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Manganese accumulation in pancreatic β-cells and its stimulation by glucose

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Electrothermal atomic-absorption spectroscopy was employed for measuring manganese in β-cell-rich pancreatic islets microdissected from *ob/ob* mice. The islet content of endogenous manganese was 80 μmol/kg dry wt., which is about half as much as found in the exocrine pancreas. The initial uptake was characterized by two components, with approximate K_m values of 35 μM and 3.7 mM respectively. After 60 min of incubation with 0.25 mM-Mn^{2+}, the intracellular concentration of manganese corresponded to an almost 25-fold accumulation compared with that of the extracellular medium. When exposed to 20 mM-D-glucose, the islets retained more manganese, owing to suppression of its mobilization. The glucose inhibition of efflux was prompt and reversible, as indicated from direct recordings of manganese in a perifusion medium. D-Glucose was an equally potent inhibitor of efflux in the presence of 15 μM- and 1.28 mM-Ca^{2+}. The inhibitory action disappeared when metabolism was suppressed by adding 0.1 mM-*N*-ethylmaleimide or by lowering the temperature from 37°C to 2°C. At a concentration of 0.25 mM, Mn^{2+} abolished the insulin-releasing action of D-glucose, exerting only moderate suppression of its metabolism. The addition of Mn^{2+} resulted in inhibition of basal insulin release in the presence of 1.28 mM-Ca^{2+}, but not in a Ca^{2+}-deficient medium. The studies indicate that the previously observed phenomenon of glucose inhibition of ^{45}Ca efflux has a counterpart in the suppression of manganese mobilization from the pancreatic islets. With the demonstration of a pronounced glucose inhibition of manganese efflux, it is evident that Mn^{2+} may represent a useful tool for exploring the mechanism of glucose-induced retention of calcium in the pancreatic β-cells.

The manganese ion has been used extensively as a blocker of Ca^{2+} conductance in various excitable cells (Rosenberger & Triggle, 1978). Although Mn^{2+} was originally reported not to permeate measurably through the membrane of crustacean muscle (Fatt & Ginsborg, 1958), subsequent studies have demonstrated its intracellular penetration into muscles (Ochi, 1970, 1976; Delahayes, 1975; Fukuda & Kawa, 1977) and other excitable cells (Baker & Glitsch, 1975; Arqueros & Daniels, 1981). Like Ca^{2+}, intracellular Mn^{2+} is sequestered by mitochondria (Vainio *et al.*, 1970; Gunter *et al.*, 1978; Pfeiffer *et al.*, 1978) and secretory granules (Daniels *et al.*, 1979; Arqueros & Daniels, 1981). Further support for a link between the bivalent cations has been provided by the demonstration that Mn^{2+} stimulates ^{45}Ca efflux from the rat pancreas (Argent *et al.*, 1972) and binds to Ca^{2+}-dependent regulatory protein (Teo & Wang, 1973; Lin *et al.*, 1974; Woolf *et al.*, 1977).

It has been reported that Mn^{2+} inhibits both the electrical activity (Dean & Matthews, 1970; Meissner *et al.*, 1980; Ribalet & Beigelman, 1980) and the release of insulin (Hermansen & Iversen, 1978) obtained after introducing glucose into a medium containing physiological concentrations of Ca^{2+}. In the present study, Mn^{2+} fluxes and related effects on secretory activity and metabolism have been studied by using microdissected pancreatic islets rich in β-cells. Since exposure to D-glucose resulted in the retention of manganese, this ion represents a useful tool for elucidating how the functionally important Ca^{2+} is regulated in the pancreatic β-cells.

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Materials and methods

Chemicals

Chemicals of analytical grade and deionized water were used. E. Merck, Darmstadt, Germany, supplied $MnCl_2,4H_2O$. Hyamine and Instafluor were obtained from Packard Instruments, Warrenville, IL, U.S.A. Sigma Chemical Co., St. Louis, MO, U.S.A., provided Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], *N*-ethylmaleimide, EGTA, 3-*O*-methyl-D-glucose and bovine serum albumin (fraction V). D-[U-^{14}C]Glucose was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and ^{125}I-labelled pig insulin was given by Farbwerke Hoechst A.G., Frankfurt/Main, Germany. Crystalline mouse insulin was a gift from Novo A/S, Copenhagen, Denmark, and insulin antibodies were prepared in guinea pigs by using crystalline pig insulin from the same source.

Animals and isolation of islets

Adult obese–hyperglycaemic mice (gene symbol *ob/ob*) of both sexes were taken from a local non-inbred colony (Hellman, 1965). Fresh pancreatic islets were isolated at 4°C by free-hand microdissection from mice starved overnight. These islets contain more than 90% β-cells known to respond appropriately to glucose and other stimulators of insulin release (Hellman, 1970; Hahn *et al.*, 1974).

