 mM- K^+	1.28 mM	2.59 ± 0.17	2.65 ± 0.20	0.06 ± 0.17
	<1 nM	1.85 ± 0.18	2.08 ± 0.22	0.23 ± 0.13
-125 mM- Na^+	1.28 mM	2.16 ± 0.14	2.18 ± 0.14	0.02 ± 0.10
	<1 nM	1.52 ± 0.21	1.95 ± 0.24	$0.43 \pm 0.16^*$

Table 2. *Effects of different agents on the islet content of manganese*

The islets were incubated for 60 min in a medium containing 1.28 mM- Ca^{2+} , 3 mM-D-glucose and 0.25 mM- Mn^{2+} and subsequently transferred for 30 min of efflux incubation to a similar Mn^{2+} -free medium modified as indicated. Mean values $\pm$ S.E.M. of five to eight experiments are shown. Differences with regard to the effects of the additives were evaluated by Student's *t* test of paired data. * $P < 0.025$.

Additive	[Ca^{2+}] (mM)	Islet content of manganese (mmol/kg dry-wt.)		
		Control (A)	Test (B)	(B) - (A)
10 μM -X537A	0.015	1.88 ± 0.20	1.54 ± 0.15	$-0.34 \pm 0.10^*$
1 mM-Tolbutamide	0.015	1.92 ± 0.15	2.31 ± 0.21	0.39 ± 0.23
0.2 mM-Tolbutamide	1.28	2.25 ± 0.15	2.70 ± 0.16	$0.45 \pm 0.15^*$
2 mM-Dibutyl cyclic AMP	1.28	2.27 ± 0.18	2.06 ± 0.17	-0.22 ± 0.28
1 mM-IBMX	1.28	2.27 ± 0.18	2.28 ± 0.21	0.01 ± 0.20

Table 3. *Effects of D-glucose on manganese contents of islet homogenates and subcellular fractions*

The islets were incubated with or without 20 mM-D-glucose for 60 min in a medium containing 1.28 mM- Ca^{2+} and 0.25 mM- Mn^{2+} . After the loading procedure, subsequent rinsing, homogenization and differential centrifugation were performed at $+4^\circ\text{C}$ in an EGTA-containing sucrose medium buffered at pH 6.0. Mean values $\pm$ S.E.M. of six experiments are shown. Differences with regard to the effect of D-glucose were evaluated by *t*-test of paired data. * $P < 0.025$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$.

Fraction	Manganese content (nmol/kg of protein)			Change (%)
	Control (A)	D-Glucose (B)	(B) - (A)	
Homogenate	6.83 ± 1.08	11.84 ± 0.80	$5.00 \pm 0.84^{***}$	+73
Nuclei + cell debris	5.08 ± 1.33	9.88 ± 1.28	$4.80 \pm 1.42^*$	+94
Mitochondria	1.73 ± 0.12	4.57 ± 0.69	$2.84 \pm 0.61^{**}$	+164
Secretory granules	2.53 ± 0.31	4.45 ± 0.26	$1.93 \pm 0.30^{****}$	+76
Microsomes	7.12 ± 0.37	6.71 ± 1.38	-0.41 ± 1.16	-6
Supernatant	8.80 ± 0.78	13.78 ± 1.47	$4.98 \pm 1.49^*$	+57

was an increased islet retention of manganese in a Ca^{2+} -deficient medium containing 0.5 mM-EGTA but not in a medium containing physiological concentrations of Ca^{2+} .

Effects of various agents on Mn^{2+} efflux

The amounts of manganese remaining in islets preloaded for 60 min with 0.25 mM- Mn^{2+} were recorded after 30 min of subsequent efflux incubation. Addition of 20 mM-D-glucose into the efflux medium resulted in a similar degree of manganese retention (0.64 ± 0.20 mmol/kg dry wt.) in the presence of 6.30 mM Ca^{2+} ($P < 0.025$; $n = 6$) as has previously been observed at extracellular Ca^{2+} concentrations of 15 μM and 1.28 mM (Rorsman *et al.*, 1982).

Table 2 shows the effects of including agents other than D-glucose in an efflux medium containing 15 μM - or 1.28 mM- Ca^{2+} . Of the agents tested only tolbutamide resembled D-glucose in tending to inhibit the efflux of Mn^{2+} . Whereas IBMX and

dibutyl cyclic AMP did not influence the content of manganese, the introduction of 10 μM -ionophore Br-X537A increased the mobilization of manganese.

