The Pancreatic β -Cell Recognition of Insulin Secretagogues

EFFECTS OF CALCIUM AND SODIUM ON GLUCOSE METABOLISM AND INSULIN RELEASE

By BO HELLMAN, LARS-ÅKE IDAHL, ÅKE LERNMARK, JANOVE SEHLIN
and INGE-BERT TÅLJEDAL

Department of Histology, University of Umeå, Umeå, Sweden

(Received 9 July 1973)

The transport and oxidation of glucose, the content of fructose 1,6-diphosphate, and the release of insulin were studied in microdissected pancreatic islets of *ob/ob* mice incubated in Krebs–Ringer bicarbonate medium. Under control conditions glucose oxidation and insulin release showed a similar dependence on glucose concentration with the steepest slope in the range 5–12 mM. The omission of Ca^{2+} , or the substitution of choline ions for Na^+ , or the addition of diazoxide had little if any effect on glucose transport. However, Ca^{2+} or Na^+ deficiency as well as diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine 1,1-dioxide) or ouabain partially inhibited glucose oxidation. These alterations of medium composition also increased the islet content of fructose 1,6-diphosphate, as did the addition of adrenaline. Phentolamine [2-*N*-(3-hydroxyphenyl)-*p*-toluidinomethyl-2-imidazoline] counteracted the effects of adrenaline and Ca^{2+} deficiency on islet fructose 1,6-diphosphate. After equilibration in Na^+ -deficient medium, the islets exhibited an increase in basal insulin release whereas the secretory response to glucose was inhibited. The inhibitory effects of Na^+ deficiency on the secretory responses to different concentrations of glucose correlated with those on $^{14}\text{CO}_2$ production. When islets were incubated with 17 mM-glucose, the sudden replacement of Na^+ by choline ions resulted in a marked but transient stimulation of insulin release that was not accompanied by a demonstrable increase of glucose oxidation. Galactose and 3-*O*-methylglucose had no effect on glucose oxidation or on insulin release. The results are consistent with a metabolic model of the β -cell recognition of glucose as insulin secretagogue and with the assumption that Ca^{2+} or Na^+ deficiency, or the addition of adrenaline or diazoxide, inhibit insulin release at some step distal to stimulus recognition. In addition the results suggest that these conditions create a partial metabolic block of glycolysis in the β -cells. Hence the interrelationship between the processes of stimulus recognition and insulin discharge may involve a positive feedback of secretion on glucose metabolism.

Grodsky *et al.* (1963) compared the insulin-releasing capacities of various sugars and suggested that only those which are metabolizable may stimulate secretion. Ashcroft *et al.* (1970, 1972, 1973) demonstrated striking correlations between rates of insulin release and glucose metabolism in the pancreatic islets. The possibility that stimulation of insulin release enhances glucose metabolism in the β -cells was rejected on the grounds that, although Ca^{2+} is essential for secretion, the omission of Ca^{2+} did not affect glucose oxidation (Ashcroft *et al.*, 1970). However, in another study Ca^{2+} deficiency increased the islet content of glucose 6-phosphate, suggesting that the glycolytic flux was inhibited (Idahl, 1971). We have therefore investigated the effects of Ca^{2+} deficiency on glucose transport, glucose oxidation and the content of fructose 1,6-diphosphate in micro-

dissected islets of *ob/ob* mice, an islet preparation that contains more than 90% β -cells. The effects of Na^+ deficiency were also studied in some detail because Na^+ may be essential for insulin release (Hales & Milner, 1968a) as well as for glucose metabolism in the pancreatic islets (Ashcroft *et al.*, 1972). Matschinsky & Ellerman (1973) reported that galactose and 3-*O*-methylglucose accelerate lactate production in rat islets, and explained this effect as being due to increased Na^+ uptake by the β -cells. Since in the same study these glucose analogues had no effect on insulin release, the conclusion was drawn that glycolysis cannot play a role in stimulus recognition. It will be shown here that 3-*O*-methylglucose and galactose stimulate neither insulin release nor glucose oxidation in the islets of *ob/ob* mice.

Materials and Methods

Chemicals

D-[6-³H]Glucose, D-[U-¹⁴C]glucose and L-[1-¹⁴C]-glucose were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and ¹²⁵I-labelled insulin from Novo A/S, Copenhagen, Denmark, and Farbwerke Hoechst A.G., Frankfurt/Main, Germany. Insulin antibodies were from Novo A/S and Wellcome Reagents Ltd., Beckenham, Kent, U.K. Crystalline mouse insulin was prepared by Novo A/S, and human serum albumin ('grade A') was from Kabi AB, Stockholm, Sweden. Non-radioactive sugars and EGTA [ethanedioxybis(ethylamine)tetra-acetic acid] were from British Drug Houses Ltd., Poole, Dorset, U.K. Diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine 1,1-dioxide) was from Schering Corp., Bloomfield, N.J., U.S.A. Adrenaline, ouabain and choline bicarbonate were from Sigma Chemical Co., St. Louis, Mo., U.S.A. In most experiments we used CaCl₂ from Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A., KHCO₃ from British Drug Houses Ltd., and other inorganic salts from E. Merck A.G., Darmstadt, Germany. Choline chloride was from British Drug Houses Ltd. Reagents of analytical grade and distilled de-ionized water were used throughout.

Methods

The methods have been described in detail previously (Bloom *et al.*, 1972; Hellman *et al.*, 1973a). Adult *ob/ob* mice were obtained from the Umeå colony and, unless otherwise stated, starved overnight. Fresh pancreatic islets were microdissected free-hand (Hellerström, 1964) and incubated at 37°C in Krebs-Ringer bicarbonate buffer (Umbreit *et al.*, 1964) equilibrated with O₂+CO₂ (95:5). Bicarbonate buffer (1 litre) contained 143.5 mequiv. of Na⁺, 5.9 mequiv. of K⁺, 5.1 mequiv. of Ca²⁺, 2.4 mequiv. of Mg²⁺, 128.3 mequiv. of Cl⁻, 25.0 mequiv. of HCO₃⁻, 2.4 mequiv. of SO₄²⁻ and 1.2 mequiv. of H₂PO₄⁻. Alterations of the ionic composition and the presence of other additives, as well as the length of incubation periods, are given in the legends to the figures and tables.