General aspects of incubations

The basal medium used for the studies of the manganese fluxes, glucose oxidation and insulin release was a Hepes buffer, pH 7.4, physiologically balanced in cations with Cl^- as the sole anion (Hellman, 1975). Incubation of the islets was usually done at 37°C in the presence of 1.28 mM-Ca^{2+}. In the manganese-uptake experiments, this was followed by washing for 30 min at 2°C in 5 ml of glucose-free medium deficient in calcium (actual concn. approx. 15 μM). The concentration of Ca^{2+} in the medium was determined with an Orion microprocessor ionalyzer (Orion Research, Cambridge, MA, U.S.A.) equipped with F2110 Ca^{2+} and K4040 calomel electrodes (Radiometer A/S, Copenhagen, Denmark). Except in the efflux experiments, the islets were allowed to equilibrate during a preincubation period of 30 min. Modification of the basic composition of the media with regard to the Ca^{2+} concentration and additives such as D-glucose are given in the legends to Figures and Tables. At the end of the experiments, the islets were plunged into melting isopentane, freeze-dried overnight and weighed on a quartz-fibre balance.

Manganese fluxes

The curves describing the uptake and efflux of manganese for islets incubated for different periods of time were determined from the experimental values by a computerized non-linear least-squares analysis based on the following exponential equations:

$$\text{Uptake: } V = A_1(1-e^{-k_1 t}) + A_2(1-e^{-k_2 t}) \quad (1)$$

$$\text{Efflux: } V = B_1 e^{-\lambda_1 t} + B_2 e^{-\lambda_2 t} \quad (2)$$

where V is the amount of manganese in the islets at time t, and k and λ are the rate constants of manganese transfer. A is the asymptotic manganese content and B the content of manganese before starting the efflux incubation.

In the uptake experiments, groups of five or six islets were incubated for different periods of time in 1 ml of Mn^{2+}-containing medium followed by the cold washing. The kinetic constants for the concentration and time dependences of uptake were derived as described by Spears *et al.* (1971) and Borle (1969) respectively. Two different techniques were used for studying the mobilization of manganese. In the first series of experiments, groups of five or six islets were loaded for 60 min with 0.25 mM-Mn^{2+} in the presence of 3 mM-glucose and then transferred to 5 ml of Mn^{2+}-free medium for further efflux incubation at 37°C in the presence or absence of 20 mM-glucose. In the other type of experiments the dynamics of the manganese efflux were recorded in a perifusion medium (Gylfe & Hellman, 1978). Groups of ten islets were loaded for 90 min with 2.5 mM-Mn^{2+} in the presence of 3 mM-glucose. The islets were then transferred to 10 μl chambers and perifused at a constant rate of approx. 40 μl/min. In each experiment, two different chambers were loaded with islets from the same animal and run in parallel. The use of two reservoirs made it possible to make sudden alterations in the composition of the medium supplied to the islets.

Measurements of manganese

Manganese was measured in a graphite-tube furnace mounted on a single-beam atomic-absorption spectrophotometer (Varian Techtron AA-6) with a peak reader module (Lundberg, 1978*a*). The time constant of the signal damping of the AA-6 readout module was decreased to 47 ms (Lundberg, 1978*b*). The spectrophotometer was operated with a spectral band width of 0.20 nm at a wavelength of 403.1 nm and a lamp current of 7 mA. To obtain sufficient sensitivity for the measurements of endogenous manganese and that appearing in the perifusates, the procedure was modified to use the 279.5 nm line, a lamp current of 4 mA and a hydrogen continuum lamp for background correction. The graphite furnace was flushed with argon (5 litres/min) and its heating rate controlled independently of the final temperature as described by Lundgren (1975). A three-step temperature pro-

gram was employed, involving drying for 65s at 150°C, ashing for 50s at 930°C (279.5nm) or 790°C (403.1nm) and atomization for 3s at 2260°C. Freeze-dried specimens, representing single islets and equal-sized pieces of exocrine pancreas, or 2μl of perifusion medium were placed directly into the graphite furnace. The reproducible calibration curves showed some deviation from linearity (Fig. 1). The coefficient of variation recorded over a period of 1 year for a standard solution of $MnCl_2$ in deionized water was ±13.67% ($n = 12$) for 1.82pmol measured at 279.5nm and ±17.87% ($n = 48$) for 18.2pmol measured at 403.1nm. The recovery of manganese added to the islets was 94.6 ± 13.4% for 1.82pmol ($n = 19$) and 106.5 ± 2.0% ($n = 6$) for 18.2pmol. The corresponding values for the recovery of manganese from the perifusion medium were 100.2 ± 3.1% ($n = 10$) and 91.7 ± 2.4% ($n = 6$) respectively.

Glucose oxidation

Groups of two islets were incubated for 60min in liquid-scintillation vials equipped with a small glass centre well (Keen *et al.*, 1963) containing 100μl of medium supplemented with 20mM-D-[U-^{14}C]-glucose (1.50Ci/mol). Blank values were obtained from incubations without islets. Metabolism was stopped with 100μl of 0.1M-HCl, and $^{14}CO_2$ was collected in 100μl of Hyamine as previously described (Hellman *et al.*, 1971).