Intracellular distribution of incorporated manganese

The intracellular distribution of manganese incorporated into the pancreatic β -cells is shown in Table 3. D-Glucose significantly stimulated Mn^{2+} uptake in all fractions except the microsomal one. However, the increase was most pronounced in the mitochondrial fraction, being as high as 164%.

Effects of Mn^{2+} on $^{45}\text{Ca}^{2+}$ fluxes

The islet uptake of La^{3+} -non-displaceable $^{45}\text{Ca}^{2+}$ in the presence of different concentrations of Mn^{2+} is shown in Fig. 2. Whereas 0.25 mM- Mn^{2+} was sufficient to suppress completely the glucose-stimulated $^{45}\text{Ca}^{2+}$ -uptake ($P < 0.001$), this concentration did not significantly inhibit the basal uptake. When the concentration was increased to 2.5 mM, Mn^{2+} also reduced the $^{45}\text{Ca}^{2+}$ uptake in a glucose-free medium ($P < 0.001$). At the latter concentration of

Mn^{2+} , the presence of 20 mM-D-glucose tended to further reduce the uptake of $^{45}Ca^{2+}$. The effects of 0.25 mM- Mn^{2+} on $^{45}Ca^{2+}$ uptake in subcellular

fractions are shown in Table 4. It is evident that the inhibitory action of Mn^{2+} on the $^{45}Ca^{2+}$ uptake was reflected in a reduction of radioactivity both in the postmicrosomal supernatant and in all particulate fractions.

Also the efflux of $^{45}Ca^{2+}$ was markedly influenced by Mn^{2+} . As shown in Fig. 3 (a), exposure to 0.25 mM- Mn^{2+} resulted in a transient stimulation of $^{45}Ca^{2+}$ efflux ($P < 0.05$) followed by an inhibitory phase after the withdrawal of manganese ($P < 0.01$). In the presence of 2.5 mM- Mn^{2+} (Fig. 3b), a pronounced stimulatory peak ($P < 0.025$) was followed by a rapid decline, resulting in a significant inhibition during the exposure to Mn^{2+} ($P < 0.01$).

Discussion

Mn^{2+} has been reported to substitute for Ca^{2+} and Mg^{2+} in various biological processes (Chiesi & Inesi, 1981). The present study provides evidence that Mn^{2+} interacts with Ca^{2+} and Mg^{2+} also in the β -cell-rich pancreatic islets from *ob/ob* mice. Whereas increasing concentrations of Mg^{2+} significantly reduced the manganese content of preloaded islets, Ca^{2+} was seemingly ineffective. However, the application of the sensitive perfusion technique to islets preloaded with $^{45}Ca^{2+}$ at a high glucose concentration made it possible to demonstrate that as little as 0.25 mM- Mn^{2+} was sufficient to stimulate $^{45}Ca^{2+}$ efflux into a Ca^{2+} -deficient medium, a condition minimizing the exchange with Ca^{2+} entering the β -cells. It has been shown with both n.m.r. and e.s.r. techniques that Mn^{2+} binds intracellularly to both Mg^{2+} and Ca^{2+} sites (Impraim *et al.*, 1979). The intracellular concentrations of magnesium are known to remain fairly stable, subject to only minor changes, with great variations of the extracellular $[Mg^{2+}]$ (Bergsten *et al.*, 1982). It is therefore likely that elevated extracellular Mg^{2+} essentially displaces manganese bound to the exterior of the

Fig. 2. Effects of different concentrations of Mn^{2+} on the intracellular uptake of $^{45}Ca^{2+}$ in the presence or absence of D-glucose

The islets were incubated with $^{45}Ca^{2+}$ for 90 min in the presence (O) or absence (●) of 20 mM-D-glucose at different concentrations of Mn^{2+} . Incubation was followed by washing with a cold solution of La^{3+} . Mean values $\pm$ s.e.m. of eight or ten experiments are shown. Differences with regard to the effects of glucose were evaluated by *t* test of paired data. * $P < 0.05$; ** $P < 0.001$.