Insulin release was studied both by incubating islets in closed vials and by determining insulin in the medium at the end of the incubation (Lernmark, 1971), and by micropertusing islets in an apparatus allowing continuous recording of the rate of secretion (Idahl, 1972). Insulin was assayed radioimmunologically with crystalline mouse insulin as a reference. Glucose transport was studied by comparing the islet uptake of D-[6-³H]glucose, which enters the β-cells by a carrier mechanism, with that of L-[1-¹⁴C]-glucose, which is restricted to the extracellular space (Hellman *et al.*, 1971a). Glucose oxidation was

measured as the production of ¹⁴CO₂ from D-[U-¹⁴C]-glucose. The radioactivity of islets or of ¹⁴CO₂ trapped in Hyamine was determined in a liquid-scintillation spectrometer by using samples of medium as external standards. Fructose 1,6-diphosphate was assayed by an enzymic fluorimetric technique employing oil wells and enzymic cycling, essentially as described by Matschinsky *et al.* (1968). All incubated islets were freeze-dried and weighed on a quartz-fibre balance.

Results

Glucose metabolism

The normal dose-response curve for glucose oxidation. Fig. 1(a) shows the dose-response relationship between glucose concentration and the rate of glucose oxidation in microdissected islets of *ob/ob* mice. For comparison the results have been plotted together with those obtained by Ashcroft *et al.* (1970) for collagenase-isolated islets of normal mice. There is almost perfect agreement between the two sets of data. The dose-response curve apparently has a sigmoidal shape with the steepest slope in the approximate concentration range 5–12 mM-glucose.

Effects of Ca²⁺ deficiency. The omission of CaCl₂ from the Krebs-Ringer buffer resulted in a significant inhibition of glucose oxidation (Table 1). When 0.5 mM-EGTA was included in the Ca²⁺-deficient medium the rate of glucose oxidation was further depressed. This effect of EGTA was probably due to the chelation of islet Ca²⁺, since the inhibition was completely reversed by 3 mM-CaCl₂. Fig. 1(b) shows how Ca²⁺ deficiency affected the dose-response relationship between the concentration and oxidation of glucose. Ca²⁺ deficiency had little effect at glucose concentrations below 5 mM, whereas at higher concentrations the rate of oxidation was significantly inhibited. As a consequence the normal sigmoidal dose-response curve was changed to one of hyperbolic appearance.

The results of fructose 1,6-diphosphate measurements are given in Table 2. The omission of Ca²⁺ doubled the concentration of fructose 1,6-diphosphate in the islets but had no effect on that in liver. Pieces of exocrine pancreas exhibited a small, though significant, increase in fructose 1,6-diphosphate after incubation in Ca²⁺-deficient medium. Table 2 also shows that the concentration of fructose 1,6-diphosphate was higher in the islets of fed *ob/ob* mice than in those of *ob/ob* mice starved overnight.

Effects of Na⁺ deficiency and excess. Table 3 summarizes how various Na⁺ concentrations affected the oxidation of glucose by pancreatic islets. Replacing about half the normal concentration of NaCl with choline chloride had no significant effect. At 6 mM-, 10 mM-, or 20 mM-glucose, a total removal of Na⁺

Fig. 1. Effects of Ca^{2+} or Na^{+} deficiency on the dose-response relationship between glucose concentration and oxidation (a) After preliminary incubation for 30min in basal medium the islets were incubated for 60min in media containing 1–30mM-D-[U- ^{14}C]glucose (0.6–17 mCi/mmol). Amounts of $^{14}\text{CO}_2$ produced are expressed as mmol of glucose equivalents oxidized/h per kg dry wt. of islets. For comparison, the data of Ashcroft *et al.* (1970) on collagenase-isolated islets of normal mice were recalculated per unit dry wt. and included in the diagram. The calculation was based on the statement by those authors that the normal mouse islets have a mean dry wt. of 0.5 μg . $\circ$, Present data on microdissected islets of *ob/ob* mice; $\bullet$, data from Ashcroft *et al.* (1970) on collagenase-isolated islets of normal mice. (b) Islets were incubated as in (a) except that Ca^{2+} - or Na^{2+} -deficient media were used. $\blacktriangle$, Ca^{2+} deficiency was produced by omitting CaCl_2 from and adding 0.5 mM-EGTA to the normal Krebs–Ringer medium. $\square$, Na^{+} deficiency was produced by substituting choline chloride for NaCl and KHCO_3 for NaHCO_3 . In all experiments islets from a single mouse were used for parallel incubations in control (a) and ion-substituted (b) media. Each point represents the mean of two to five separate experiments.

Table 1. Effects of Ca^{2+} deficiency, diazoxide or ouabain on glucose oxidation by pancreatic islets

Islets were incubated for 30 min in glucose-free Krebs–Ringer bicarbonate medium modified as indicated. ‘No Ca^{2+} ’ indicated that CaCl_2 was replaced by NaCl , ‘plus extra CaCl_2 ’ that 3 mM- CaCl_2 was added in addition to the normal 2.55 mM. The islets were then incubated for 60 min in the same types of media supplemented with 3–15 mM-D-[U- ^{14}C]glucose (1.1–5.7 mCi/mmol). Amounts of $^{14}\text{CO}_2$ produced are expressed as mmol of glucose equivalents oxidized/h per kg dry wt. of islets. Results are given as mean values $\pm$ s.e.m. for each medium (Primary data) as well as for the differences between parallel test and control incubations, with numbers of separate experiments in parentheses.

Modification of medium	[Glucose] (mM)	Rate of oxidation (mmol/h per kg dry wt. of islets)		P
		Primary data	Test minus control	
None (control)	10	31.1 $\pm$ 1.8 (20)	—	
No Ca^{2+}	10	26.4 $\pm$ 1.7 (9)	–5.5 $\pm$ 1.8 (9)	<0.02
No Ca^{2+} + EGTA (0.5 mM)	10	22.4 $\pm$ 2.3 (7)	–12.3 $\pm$ 1.6 (7)	<0.001
EGTA (0.5 mM) + extra CaCl_2 (3 mM)	10	33.9 $\pm$ 1.9 (10)	0.6 $\pm$ 2.5 (10)	
Diazoxide (125 $\mu\text{g}/\text{ml}$)	10	22.8 $\pm$ 2.9 (7)	–4.4 $\pm$ 0.8 (7)	<0.005
None (control)	3	6.1 $\pm$ 0.5 (6)	—	
Ouabain (0.1 mM)	3	5.2 $\pm$ 0.7 (6)	–0.9 $\pm$ 0.6 (6)	
None (control)	15	45.0 $\pm$ 1.9 (6)	—	
Ouabain (0.1 mM)	15	37.6 $\pm$ 2.9 (6)	–7.4 $\pm$ 2.7 (6)	<0.05

resulted in a marked inhibition of glucose oxidation whether the substituting ion was choline, K^{+} , choline plus K^{+} , or choline plus Li^{+} . No further inhibition of glucose oxidation was obtained by withdrawing CaCl_2 from and adding EGTA to the Na^{+} -free medium. Glucose oxidation was strongly inhibited when an

excess of 107 mM- NaCl was added to the normal Krebs–Ringer buffer. The inhibitory effect of excess of Na^{+} was probably not due to the increase of medium osmoticity or ionic strength, since 107 mM-choline chloride had no obvious effect on glucose oxidation.