Insulin release

Batches of two islets were incubated for 60min in 200μl of albumin-containing (1mg/ml) medium, and insulin was measured radioimmunologically by using crystalline mouse insulin as a reference. Free and antibody-bound insulin were separated by precipitation with ethanol (Heding, 1966). The values for insulin release are given with correction for a moderate interference with the radioimmunoassay (approx. 15%) for the highest manganese concentration encountered.

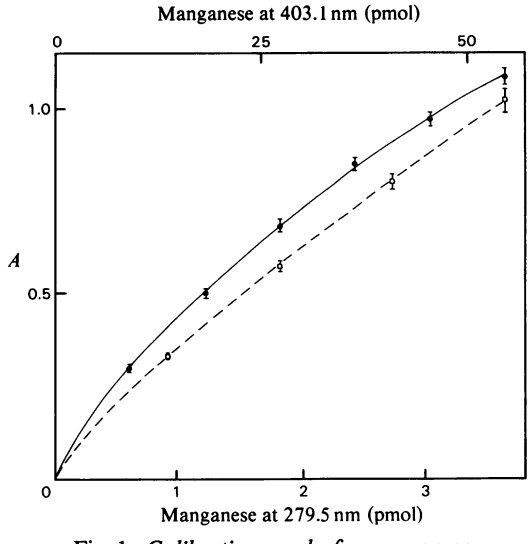

Fig. 1. *Calibration graphs for manganese*
Manganese was measured with different sensitivities at two wavelengths by using electrothermal atomic-absorption spectroscopy. The less sensitive approach (403.1nm, ●) was used when measuring the amounts incorporated into the islets, whereas the other approach (279.5nm, □) was utilized for the minute amounts present in perifusates or in islets not exposed to manganese. Mean values ± S.E.M. for 48 and 12 experiments respectively are shown.

Results

Islet content of manganese after incubation in the absence of Mn^{2+}

The endogenous concentrations of manganese were recorded after incubation of the islets in media lacking detectable amounts of the element. After 90min of incubation in the presence of 1.28mM-Ca^{2+} and 3mM-glucose, the islets contained 0.08 ± 0.01mmol/kg dry wt. (mean value ± S.E.M. for 15 experiments). Similarly treated pieces of exocrine pancreas contained 0.16 ± 0.01mmol/kg dry wt. ($P < 0.001$). An increase of the glucose concentration to 20mM during the final 30min of incubation did not significantly influence the amounts of manganese found in the islets.

Islet uptake of manganese

The manganese contents of islets incubated for various periods of time with 0.25mM-Mn^{2+} are shown in Fig. 2. No steady state was reached during the observation period of 90min. By plotting dV/dt semilogarithmically, two distinct components of manganese uptake were displayed. The rate constants were 0.279min^{-1} and $4.75 \times 10^{-4}min^{-1}$ for the fast and slow phases respectively. Corresponding values for the asymptotic manganese contents were 1.7 and 83.4mmol/kg dry wt. The concentration-dependence of the initial uptake was studied by incubating the islets for 3min with different concentrations of Mn^{2+} (Fig. 3). As indicated from the inset, two different uptake components could be demonstrated with a Hanes–Woolf plot (Segel, 1975). For the high-affinity uptake K_m was estimated as 35μM, with a $V_{max.}$ of 0.6mmol/kg dry wt., and for the low-affinity uptake K_m was 3.7mM, with a $V_{max.}$ of 10.8mmol/kg dry wt. The effect of D-glucose on the Mn^{2+} uptake at physiological Ca^{2+} concentrations is shown in Table 1. D-Glucose did not significantly stimulate the initial

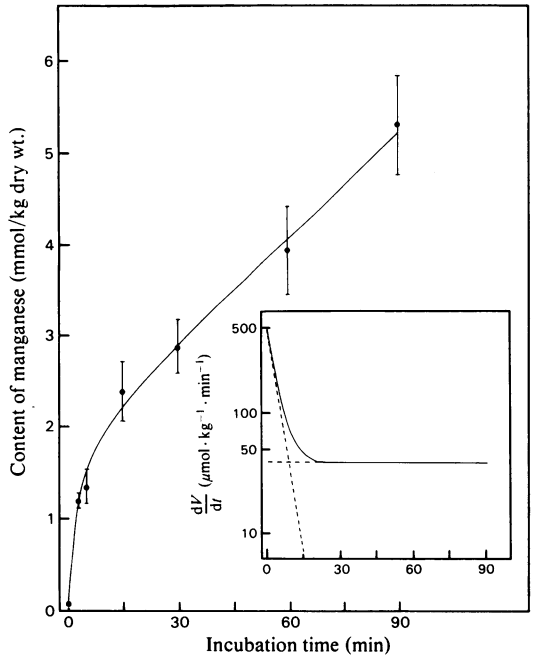

Fig. 2. *Dynamics of the islet Mn^{2+} uptake*
After 30 min of preincubation the islets were loaded for 3–90 min with 0.25 mM-Mn^{2+} in glucose-free medium containing 1.28 mM-Ca^{2+}. After loading, the islets were washed for 30 min at +2°C in glucose-free medium deficient in Ca^{2+} (15 μM). The uptake curve was determined by a computerized non-linear-regression analysis of the experimental data. Mean values ± S.E.M. for four experiments are shown. The inset shows dV/dt of the uptake curve plotted semilogarithmically.