Table 4. Effects of Mn^{2+} on $^{45}Ca^{2+}$ contents of homogenates and subcellular fractions

The islets were incubated for 60 min in a medium containing 1.28 mM- $^{45}Ca^{2+}$, 20 mM-D-glucose and 0 or 0.25 mM- Mn^{2+} . After incubation, subsequent rinsing, homogenization and differential centrifugation were performed at $+4^{\circ}C$ in an EGTA-containing medium buffered at pH 6.0. Mean values $\pm$ s.e.m. of six experiments are shown. Differences with regard to the effects of Mn^{2+} were evaluated by Student's *t* test of paired data. * $P < 0.05$; ** $P < 0.025$; *** $P < 0.005$.

Fraction	$^{45}Ca^{2+}$ content (mmol/kg of protein)			Change (%)
	Control (A)	Test (B)	(B) - (A)	
Homogenate	12.65 $\pm$ 2.26	6.70 $\pm$ 1.16	-5.95 $\pm$ 1.16***	-47
Nuclei + cell debris	9.55 $\pm$ 2.61	3.88 $\pm$ 1.02	-5.67 $\pm$ 1.65**	-59
Mitochondria	7.52 $\pm$ 1.80	2.30 $\pm$ 0.32	-5.22 $\pm$ 1.59**	-69
Secretory granules	6.97 $\pm$ 1.61	3.35 $\pm$ 0.68	-3.62 $\pm$ 1.12**	-52
Microsomes	3.08 $\pm$ 0.62	1.92 $\pm$ 0.69	-1.17 $\pm$ 0.44**	-38
Supernatant	15.50 $\pm$ 1.89	9.92 $\pm$ 1.60	-5.58 $\pm$ 1.77*	-36

Fig. 3. Effects of Mn^{2+} on the efflux of $^{45}Ca^{2+}$ in media deficient in Ca^{2+}

The experiments were performed in the parallel channels of a perfusion apparatus with islets loaded for 90 min with $1.28\text{ mM-}^{45}Ca^{2+}$ in the presence of 20 mM-D-glucose . The islets were perfused with a Ca^{2+} -deficient medium (approximately $15\text{ }\mu\text{M}$) containing 3 mM-D-glucose and $1\text{ mg of albumin/ml}$. Mn^{2+} ($\bullet$) was introduced at a concentration of 0.25 mM (a) or 2.5 mM (b) during the period indicated by the horizontal black bar. $\circ$, Control experiments without exposure to Mn^{2+} . The efflux of $^{45}Ca^{2+}$ is given as a percentage of that noted in the individual experiments during the 45th or 60th min of perfusion. Mean values $\pm$ S.E.M. of four to five experiments are shown.

β -cells, leaving the intracellular stores more or less intact. In support of this idea, 0.5 mM-EGTA was found to remove similar amounts of manganese as did the highest tested concentration of Mg^{2+} .

The present study confirms the previous observation of an inhibitory action of D -glucose on Mn^{2+} efflux (Rorsman *et al.*, 1982). The rate of Ca^{2+} influx is known to be dependent on the extracellular concentration of the ion, more Ca^{2+} entering into the β -cells at high than at low concentrations (Abrahamsson *et al.*, 1981). Nevertheless, the effect of D -glucose on Mn^{2+} efflux was of the same magnitude despite a 400-fold variation of the extracellular Ca^{2+} concentration. This observation suggests that the glucose-induced retention of manganese is accounted for by mechanisms other than increased competition with Ca^{2+} for the outward transport across the plasma membrane. It is pertinent to note that an increase in the K^+ concentration, which mimics the effect of glucose in stimulating the influx of Ca^{2+} after opening of voltage-dependent channels (Andersson *et al.*, 1980), failed to affect the rate of Mn^{2+} efflux.

In contrast with the observations made after

elevation of K^+ , the effect of tolbutamide resembled that of D -glucose in inhibiting the Mn^{2+} efflux. Although this sulphonylurea and an increased extracellular K^+ share the ability to facilitate Ca^{2+} influx by depolarizing the β -cells (Henquin, 1980; Hellman, 1981), the effects on the intracellular distribution of Ca^{2+} differ. The calcium incorporated in response to excessive K^+ is deposited into more readily mobilizable pools than that taken up during exposure to D -glucose or tolbutamide (Hellman, 1981; Andersson *et al.*, 1982). Also the absence of effects of dibutyryl cyclic AMP and IBMX can be reconciled with the existence of a relationship between inhibition of Mn^{2+} efflux and the sequestration of this ion into pools of low mobility. Increase of cyclic AMP leads to stimulated efflux of $^{45}Ca^{2+}$ (Gylfe & Hellman, 1981), which can be accounted for by reduced incorporation of $^{45}Ca^{2+}$ into the mitochondria (Hahn *et al.*, 1980).