Table 2. *Effects of Ca²⁺ deficiency, adrenaline, diazoxide, ouabain and phentolamine on the content of fructose 1,6-diphosphate in islets, exocrine pancreas and liver*

Islets from female *ob/ob* mice were incubated for 40 min (series I and III) or 60 min (series II) in Krebs–Ringer bicarbonate medium containing 17 mM-glucose and modified as indicated. In series I the animals had been starved overnight, whereas in series II and III they were given free access to food. Pieces of exocrine pancreas or liver were taken from phenotypically normal (lean) mice with free access to food and were incubated with 17 mM-glucose for 60 min. Results are given as mean values $\pm$ S.E.M. for each medium (Primary data) as well as for the differences between parallel test and control incubations. Statistical significances were judged from these differences. In addition, the statistical significances for differences between parallel test incubations were calculated in some cases: *, versus no Ca²⁺; †, versus adrenaline; ‡, versus diazoxide. The numbers of experiments are stated in parentheses.

Modification of medium	Content of fructose 1,6-diphosphate (μ mol/kg dry wt. of tissue)		P
	Primary data	Test minus control	
Series I: islets of starved <i>ob/ob</i> mice			
None (control)	175 $\pm$ 11 (23)	—	
No Ca ²⁺	371 $\pm$ 54 (11)	215 $\pm$ 52 (11)	<0.005
Adrenaline (2.0 μ g/ml)	293 $\pm$ 36 (12)	102 $\pm$ 28 (12)	<0.005
Phentolamine (10 μ g/ml)	165 $\pm$ 18 (11)	9 $\pm$ 23 (11)	
No Ca ²⁺ + phentolamine (10 μ g/ml)	252 $\pm$ 28 (11)	95 $\pm$ 29 (11)	<0.01
		-119 $\pm$ 44 (11)*	<0.025
Adrenaline (2.0 μ g/ml) + phentolamine (10 μ g/ml)	183 $\pm$ 13 (12)	-8 $\pm$ 14 (12)	
		-110 $\pm$ 27 (12)†	<0.005
Series II: islets of fed <i>ob/ob</i> mice			
None (control)	260 $\pm$ 18 (35)	—	
No Ca ²⁺	441 $\pm$ 57 (11)	206 $\pm$ 57 (11)	<0.005
Adrenaline (2.0 μ g/ml)	483 $\pm$ 60 (24)	211 $\pm$ 52 (24)	<0.001
Diazoxide (125 μ g/ml)	405 $\pm$ 42 (35)	145 $\pm$ 32 (35)	<0.001
Ouabain (10 μ M)	310 $\pm$ 34 (12)	58 $\pm$ 22 (12)	<0.025
No Ca ²⁺ + diazoxide (125 μ g/ml)	470 $\pm$ 94 (11)	235 $\pm$ 78 (11)	<0.02
Adrenaline (2.0 μ g/ml) + diazoxide (125 μ g/ml)	413 $\pm$ 41 (12)	121 $\pm$ 32 (12)	<0.005
Ouabain (10 μ M) + diazoxide (125 μ g/ml)	431 $\pm$ 45 (12)	179 $\pm$ 51 (12)	<0.005
Phentolamine (10 μ g/ml) + diazoxide (125 μ g/ml)	348 $\pm$ 60 (12)	57 $\pm$ 56 (12)	
		-121 $\pm$ 89 (12)‡	
Adrenaline (2.0 μ g/ml) + ouabain (10 μ M)	338 $\pm$ 44 (12)	85 $\pm$ 31 (12)	<0.02
Series III: islets of fed <i>ob/ob</i> mice			
None (control)	311 $\pm$ 45 (12)	—	
Adrenaline (0.02 μ g/ml)	457 $\pm$ 94 (12)	146 $\pm$ 61 (12)	<0.05
Adrenaline (0.2 μ g/ml)	559 $\pm$ 80 (12)	248 $\pm$ 70 (12)	<0.01
Adrenaline (2.0 μ g/ml)	617 $\pm$ 146 (12)	306 $\pm$ 129 (12)	<0.05
Series IV: exocrine pancreas of fed lean mice			
None (control)	42 $\pm$ 3 (12)	—	
No Ca ²⁺	59 $\pm$ 5 (12)	17 $\pm$ 5 (12)	<0.005
Series V: liver of fed lean mice			
None (control)	44 $\pm$ 4 (23)	—	
No Ca ²⁺	48 $\pm$ 4 (12)	-1 $\pm$ 5 (12)	
Adrenaline (2.0 μ g/ml)	40 $\pm$ 4 (11)	1 $\pm$ 3 (11)	
Diazoxide (125 μ g/ml)	45 $\pm$ 5 (10)	8 $\pm$ 4 (10)	<0.05

In comparison with the marked inhibitory effects of Na⁺ deficiency on glucose oxidation at glucose concentrations above 6 mM, the same ionic substitutions seemed to have comparatively less effect at 3 mM-glucose (Table 3). Fig. 1(b) shows the effect of replacing Na⁺ with choline ions and K⁺ at a large number of glucose concentrations. Inhibition of oxidation was more pronounced than that caused by

Ca²⁺ deficiency and, again, was particularly striking at glucose concentrations higher than 5–6 mM. As a result the omission of Na⁺ appeared to eliminate the sigmoidal shape of the dose–response curve.

The inhibition of glucose oxidation by Na⁺ deficiency was accompanied by a considerable increase of glucose 6-phosphate and fructose 1,6-diphosphate in the pancreatic islets (Table 4).