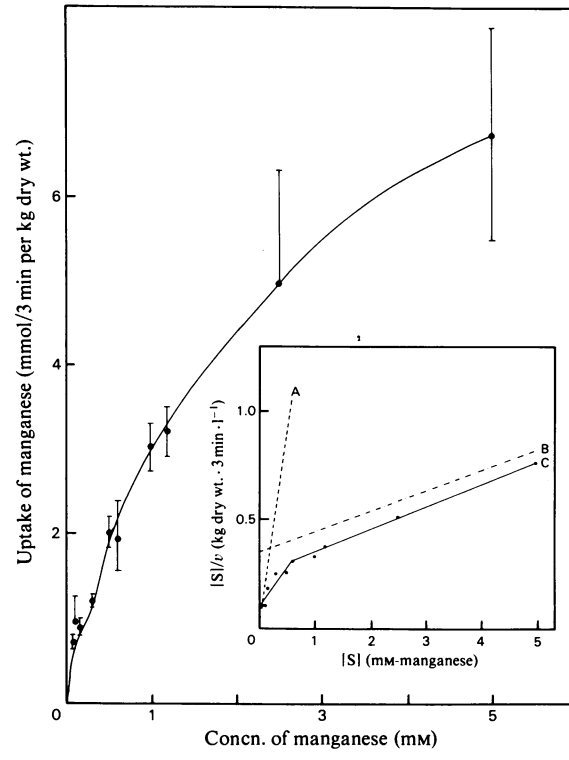

Fig. 3. *Concentration-dependence of the islet uptake of Mn^{2+}*
After 30 min of preincubation the islets were loaded for 3 min with 0.075–5.0 mM-Mn^{2+} in glucose-free medium containing 1.28 mM-Ca^{2+}. After loading, the islets were washed for 30 min at 2°C in glucose-free medium deficient in Ca^{2+} (15 μM). Mean values ± S.E.M. for four to seven experiments are shown. The inset shows a Hanes–Woolf plot of the mean values, giving $V_{max.}$ as the inverse of the slope and K_m as the negative intercept on the abscissa. A, B and C represent the low-K_m–low-$V_{max.}$, the high-K_m–high-$V_{max.}$ and the sum of the two components respectively.

(3 min) uptake when Mn^{2+} was present at concentrations intended to reveal the high- and low-affinity components. At 0.05 mM-Mn^{2+} some inhibition was noted. The lack of a stimulatory effect of D-glucose on the initial uptake differed from that observed after incubation of islets for 60 min. In the latter case, exposure to 20 mM-D-glucose increased the amounts of manganese incorporated compared with controls incubated with an equimolar concentration of 3-*O*-methyl-D-glucose. Table 2 indicates the effects of including 0.5 mM-EGTA in the cold medium used for the final washing of the islets in the uptake experiments. Whereas the presence of the chelator resulted in a significant decrease of the islet content of manganese, it did not affect the additional amounts of manganese taken up in response to D-glucose during 60 min of incubation.

Mobilization of incorporated islet manganese

Fig. 4 shows the amounts of manganese remaining in pre-loaded islets after different periods of efflux at 37°C in medium lacking Mn^{2+} but containing 1.28 mM-Ca^{2+}. After an initial rapid loss, the islet content of manganese approached a steady state. The inset in Fig. 4 shows that the disappearance curve resembled that for the uptake in displaying two distinct phases manifested as linear functions in a semilogarithmic plot. The presence of 20 mM-D-glucose decreased the amounts of manganese mobilized during the second phase, leading to a prolongation of the time necessary for reaching the steady state. The rate constants for the rapid phases were 0.271 and 0.261 min^{-1} with and without D-glucose. The corresponding values for the slow phases were $2.62 \times 10^{-3} min^{-1}$ in the presence of

Table 1. *Effect of glucose on the islet uptake of manganese*

After 30min of preincubation in a medium containing 1.28mM-Ca^{2+} and 20mM-3-*O*-methyl-D-glucose or -D-glucose, the islets were incubated for 3 or 60min in media of similar composition with 0.05mM-, 0.25mM- or 2.50mM-Mn^{2+}. After the loading with Mn^{2+} the islets were washed for 30min at +2°C in glucose-free medium deficient in Ca^{2+} (15μM). Mean values ± S.E.M. for the indicated number of experiments are shown. Statistical significances are shown for the effect of D-glucose: $*P < 0.05$; $***P < 0.001$.