The equimolar substitution of Na^+ with choline⁺ was associated with a retention of manganese in a Ca^{2+} -deficient medium supplemented with 0.5 mM-EGTA . It is therefore probable that Na^+/Mn^{2+}

exchange participates in the extrusion of Mn^{2+} from the pancreatic β -cells. The fact that Na^+ -removal did not inhibit Mn^{2+} efflux in the presence of 1.28 mM- Ca^{2+} is probably the result of an increased intracellular concentration of Ca^{2+} exchanging with the manganese stored in the β -cells. Removal of Na^+ has opposite effects on the efflux of $^{45}Ca^{2+}$ in the absence or presence of extracellular Ca^{2+} , some stimulation being observed during the latter condition (Hellman *et al.*, 1980). The action of the sulphonylurea compound tolbutamide in enhancing the retention of manganese is in definite contrast with the mobilizing effect of the ionophore Br-X537A. This difference adds to the previous arguments (Hellman, 1981) against the idea that the insulin-releasing effect of the sulphonylurea is due to an ionophoretic action (Couturier & Malaisse, 1980).

Whereas Mn^{2+} is known to block the voltage-dependent Ca^{2+} channel of the plasma membrane (Rosenberger & Triggle, 1978) it may also affect the intracellular Ca^{2+} transport. At a concentration of 0.25 mM, Mn^{2+} abolished the glucose-stimulated uptake of $^{45}Ca^{2+}$ into the islets but had only minor effects on the basal uptake. The inhibition of the glucose-stimulated $^{45}Ca^{2+}$ -uptake noted in the presence of 0.25 mM- Mn^{2+} was reflected in a diminished incorporation of the isotope into the mitochondrial as well as the other subcellular fractions. A 10-fold concentration of Mn^{2+} produced a further decrease in the basal uptake of $^{45}Ca^{2+}$. Under the latter condition, the presence of 20 mM-D-glucose resulted in a reversed pattern of $^{45}Ca^{2+}$ uptake. This paradoxical inhibitory action of D-glucose on the $^{45}Ca^{2+}$ uptake might result from the sugar-induced accumulation of intracellular manganese, which interferes with the incorporation of $^{45}Ca^{2+}$ into the organelles.

The significance of the intracellular organelles for the trapping of manganese during glucose stimulation was evident from the subcellular fractionation experiments. Contrary to what has been observed in other tissues (Beliles, 1975) there was a poor incorporation *in situ* into the β -cell mitochondria during basal conditions. The manganese concentration of the mitochondrial fraction was only 25% of that in the homogenate when expressed in relation to the protein content. However, after glucose stimulation the manganese concentration of the mitochondrial fraction rose by 164%, which is more than twice as much as noted for the secretory granules. As a matter of fact, the glucose effect on the mitochondria can be expected to be even more impressive, since this fraction is contaminated with secretory granules (Kohnert *et al.*, 1979).

Previous studies have demonstrated a pronounced glucose stimulation of the net uptake of $^{45}Ca^{2+}$ in identically prepared mitochondrial fractions from

the islets of the *ob/ob* mice (Kohnert *et al.*, 1979; Hahn *et al.*, 1980; Andersson *et al.*, 1982). Whereas the influxes of Mn^{2+} and Ca^{2+} into the mitochondria are considered to be mediated by the same carrier, there is evidence that the effluxes of the ions from the mitochondria represent separate processes with different biochemical features (Gunter *et al.*, 1978; Hunter *et al.*, 1980). With the demonstration of a marked glucose action on the accumulation of both ^{45}Ca and manganese it seems likely that the primary effect of glucose is to stimulate Ca^{2+} influx into the mitochondria. Increased mitochondrial accumulation should be considered as an attractive alternative to the idea of inhibition at the plasma membrane in the attempts to explain the glucose-induced inhibition of $^{45}Ca^{2+}$ efflux from pancreatic β -cells.

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