Table 3. *Effects of monovalent cations on glucose oxidation by pancreatic islets*

Rate of oxidation (mmol/h per kg dry wt. of islets)					Comparisons between groups	
Group	Salt omitted	Compound added to replace omitted salt, or added to basal Krebs-Ringer buffer	[Glucose] (mm)	Primary data	Groups	Difference
1	None	None	3	4.4±0.6 (6)	2-1	-2.1±0.5* (6)
2	NaCl, NaHCO ₃	Choline chloride, choline bicarbonate	3	2.3±0.2 (6)		
3	None	None	6	17.0±1.0 (5)	4-3	-10.4±1.3** (5)
4	NaCl, NaHCO ₃	Choline chloride, choline bicarbonate	6	6.6±0.6 (5)		
5	None	None	10	26.2±2.8 (7)	6-5	-16.4±2.6*** (7)
6	NaCl, NaHCO ₃	Choline chloride, choline bicarbonate	10	9.8±1.0 (7)		
7	CaCl ₂	None	10	14.1±2.6 (7)	7-5	-12.1±3.6* (7)
8	NaCl, NaHCO ₃ , CaCl ₂	Choline chloride, choline bicarbonate	10	9.7±1.1 (7)	8-5	-16.5±2.3*** (7)
9	None	None	20	51.7±3.0 (6)	10-9	-35.1±3.2*** (6)
10	NaCl, NaHCO ₃	Choline chloride, choline bicarbonate	20	16.6±2.0 (6)		
11	None	None	10	27.1±2.1 (7)	12-11	-13.1±1.3*** (7)
12	NaCl, NaHCO ₃	Choline chloride, KHCO ₃	10	14.0±1.2 (7)		
13	NaCl, NaHCO ₃	LiCl, KHCO ₃	3	3.4±0.7 (6)	13-1	-1.0±1.1 (6)
14	NaCl, NaHCO ₃	KCl, KHCO ₃	3	2.8±0.4 (6)	14-1	-1.6±0.6 (6)
15	None	None	10	30.2±2.7 (6)	16-15	-14.9±2.7** (6)
16	NaCl, NaHCO ₃	LiCl, KHCO ₃	10	15.3±1.7 (6)		
17	NaCl, NaHCO ₃	KCl, KHCO ₃	10	12.3±1.0 (6)	17-15	-17.9±2.5*** (6)
18	None	None	10	33.8±4.4 (6)	19-18	7.9±7.2 (6)
19	NaCl (68 mm)	Choline chloride (68 mm)	10	41.7±5.3 (6)		
20	None	Choline chloride (57 mm)	10	28.0±3.3 (6)	21-20	-1.8±1.7 (6)
21	None	NaCl (57 mm)	10	26.2±2.7 (6)		
22	None	Choline chloride (107 mm)	10	24.2±3.9 (5)	23-22	-16.8±1.6*** (5)
23	None	NaCl (107 mm)	10	7.4±2.5 (5)		

Islets were incubated for 30 min in glucose-free Krebs-Ringer bicarbonate medium modified as indicated. When CaCl₂ was omitted, the medium also contained 0.5 mM-EGTA. The islets were then incubated for 60 min in the same types of media supplemented with 3–20 mM-D-[U-¹⁴C]glucose (0.9–5.7 mCi/mmol). Amounts of ¹⁴CO₂ produced are expressed as mmol of glucose equivalents oxidized/h per kg dry wt. of islets. Results are given as mean values±s.e.m. for each medium (Primary data) as well as for the differences between parallel incubations in different types of media, with numbers of experiments in parentheses. **P*<0.05; ***P*<0.01; ****P*<0.001.

Table 4. *Effects of sodium deficiency on insulin release and on the concentrations of glucose 6-phosphate and fructose 1,6-diphosphate in microdissected islets*

Control media contained Krebs–Ringer bicarbonate buffer, whereas in the test media NaCl had been replaced by choline chloride and NaHCO_3 by KHCO_3 . Both media contained 1 mg of serum albumin/ml. After preliminary incubation for 40 min in glucose-free test or control medium, the islets were incubated for 20 or 60 min in the same types of media supplemented with 20 mM-glucose. Six different experiments were performed. In each of these the concentrations of glucose 6-phosphate and fructose 1,6-diphosphate were measured in the same islets which were also used for studying insulin release. Islet concentrations of substrates are presented as $\mu\text{mol/kg}$ dry wt. of islets, and insulin release as $\text{ng}/\mu\text{g}$ dry wt. of islets per 20 or 60 min. Mean values $\pm$ S.E.M. are given for each medium as well as for the differences between parallel test and control incubations. * $P < 0.02$; ** $P < 0.01$.

Parameter studied	Control	Na^+ deficiency	Effect of Na^+ deficiency
Incubation for 20 min			
Glucose 6-phosphate	298 ± 33	490 ± 50	$192 \pm 54^*$
Fructose 1,6-diphosphate	194 ± 33	471 ± 77	$277 \pm 68^{**}$
Insulin release	1.77 ± 0.30	1.46 ± 0.13	-0.31 ± 0.28
Incubation for 60 min			
Glucose 6-phosphate	376 ± 31	564 ± 48	$188 \pm 43^{**}$
Fructose 1,6-diphosphate	210 ± 36	319 ± 36	109 ± 51
Insulin release	4.88 ± 1.16	3.80 ± 0.56	-1.08 ± 0.66

Table 5. *Reversibility of the metabolic inhibition caused by Na^+ or Ca^{2+} deficiency*

Islets were incubated for 30 or 90 min (first period) in Na^+ - or Ca^{2+} -free media composed as in Fig. 1(b). They were then incubated for 60 min (second period) with 10 mM-D-[U- ^{14}C]glucose (1.7 mCi/mmol) in the same types of ion-substituted medium or in normal Krebs–Ringer buffer. Amounts of $^{14}\text{CO}_2$ produced are expressed as mmol of glucose equivalents oxidized/h per kg dry wt. of islets. Results are given as mean values $\pm$ S.E.M. for each group as well as for the differences between parallel test and control groups, with numbers of experiments in parentheses. * $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$.

Group	Medium composition		Rate of oxidation (mmol/h per kg dry wt. of islets)	
	First period	Second period	30 min first period	90 min first period
1	No Na^+	No Na^+	12.6 ± 1.1 (7)	14.3 ± 3.4 (5)
2	No Na^+	Krebs–Ringer	25.3 ± 2.0 (7)	28.5 ± 4.3 (5)
3	No Ca^{2+}	No Ca^{2+}	19.1 ± 2.4 (7)	22.5 ± 1.9 (5)
4	No Ca^{2+}	Krebs–Ringer	24.7 ± 2.4 (7)	27.3 ± 2.4 (5)
2–1			$12.7 \pm 2.1^{***}$ (7)	$14.2 \pm 2.9^{**}$ (5)
4–3			$5.6 \pm 1.3^{**}$ (7)	$4.7 \pm 1.3^*$ (5)

All of the above effects of Na^+ deficiency were recorded after 30–40 min of preliminary incubation in ion-substituted media. In one further series of experiments the islets were subjected to preliminary incubation for 30 min in normal Krebs–Ringer bicarbonate medium containing 17 mM-glucose. To study the immediate effect of Na^+ deficiency on glucose oxidation, the islets were then incubated for 20 min with 17 mM-D-[U- ^{14}C]glucose (1.7 mCi/mmol) in normal medium or in medium containing choline chloride and choline bicarbonate instead of NaCl and NaHCO_3 . During this period the control islets oxidized 10.1 ± 1.5 mmol of glucose equivalents/kg dry wt. (mean value $\pm$ S.E.M. for five experiments), whereas the corresponding values for islets incubated in Na^+ -deficient medium were 9.2 ± 1.4 . Thus the rate of glucose oxidation was not significantly altered within the first 20 min of incubation in Na^+ -deficient

medium. The oxidation rate recorded for control islets in these experiments was lower than one-third of that observed during incubations with 17 mM-glucose for 1 h (Fig. 1). This is because each period of $^{14}\text{CO}_2$ production includes an initial lag phase that affects the results proportionally more in the 20-min incubations.