		Islet content of manganese (mmol/kg dry wt.)		
Incubation time (min)	Mn^{2+} concn. (mM)	3-*O*-Methylglucose (*A*)	D-Glucose (*B*)	(*B*)−(*A*)
3	0.05	0.59 ± 0.08 (5)	0.36 ± 0.05 (5)	−0.24 ± 0.09*
3	0.25	1.19 ± 0.08 (7)	1.44 ± 0.29 (7)	0.25 ± 0.30
3	2.50	4.02 ± 0.52 (6)	4.92 ± 0.73 (6)	0.90 ± 0.92
60	0.25	3.45 ± 0.40 (7)	6.19 ± 0.45 (7)	2.74 ± 0.60***

Table 2. *Effect of EGTA-washing on the islet manganese incorporated in response to D-glucose*

After 30min of preincubation the islets were loaded for 60min with 0.25mM-Mn^{2+} in the presence or absence of 20mM-D-glucose in medium containing 1.28mM-Ca^{2+}. After loading the islets were washed for 30min at 2°C in glucose-free and Ca^{2+}-deficient medium supplemented or not with 0.5mM-EGTA. Mean values ± S.E.M. for five experiments are shown. Statistical significances are shown for the effect of D-glucose and for washing with EGTA: $*P < 0.05$; $**P < 0.01$.

	Islet content of manganese (mmol/kg dry wt.)		
Additive	Washing without EGTA (*A*)	Washing with EGTA (*B*)	(*B*)−(*A*)
None	4.68 ± 0.46	2.92 ± 0.23	−1.76 ± 0.51**
20mM-D-glucose	7.02 ± 0.34**	4.75 ± 0.67*	−2.27 ± 1.10*

Table 3. *Effect of glucose on the mobilization of islet manganese under different experimental conditions*

The islets were loaded for 60min with 0.25mM-Mn^{2+} in the presence of 3mM-D-glucose in medium containing 1.28mM-Ca^{2+}. The remaining manganese was measured after 30min of subsequent efflux incubation in the absence of Mn^{2+} in media of different Ca^{2+} concentrations supplemented or not (control) with 20mM-D-glucose, 0.5mM-EGTA and 0.1mM-*N*-ethylmaleimide (NEM) as indicated. Mean values ± S.E.M. for *n* experiments are shown. Statistical significances were judged from the differences between paired incubations performed in the presence or absence of D-glucose: $*P < 0.05$; $**P < 0.01$.

				Islet content of manganese (mmol/kg dry wt.)		
Temperature (°C)	Ca^{2+} (mM)	Additives	*n*	Control (*A*)	D-Glucose (*B*)	(*B*)−(*A*)
37	0.015		5	3.32 ± 0.32	3.88 ± 0.46	0.57 ± 0.19*
37	1.28		11	2.75 ± 0.19	3.41 ± 0.32	0.66 ± 0.20**
37	0.015	0.1mM-NEM	5	3.83 ± 0.48	3.44 ± 0.62	−0.39 ± 0.33
2	0.015		6	5.23 ± 0.76	4.76 ± 0.72	−0.47 ± 0.28
2	~10^{-6}	0.5mM-EGTA	5	3.85 ± 0.43	3.59 ± 0.38	−0.26 ± 0.14

D-glucose and approximately zero in the absence of the sugar. D-Glucose inhibited Mn^{2+} efflux also in the presence of 15μM-Ca^{2+}, and this inhibitory effect was abolished by 0.1mM-*N*-ethylmaleimide or by lowering the temperature from 37°C to 2°C (Table 3). The dynamics of the efflux were also studied by continuous recordings of the manganese appearing in the medium during perifusion of the islets (Fig. 5). After addition of 20mM-D-glucose there was a prompt inhibition of the manganese efflux, which

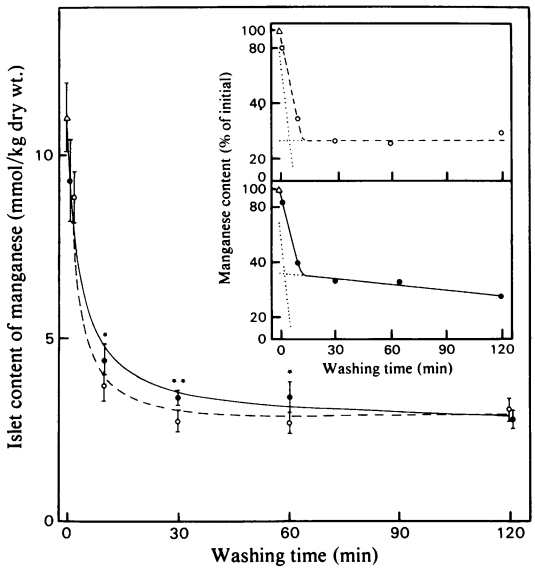

Fig. 4. *Islet content of manganese after different periods of efflux*

The islets were loaded for 60min with 0.25mM-Mn^{2+} in the presence of 3mM-D-glucose in medium containing 1.28mM-Ca^{2+}. The manganese remaining was measured after 1–120min of subsequent efflux incubation in the absence of Mn^{2+} in similarly composed medium supplemented (●) with 20mM-D-glucose or not (○). The efflux curves were determined by a computerized non-linear-regression analysis of the experimental data. The manganese content before the efflux incubation (11.09 ± 0.91 mmol/kg dry wt.) is shown as a triangle (△). Mean values ± S.E.M. for six to eleven experiments are shown. The inset shows a semi-logarithmic presentation of the data. Statistical significances were judged from the differences between paired incubations performed in the presence or absence of D-glucose: $*P<0.05$; $**P<0.01$.

was readily reversible with the omission of the sugar. Equimolar amounts of 3-*O*-methyl-D-glucose did not influence the amounts of manganese appearing in the perifusion medium.