Reversibility of metabolic inhibition. To test whether the inhibitory effects of Na^+ or Ca^{2+} deficiency on glucose metabolism are reversible, islets were incubated in the same types of ion-substituted media as used in Fig. 1(b). Glucose oxidation was then measured during incubation for 60 min in normal Krebs–Ringer buffer or during continued incubation in the ion-substituted media. Table 5 shows that glucose oxidation reverted to the normal rate even after as long as 90 min of preliminary incubation in the absence of Na^+ or Ca^{2+} .

Effects of adrenaline, diazoxide, ouabain and phentolamine. The addition to normal medium of 0.02–2.0 μ g of adrenaline/ml, 125 μ g of diazoxide/ml, or 10 μ M-ouabain resulted in an increase of islet fructose 1,6-diphosphate (Table 2). Phentolamine [2-*N*-(3-hydroxyphenyl)-*p*-toluidinomethyl-2-imidazoline] (10 μ g/ml) significantly counteracted the effects on this metabolite of Ca^{2+} deficiency and adrenaline, and tended to inhibit that of diazoxide. Combinations of 125 μ g of diazoxide/ml with Ca^{2+} deficiency, 2.0 μ g of adrenaline/ml or 10 μ M-ouabain, or of 2.0 μ g of adrenaline/ml with 10 μ M-ouabain produced no increase of islet fructose 1,6-diphosphate above that obtained with any one of these treatments alone. As shown in Table 1, 125 μ g of diazoxide/ml or 100 μ M-ouabain also inhibited glucose oxidation by the islets.

Effects of galactose and 3-O-methylglucose. Matschinsky & Ellerman (1973) reported that galactose

and 3-O-methylglucose strongly stimulated lactate production from rat islets. They postulated that this effect was due to the sugars being co-transported with Na^+ , resulting in high intracellular Na^+ concentrations that were in turn thought to activate glycolysis. The effects of these sugars on glucose oxidation were therefore studied. As shown in Table 6, neither galactose nor 3-O-methylglucose stimulated the production of $^{14}\text{CO}_2$ from the islets of *ob/ob* mice.

Glucose transport

To study the transport of D-glucose into β -cells, the islet uptake of D-[6- ^3H]glucose was compared with the simultaneous uptake of L-[1- ^{14}C]glucose. Previous studies at 8°C indicated that D-glucose enters the β -cells by a carrier system, whereas L-glucose is excluded from the cells and restricted to the extracellular space (Hellman *et al.*, 1971a). Fig. 2 shows

Table 6. *Effects of galactose and 3-O-methylglucose on glucose oxidation*

After preliminary incubation for 30 min in glucose-free Krebs–Ringer bicarbonate medium containing 3 mg of serum albumin/ml, the islets were incubated for 60 min in the same type of medium supplemented with 2 mM- or 10 mM-D-[U- ^{14}C]glucose (8.5 and 1.7 mCi/mmol). Control incubations were performed without further additions to the media, but test media also contained 10 mM-galactose or 10 mM-3-O-methylglucose. Amounts of $^{14}\text{CO}_2$ produced are expressed as mmol of glucose equivalents oxidized/h per kg dry wt. of islets. Results are given as mean values $\pm$ S.E.M. for each medium as well as for the differences between parallel test and control incubations, with numbers of experiments in parentheses.

Test compound	[Glucose] (mM)	Rate of oxidation (mmol/h per kg dry wt. of islets)		
		Control (a)	Test (b)	(b) – (a)
Galactose (10 mM)	2	4.7 $\pm$ 0.5 (5)	3.8 $\pm$ 0.7 (5)	–0.9 $\pm$ 1.0
Galactose (10 mM)	10	29.5 $\pm$ 3.6 (4)	30.5 $\pm$ 2.8 (4)	1.1 $\pm$ 3.3
3-O-Methylglucose (10 mM)	10	22.7 $\pm$ 3.9 (5)	17.4 $\pm$ 3.6 (5)	–5.3 $\pm$ 5.9

Table 7. *Islet uptake of D-glucose in excess of the L-glucose space*

Islets were incubated for 30 min in Krebs–Ringer bicarbonate buffer ('control') or in the same buffer modified as indicated ('test'). 'No Na^+ ' means that NaCl was replaced by choline chloride and NaHCO_3 by KHCO_3 ; 'No Ca^{2+} ' that CaCl_2 was replaced by NaCl. The islets were then incubated for 45 s in the same types of media supplemented with D-[6- ^3H]glucose at the indicated concentrations (0.4–30 mCi/mmol) as well as 2.5 mM-L-[1- ^{14}C]glucose (3 mCi/mmol). Uptake of D-glucose in excess of the L-glucose space is given as the mean values $\pm$ S.E.M. for each medium. In addition, the mean values $\pm$ S.E.M. for differences between parallel test and control incubations are presented, with numbers of experiments in parentheses. * $P < 0.05$.

Modification of medium	D-Glucose in medium (mM)	Glucose uptake (mmol of D-glucose/kg dry wt. of islets)		
		Control (a)	Test (b)	(b) – (a)
No Na^+	0.5	0.42 $\pm$ 0.02 (7)	0.41 $\pm$ 0.05 (7)	–0.01 $\pm$ 0.04 (7)
No Na^+	15	11.9 $\pm$ 0.6 (8)	9.4 $\pm$ 0.9 (8)	–2.5 $\pm$ 1.0* (8)
No Na^+	40	34.5 $\pm$ 3.0 (6)	33.1 $\pm$ 2.4 (6)	–1.3 $\pm$ 3.5 (6)
No Ca^{2+}	2	1.67 $\pm$ 0.16 (7)	1.57 $\pm$ 0.05 (7)	–0.10 $\pm$ 0.14 (7)
No Ca^{2+}	10	8.20 $\pm$ 0.57 (6)	8.01 $\pm$ 0.71 (6)	–0.19 $\pm$ 0.78 (6)
No Ca^{2+} + 0.5 mM-EGTA	10	8.20 $\pm$ 0.57 (6)	7.26 $\pm$ 0.51 (6)	–0.94 $\pm$ 0.53 (6)
Diazoxide (125 μ g/ml)	2	1.67 $\pm$ 0.16 (7)	1.80 $\pm$ 0.16 (7)	0.13 $\pm$ 0.09 (7)
Diazoxide (125 μ g/ml)	10	8.20 $\pm$ 0.57 (6)	7.68 $\pm$ 0.46 (6)	–0.52 $\pm$ 0.48 (6)

Fig. 2. Islet uptake of D-glucose with time

(a) After preliminary incubation for 30 min in basal medium, the islets were incubated for 45 s to 45 min in medium containing 0.5 mM-D-[6-³H]glucose (30 mCi/mmol) and 2.5 mM-L-[1-¹⁴C]glucose (3 mCi/mmol). The points represent the uptake of D-glucose in excess of the L-glucose space and are the mean values of three to five different experiments. Bars denote $\pm$ S.E.M. (b) Islets were incubated as in (a) except that 5.0 mM-D-[6-³H]glucose (3 mCi/mmol) was used. Mean values $\pm$ S.E.M. for six or seven different experiments.

the time-course at 37°C of D-glucose uptake in excess of the L-glucose space. With both 0.5 mM- and 5 mM-D-glucose the time curve was biphasic with a fast component that predominated during the first 1–2 min, and a subsequent slow uptake that progressed at a constant rate for at least 45 min. The initial phase probably represents transmembrane transport, whereas the slower second phase may be due to the accumulation of ³H-labelled glucose metabolites in the β -cells. In support of this interpretation the sharp bend of the time curve corresponds closely to the uptake values that would be expected at completed equilibration of D-glucose across the β -cell plasma membrane. Assuming that the intracellular water of incubated islets is 1.2 litre/kg dry wt.

Fig. 3. Effect of Na⁺ deficiency on the dose-response relationship between glucose concentration and insulin release

Islets were subjected to preliminary incubation for 30 min in normal Krebs–Ringer bicarbonate medium or in Na⁺-deficient media. Na⁺ deficiency was produced by substituting choline chloride for NaCl, and choline bicarbonate or KHCO₃ for NaHCO₃. The islets were then incubated for 60 min in the same types of media to which were added glucose as indicated. Amounts of insulin released during the 60-min period are given as mean values $\pm$ S.E.M. for 7–15 different experiments. In each experiment control incubations were performed parallel to those in Na⁺-deficient media. Statistical analysis of the differences between paired test and control incubations revealed that insulin release was significantly enhanced ($P < 0.01$) at 0 mM-glucose in both types of Na⁺-deficient media. At 20 mM-glucose the replacement of Na⁺ by choline ions and K⁺ significantly ($P < 0.025$) inhibited insulin release. $\circ$, Control; $\blacksquare$, choline chloride and choline bicarbonate; $\bullet$, choline chloride and KHCO₃.

(Hellman *et al.*, 1971b), the intracellular concentration of D-glucose at the end of the initial uptake phase can be estimated as 0.41 mM during incubation with 0.5 mM-glucose, and 5.2 mM during incubation with 5 mM-glucose. Fig. 2 also shows that at 45 s, the shortest incubation time that we could use with reasonable accuracy, the uptake of D-glucose in excess of the L-glucose space was well below that expected at equilibrium between the extracellular and intracellular D-glucose. The effect of Na⁺ or Ca²⁺ deficiency on D-glucose transport was therefore studied in incubations for 45 s.

As shown in Table 7, the same ionic manipulations or concentration of diazoxide that inhibited glucose oxidation (Fig. 1b; Table 1) had only little or no

effect on glucose transport. In control as well as in test media the estimated rates of glucose transport were several times larger than the normal rates of glucose oxidation at all glucose concentrations tested. Since we did not measure the glucose uptake during incubations shorter than 45s, the values in Table 6 may not represent true initial rates. However, they are probably valid as minimum estimates of the initial rates and as such conform with our previous conclusion that the transport system is far from saturation within the physiological range of glucose concentrations (Hellman *et al.*, 1971a).

Insulin release

Effects of Na^+ deficiency. In addition to demonstrating increased concentrations of glucose 6-phosphate and fructose 1,6-diphosphate in islets incubated without Na^+ , Table 4 shows that there was a tendency to inhibited insulin release from the same islet specimens. Because the release data in Table 4 are attended by comparatively large random errors, we performed a more detailed study on insulin release at various glucose concentrations. Fig. 3 shows that the replacement in Krebs-Ringer bicarbonate buffer of Na^+ by choline ions alone or by choline ions and K^+ markedly altered the curve relating insulin release to glucose concentration. Although these ion substitutions enhanced basal release in the absence of glucose, they inhibited release in the presence of 20mM-glucose. As a result the glucose-induced secretory responses were considerably diminished in

Fig. 4. Effect of glucose on the dynamics of insulin release in normal and Na^+ -deficient medium

Islets were perfused with 3mM-glucose in normal Krebs-Ringer bicarbonate medium ($\circ$) or in medium containing choline chloride and choline bicarbonate instead of NaCl and NaHCO_3 ($\blacksquare$). After 60min the glucose concentration was suddenly raised to 17mM. Perfusion with 17mM-glucose is indicated by the solid bar. The points represent the average rate of insulin release over each sampling period.

Fig. 5. Effect of sudden Na^+ withdrawal on the dynamics of insulin release

Islets were perfused with normal Krebs-Ringer bicarbonate medium containing 17mM-glucose. After 60min the medium was suddenly changed to one also containing 17mM-glucose but with choline chloride and choline bicarbonate instead of NaCl and NaHCO_3 . Perfusion with Na^+ -deficient medium is indicated by the solid bar. The points represent the average rate of insulin release over each sampling period.

Na^+ -deficient media. This effect of Na^+ deficiency was also observed in micro-perfusion experiments (Fig. 4); basal secretion was increased and the normal response to 17mM-glucose was suppressed in islets that had been perfused with Na^+ -deficient medium for about 1 h. However, when the islets were perfused with 17mM-glucose in normal medium, the sudden withdrawal of all Na^+ in the medium caused a prompt enhancement of insulin release (Fig. 5). After a few min the secretory rate fell again, resulting in a sharp peak of insulin release.

Effects of galactose and 3-O-methylglucose. Galactose has been reported both to stimulate (Landgraf *et al.*, 1971) and not to stimulate (Grodsky *et al.*, 1963; Matschinsky & Ellerman, 1973) insulin release as well as to potentiate glucose-induced insulin release in the rat but not in the mouse (Ashcroft *et al.*, 1972). Since, in addition, galactose and 3-O-methylglucose were thought to accelerate rat islet glycolysis by a Na^+ -dependent mechanism (Matschinsky & Ellerman, 1973), we decided to investigate the effects of these sugars on insulin release from the islets of *ob/ob* mice. Table 8 shows that neither 10mM-galactose nor 20mM-3-O-methylglucose stimulated insulin release significantly whether the sugars were tested alone or in combination with glucose.