Effect of Mn^{2+} on the islet metabolism of D-*glucose*

The effect of Mn^{2+} on the islet oxidation of D-[U-^{14}C]glucose is shown in Table 4. When present

Table 4. *Effect of manganese on islet oxidation of glucose*

After 30min of preincubation in non-radioactive medium containing 1.28mM-Ca^{2+} and 3mM-D-glucose the islets were incubated for 60min in similarly composed media supplemented with 20mM-D-[U-^{14}C]glucose. Different concentrations of Mn^{2+} were present during the preincubation and incubation periods as indicated. The amounts of $^{14}CO_2$ produced were expressed as mmol of glucose equivalents oxidized. Mean values ± S.E.M. for indicated numbers of experiments are shown. Statistical significances are shown for comparison with islets not exposed to Mn^{2+}: $*P<0.05$; $**P<0.01$; $***P<0.001$.

Mn^{2+} concn. (mM)		Glucose oxidized
Preincubation	Incubation	(mmol/h per kg dry wt.)
0	0	24.9 ± 2.2 (10)
0	0.25	17.1 ± 2.2* (5)
0	1.00	8.8 ± 2.6*** (5)
0	2.50	9.1 ± 2.7*** (5)
0.25	0	24.5 ± 4.2 (5)
1.00	0	13.5 ± 2.0* (4)
2.50	0	10.4 ± 4.4** (5)

Table 5. *Effect of manganese on insulin release*

After 30min of preincubation in media containing albumin (1mg/ml) and 15μM- or 1.28mM-Ca^{2+} the islets were incubated for 60min in similarly composed media supplemented or not with 20mM-D-glucose and 0.25mM- or 2.5mM-Mn^{2+} as indicated. Mean values ± S.E.M. for seven experiments are shown. Statistical significances are given for the effects of Mn^{2+} and D-glucose: $*P<0.05$; $**P<0.01$; $***P<0.001$.

Ca^{2+} concn. (mM)	Mn^{2+} concn. (mM)	Insulin release (ng/h per μg dry wt.) Control (*A*)	20mM-D-glucose (*B*)	(*B*) − (*A*)
0.015	0	0.45 ± 0.16	0.39 ± 0.15	−0.06 ± 0.22
0.015	0.25	0.47 ± 0.13	0.48 ± 0.38	0.01 ± 0.40
0.015	2.50	0.79 ± 0.44	0.32 ± 0.13	−0.47 ± 0.46
1.28	0	0.64 ± 0.16	1.86 ± 0.25	1.22 ± 0.30**
1.28	0.25	0.21 ± 0.06*	0.40 ± 0.14***	0.19 ± 0.15
1.28	2.50	0.09 ± 0.02**	0.07 ± 0.02***	−0.02 ± 0.03

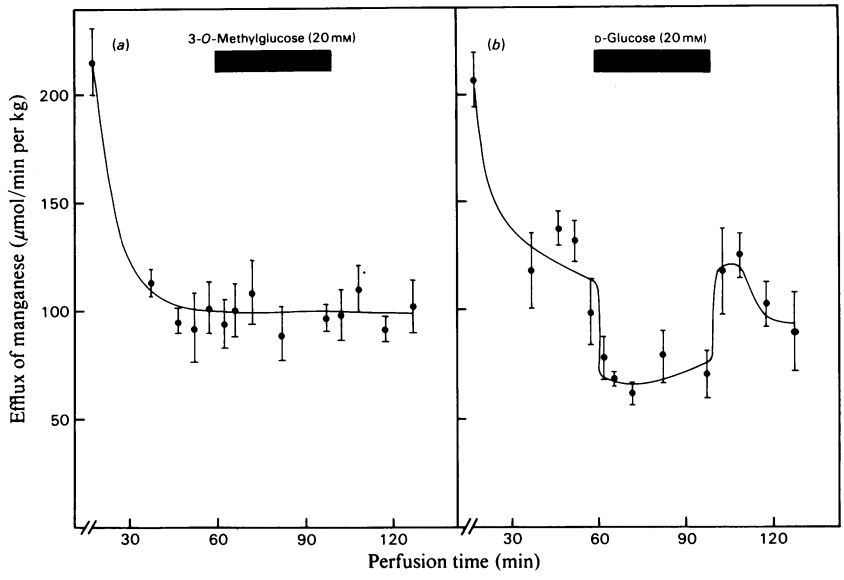

Fig. 5. *Effect of glucose on islet manganese efflux*

The experiments were performed in the parallel channels of a perifusion apparatus with islets loaded for 90 min with 2.5 mM-Mn^{2+} in the presence of 3 mM-D-glucose and 1.28 mM-Ca^{2+}. During the perifusion, the islets were exposed to similarly composed medium lacking Mn^{2+} but supplemented with 20 mM-3-*O*-methyl-D-glucose (*a*) or 20 mM-D-glucose (*b*) during the periods indicated by the horizontal black bars. Mean values ± S.E.M. for four or five experiments are shown.

during the recordings of the oxidation rates, Mn^{2+} was moderately inhibitory (31%) at 0.25 mM and exhibited pronounced inhibition at concentrations of 1.0 mM and 2.5 mM. The effect became less prominent when exposure to Mn^{2+} was followed by brief washing and glucose oxidation was measured in medium lacking this ion. Under the latter conditions 0.25 mM-Mn^{2+} did not affect the rate of $^{14}CO_2$ production.

Effect of Mn^{2+} on basal and glucose-stimulated insulin release

Table 5 shows the effect of Mn^{2+} on the amounts of insulin released into media containing 15 μM- or 1.28 mM-Ca^{2+}. Whereas manganese was without effect on the release of insulin into a medium deficient in Ca^{2+}, 0.25 mM-Mn^{2+} was sufficient for suppressing the stimulatory effect of D-glucose otherwise seen in the presence of the physiological Ca^{2+} concentration. Moreover, in the latter case the exposure to Mn^{2+} resulted also in an inhibition of insulin release obtained in the absence of D-glucose.

Discussion

Manganese is known to be a normal constituent of animal cells. It was therefore important to explore to what extent endogenous manganese might contribute to the amounts recorded in the experiments. The amounts of manganese in the β-cell-rich pancreatic islets of the *ob/ob* mice were of the same magnitude as reported by Havu *et al.* (1977) to be present in islets from other rodents. The inability of D-glucose to affect the endogenous amounts suggests that this manganese represents a stable pool. With the observation that the exocrine pancreas contained higher concentrations of manganese than the islets, it seems less likely that this metal participates in the storage of insulin in the β-granules.

The uptake and efflux of manganese both displayed a slow and a rapid phase. These transport processes did not differ to any great extent with regard to the rate constants for either phase. The demonstration of two components for the uptake and efflux of manganese is analogous to that reported for ^{45}Ca fluxes in various cells (Borle, 1969, 1981), including the pancreatic β-cells (Naber *et al.*, 1977). Furthermore, radioisotope studies have indicated that the mobilization of ^{54}Mn from isolated cells resembles that of ^{45}Ca (Impraim *et al.*, 1979). Whereas the rapid phase is usually interpreted to represent a readily exchangeable pool associated with the cell surface, the slow phase can be assumed to be of intracellular origin. It is pertinent to note that the action of D-glucose appeared to be restricted

to the slow phase, no effect being seen on the initial rapid uptake and efflux.

The starting value for the slow component of manganese efflux (extrapolated) is in general agreement with the theoretical value calculated for the slow component in the uptake experiments in which the islets were subjected to cold washing. This observation supports the idea that the wash procedure predominantly removes superficial manganese. A comparison of the manganese contents of islets immediately after loading with 0.25 mM-Mn^{2+} in the presence of 3 mM-D-glucose (Fig. 4) with that remaining after 30 min of the cold-washing procedure (Table 3) indicates a loss of more than 50% or about 6 mmol/kg dry wt. The amounts of manganese bound to the cell surface may even exceed this value, since the inclusion of EGTA in the cold washing medium resulted in an additional loss of 1.5 mmol/kg dry wt. The observation of substantial amounts of superficial manganese is far from surprising, since e.p.r. measurements have indicated that as much as 40% of the manganese associated with Lettrée cells is located on the cell surface (Impraim *et al.*, 1979). The abundance of superficial binding sites for cations in the *ob/ob*-mouse islets is apparent from the observations that this preparation can accommodate large amounts of La^{3+}-displaceable ^{45}Ca (Hellman *et al.*, 1976, 1979) and ^{171}Tm (Flatt *et al.*, 1981).

There is now considerable evidence that the entry of Ca^{2+} into the pancreatic β-cells is similar to that in other excitable cells in utilizing a specific calcium channel distinct from that carrying the early sodium current (Donatsch *et al.*, 1977; Hellman *et al.*, 1979). Although Mn^{2+} is one among several bivalent cations which antagonizes the intracellular uptake of Ca^{2+}, it may also act as a substitute for intracellular Ca^{2+} in being able to permeate into the specific calcium channel (Fukuda & Kawa, 1977; Rosenberger & Triggle, 1978). The present studies provide ample support for a large driving force for the entry of Mn^{2+} into the pancreatic β-cells by demonstrating a rapid incorporation of the cation into islets subsequently subjected to cold washing. When the islets were incubated for 60 min with 0.25 mM-Mn^{2+} in the presence of D-glucose, they incorporated up to 7 mmol of manganese/kg dry wt. (Table 2), a value corresponding to 6 mM when expressed in terms of intracellular water (Hellman *et al.*, 1971). The latter concentration implies an almost 25-fold accumulation of manganese as compared with that in the extracellular medium.