Table 8. *Effects of galactose and 3-O-methylglucose on insulin release*

After preliminary incubation for 40 min in glucose-free Krebs–Ringer bicarbonate medium containing 5 mg of serum albumin/ml, islets from fed mice were incubated for 60 min in the same type of medium supplemented with glucose as indicated. Control incubations were performed without further additions to the media, but test media also contained 20 mM-3-O-methylglucose or 10 mM-galactose. Amounts of insulin released are given as mean values $\pm$ S.E.M. for the numbers of experiments in parentheses.

[Glucose] (mM)	Insulin released (ng/h per μ g dry wt. of islets)	
	Control	20 mM-3-O-Methyl- glucose
0	0.78 $\pm$ 0.20 (6)	1.06 $\pm$ 0.32 (5)
5	2.59 $\pm$ 0.87 (5)	3.87 $\pm$ 1.09 (6)
10	8.90 $\pm$ 1.40 (6)	10.10 $\pm$ 1.74 (6)
20	14.87 $\pm$ 2.00 (6)	13.81 $\pm$ 2.04 (6)
	Control	10 mM-Galactose
0	1.77 $\pm$ 0.38 (6)	1.51 $\pm$ 0.33 (6)
10	8.25 $\pm$ 1.44 (6)	7.42 $\pm$ 1.64 (6)

Discussion

Glucose metabolism

The relationship between the concentration and oxidation of glucose in collagenase-isolated islets of normal mice was reported by Ashcroft *et al.* (1970). Allowing for random error, our data for micro-dissected islets of *ob/ob* mice are superimposable on those of Ashcroft *et al.* (1970), suggesting that the regulation of glucose oxidation is principally the same in the two islet preparations. Both sets of data indicate that the dose–response relationship is sigmoidal with its steepest slope in the approximate range 5–12 mM-glucose. Our results suggest that the curve has a smooth bend, rather than an abrupt threshold (Ashcroft *et al.*, 1970), at about 5 mM-glucose and tends towards V_{max} between 20 mM- and 30 mM-glucose. Also in agreement with Ashcroft *et al.* (1972) we find that the omission of Na^+ ions from the physiological buffer results in a marked inhibition of glucose oxidation at glucose concentrations of about 10 mM. Moreover, the results reported here show that the omission of Na^+ inhibits glucose oxidation throughout a wide range of glucose concentrations in such a way as to apparently eliminate the bend at 5 mM-glucose and hence the sigmoidal shape of the dose–response curve.

The detailed effects of Na^+ deficiency on islet glucose metabolism are not clear, but measurements of water (Ashcroft *et al.*, 1972) and lactate (Matschinsky & Ellerman, 1973) productions indicated that the

glycolytic flux may be retarded. Such a retardation could be due to inhibition of glucose entry into the β -cells or of some subsequent metabolic step. The glucose-transporting system in β -cell plasma membranes has such a great capacity (Hellman *et al.*, 1971a) that it must be highly dependent on Na^+ to explain the observed effects of Na^+ deficiency on glucose metabolism. In a preliminary study using washing of the islets to remove extracellular and contaminating radioactivity, a moderate although significant decrease in D-glucose uptake was observed in Na^+ -deficient medium (Hellman *et al.*, 1971c). The present results obtained with a technically superior double-label method showed that Na^+ deficiency had little if any effect on glucose uptake during 45 s. In spite of the short incubation time a slight inhibition could be due to a certain fraction of the glucose taken up being metabolized in a Na^+ -dependent manner. We are therefore inclined to believe that the marked effects of Na^+ deficiency on glucose metabolism are not due to inhibited transport but to a block in glucose degradation. This assumption is supported by the demonstration that the inhibition of glucose oxidation was accompanied by increased concentrations of glucose 6-phosphate and fructose 1,6-diphosphate in the islets. Matschinsky & Ellerman (1973) proposed that 3-O-methylglucose stimulates islet glycolysis by being co-transported with Na^+ and thereby giving rise to a high intracellular Na^+ concentration. It is therefore noteworthy that 3-O-methylglucose is apparently transported by the same system as D-glucose in the β -cells (Hellman *et al.*, 1973b) and thus probably does not depend to any great extent on Na^+ ions. Further, excess of Na^+ did not stimulate but inhibited glucose oxidation in the present study.

An inhibitory effect of Na^+ deficiency on glucose degradation could arise because some metabolic step is Na^+ -dependent, or there may be some indirect reason. Ashcroft *et al.* (1972) suggested that inhibition of glucose utilization may result from a decreased turnover of ATP owing to inhibition of $Na^+ + K^+$ -activated ATPase (adenosine triphosphatase). This idea conforms with our observations that glucose oxidation was inhibited and fructose 1,6-diphosphate was increased by ouabain, an inhibitor of $Na^+ + K^+$ -activated ATPase. Matschinsky & Ellerman (1973) reported that ouabain inhibits lactate production in rat islets. These effects of ouabain would not be expected if glycolysis were stimulated predominantly by the intracellular Na^+ ions as such. Thus the results lend indirect support to the idea that the rate of glycolysis in β -cells, as in other cells, may depend on the ratio of ATP to ADP, AMP and P_i (Hellman *et al.*, 1969).

Idahl (1971) observed that the omission of Ca^{2+} increased the islet content of glucose 6-phosphate and suggested that glycolysis was retarded. This idea is

supported by the results reported here showing that Ca^{2+} deficiency inhibited glucose oxidation as well as increasing the islet content of fructose 1,6-diphosphate. Ashcroft *et al.* (1970) observed no effect of Ca^{2+} deficiency on glucose oxidation in collagenase-isolated normal mouse islets but, however, they apparently did not design the experiments with the primary objective of detecting such an effect. Those authors concluded as their principal finding that the omission of Ca^{2+} does not abolish the enhancement of glucose oxidation elicited by a high glucose concentration, a conclusion that agrees well with our results. Even in Ca^{2+} -free medium containing EGTA, the rate of oxidation was markedly dependent on glucose concentration.

Malaisse-Lagae & Malaisse (1971) and Malaisse *et al.* (1973) suggested that the net uptake of Ca^{2+} by pancreatic β -cells is inhibited by adrenaline or diazoxide. Hellman *et al.* (1970), although not Ashcroft *et al.* (1970), observed that adrenaline inhibits glucose oxidation by the islets. As shown here, diazoxide also inhibited glucose oxidation. Moreover, adrenaline or diazoxide increased the islet content of fructose 1,6-diphosphate as did the omission of Ca^{2+} . Phentolamine counteracted the effect of adrenaline, and perhaps also that of diazoxide, as if these effects were mediated by α -adrenergic stimulation. However, in view of the observation that phentolamine also counteracted the effect of Ca^{2+} deficiency, it seems rather unlikely that the metabolic effects of adrenaline or diazoxide were only mediated by inhibited Ca^{2+} uptake.