Exposure to 20 mM-D-glucose resulted in an increased net accumulation of manganese in the pancreatic islets. The manganese incorporated in response to D-glucose remained unaffected during washing with EGTA, indicating its location in intracellular stores. Whereas D-glucose was without effect under conditions supposed to reflect the initial Mn^{2+} uptake, the presence of the sugar resulted in a significant inhibition of the mobilization of manganese from preloaded islets. Since D-glucose is also known to inhibit the efflux of ^{45}Ca (Malaisse *et al.*, 1973; Kikuchi *et al.*, 1978; Gylfe & Hellman, 1978; Gylfe *et al.*, 1978; Abrahamsson *et al.*, 1980), the elucidation of the mechanisms involved might aid our understanding of how Ca^{2+} is regulated in the pancreatic β-cells.

The decreased mobilization of manganese is not necessarily due to interference with its transport across the plasma membrane. It may also be due to glucose-induced retention of manganese in such organelles where the transport of Mn^{2+} and Ca^{2+} is mediated by a common carrier. Whereas the inhibitory effect of D-glucose on ^{45}Ca efflux can be demonstrated readily in a medium deficient in Ca^{2+}, it tends to be masked by a stimulatory component in the presence of physiological concentrations of Ca^{2+} (Kikuchi *et al.*, 1978; Abrahamsson *et al.*, 1980). However, D-glucose was equally potent in inhibiting the efflux of manganese irrespective of whether the concentration of extracellular calcium was 15 μM or 1.28 mM (Table 3). The ability of manganese to suppress the entry of Ca^{2+} through potential-dependent channels (Rosenberger & Triggle, 1978) may explain why the manganese efflux was clearly inhibited also in the presence of physiological concentrations of Ca^{2+}. With the demonstration that the glucose action on manganese efflux comprises only an inhibitory component, Mn^{2+} offers certain advantages for the exploration of the ionic mechanisms associated with the glucose stimulation of insulin release.

The inhibitory effect of D-glucose on manganese efflux could not be reproduced with its non-metabolizable analogue 3-*O*-methyl-D-glucose. Moreover, inhibition of efflux was not observed under conditions known to suppress the β-cell metabolism, such as lowering of the temperature (Frankel *et al.*, 1978) or exposure to 0.1 mM-*N*-ethylmaleimide (Hellman, 1979). Being dependent on the metabolism, the retention of manganese equals that observed for intracellular ^{45}Ca (Hellman, 1979). The glucose-stimulated incorporation of La^{3+}-non-displaceable ^{45}Ca differs from the basal uptake in being suppressed by metabolic inhibitors. It is also noteworthy that inhibition of metabolism results in a mobilization of the ^{45}Ca incorporated in response to D-glucose when the remaining ^{45}Ca is unaffected. When evaluating the effect of D-glucose on the uptake of Mn^{2+}, this ion was introduced in a concentration of 0.25 mM. As indicated from the oxidation experiments, this concentration of Mn^{2+} still allows a significant metabolism of glucose. In the perifusion experiments the islets were preloaded in the presence of as much as 2.5 mM-Mn^{2+} to reach

concentrations sufficient for direct recordings of the manganese in the efflux medium. D-Glucose was inhibitory also under these conditions, indicating that most of the manganese interfering with the metabolism had been washed away before the introduction of the sugar.

Mn^{2+} has been reported to have dual effects on the insulin release from isolated perfused dog pancreas (Hermansen & Iversen, 1978). Whereas 0.25 mM of this ion was found to inhibit the glucose-stimulated insulin release in the presence of physiological concentrations of Ca^{2+}, 0.5 mM proved to be stimulatory when the medium was depleted of Ca^{2+}. The present study adds to the observation of Mn^{2+} inhibition in the presence of physiological concentrations of Ca^{2+} by demonstrating that this inhibition includes not only the effects of glucose but also the basal release seen in the absence of the sugar. Moreover, the glucose-stimulated insulin release was found to be abolished at a concentration of Mn^{2+} (0.25 mM) when the glucose oxidation was only moderately suppressed. In variance with observations made with perfused dog pancreas (Hermansen & Iversen, 1978), Mn^{2+} failed to stimulate the release of insulin into a Ca^{2+}-deficient medium supplemented with 20 mM-D-glucose. However, in support for the idea that removal of Ca^{2+} alters the effects of Mn^{2+}, increasing concentrations of this ion did not suppress the basal release of insulin, as otherwise noted in the presence of 1.28 mM-Ca^{2+}. Previous studies have indicated that occupancy of the binding sites for cations in the β-cell membrane can suppress insulin release without affecting the transmembrane Ca^{2+} fluxes (Hellman *et al.*, 1979; Flatt *et al.*, 1980). Intracellular Mn^{2+} may therefore promote insulin release, although this effect is superseded by an inhibitory action of the manganese bound to the exterior of the β-cells.

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