Insulin release

Although there is universal agreement that Ca^{2+} is essential for glucose-stimulated insulin release, the importance of Na^+ has been the subject of a certain amount of controversy. Hales & Milner (1968a) found that the replacement of Na^+ by choline, K^+ or Li^+ ions led to an almost total suppression of the glucose-stimulated secretory response of rabbit pancreas. On the other hand, Malaisse *et al.* (1971) reported that choline ions could effectively proxy for Na^+ in enabling pieces of rat pancreas to release insulin in response to glucose; in choline-substituted media the glucose-induced insulin release was even enhanced as compared with that observed in normal medium. The results reported here support the view that Na^+ plays an important role in the stimulation of insulin release by glucose. The glucose-induced secretory response was suppressed in islets that had been equilibrated with Na^+ -free media. In addition, the perfused islets of *ob/ob* mice responded with a marked and transient enhancement of insulin release when Na^+ was suddenly withdrawn from a medium of high glucose concentration. Hales & Milner (1968a) observed a similar effect on transferring pieces of

rabbit pancreas from normal to Na^+ -free media containing 3.3 mM-glucose.

Interrelationships between glucose metabolism and insulin release

Grodsky *et al.* (1963) suggested that glucose is recognized as an insulin secretagogue by being metabolized in the pancreatic β -cells. In support of this hypothesis correlations have been demonstrated between the rate of insulin release and various parameters of glucose metabolism in the pancreatic islets (e.g. Ashcroft *et al.*, 1970, 1972, 1973; Hellerström & Gunnarsson, 1970; Idahl, 1973). The results reported here for the β -cell-rich islets of *ob/ob* mice demonstrate some further correlations of this kind; note first the similarity between the control curves for glucose oxidation and insulin release in Figs. 1(a) and 3. The possibility that such correlations are due to glucose metabolism being driven by the secretory activity was rejected by Ashcroft *et al.* (1970) on the grounds that omitting Ca^{2+} abolished the secretory response without inhibiting glucose oxidation. Since we find that Ca^{2+} deficiency or the addition of adrenaline or diazoxide, each of which conditions inhibits insulin release, inhibits glucose oxidation and increases the islet content of fructose 1,6-diphosphate, it is conceivable that insulin release could drive glycolysis to a certain extent. However, the effects of these treatments on glucose oxidation are small in comparison with the accompanying abolishment of glucose-induced insulin release (Grodsky & Bennett, 1966; Hales & Milner, 1968b; Milner & Hales, 1969; Lernmark & Hellman, 1970). Our results therefore conform with the idea that Ca^{2+} deficiency, adrenaline or diazoxide inhibits the secretory process at some step distal to glucose recognition (Milner & Hales, 1969). Yet the possibility of a feedback of insulin release on glucose metabolism may be of interest in attempts to explain the kinetics of glucose metabolism and insulin release as functions of glucose concentration. In Ca^{2+} -free medium the dose-response curve for glucose oxidation was apparently changed from a sigmoidal to a hyperbolic shape, suggesting that the normal acceleration of glucose metabolism above 5 mM-glucose may in part be the result of stimulated secretion.

Milner & Hales (1970) suggested that Na^+ deficiency inhibits insulin release because intracellular Na^+ is important for Ca^{2+} uptake and hence for stimulus-secretion coupling in the β -cells. However, the metabolic effects of Na^+ deficiency reported here could scarcely be due to a decreased Ca^{2+} uptake alone, as Na^+ deficiency inhibited glucose oxidation more than did the omission of Ca^{2+} . For the same reason the effects of Na^+ deficiency on glucose metabolism were probably not solely secondary to the inhibition of insulin release. Therefore, if one assumes

that glucose recognition is metabolic in nature it seems logical to assume also that the effects of Na^+ deficiency on insulin release could at least in part result from inhibited glucose recognition. This possibility is emphasized by the close correlation that appeared to exist between the effects of Na^+ deficiency on glucose oxidation and insulin release (Fig. 6). Note that such a correlation does not prove the dependence of insulin release on glucose metabolism; it might well be that the secretory and metabolic responses of the β -cells have a similar requirement for Na^+ without being causally related.

A role of Na^+ in stimulus recognition would not exclude the involvement of Na^+ in stimulus-secretion coupling as well. Na^+ deficiency may inhibit secretion in response to insulin secretagogues other than glucose (Hales & Milner, 1968a). Moreover, Hales & Milner (1968a) reported, and it is confirmed here, that insulin release is initially enhanced on the withdrawal of Na^+ . Milner & Hales (1970) suggested that this effect arises because extracellular Na^+ somehow

inhibits Ca^{2+} uptake by the β -cells. Studies on the membrane potential of islet cells (Dean & Matthews, 1970) as well as on the $^{45}\text{Ca}^{2+}$ uptake by isolated islets (Hellman *et al.*, 1971d) support this view. The measurements of $^{14}\text{CO}_2$ production reported here indicate that the immediate enhancement of insulin release by Na^+ withdrawal is probably not due to an increase of the oxidative glucose metabolism.

Matschinsky & Ellerman (1973) concluded that the recognition of glucose as insulin secretagogue cannot depend on glycolysis because 3-O-methylglucose or galactose stimulated lactate production but not the release of insulin from rat islets. We have previously reported that 3-O-methylglucose is not oxidized to CO_2 by the islets of *ob/ob* mice (Hellman *et al.*, 1973b). As shown here, neither 3-O-methylglucose nor galactose had any significant effect on the production of $^{14}\text{CO}_2$ from D-[U- ^{14}C]glucose or on insulin release. These negative results cannot help to decide whether direct receptors for the glucose molecule may play a role in stimulus recognition (cf. Hellman *et al.*, 1972; Ashcroft *et al.*, 1973; Sehlin, 1973). In the present context it is significant, however, that our observations with galactose and 3-O-methylglucose are entirely consistent with a cause-effect relationship between islet glucose metabolism and insulin release.

This work was supported by the Swedish Medical Research Council (Grant 12x-2288). The paper represents part 9 of the series: 'The pancreatic β -cell recognition of insulin secretagogues'.

Fig. 6. Relationship between the effects of Na^+ deficiency on metabolic and secretory responses to glucose

From the curves presented in Fig. 3 we read off the glucose-induced increments of insulin release above the rate at 3 mM-glucose. This was done at intervals of 1 mM, yielding 17 estimates of secretory responses for the control curve as well as for that representing incubations in Na^+ -deficient media. At each of the 17 glucose concentrations the difference between the increments of the control and test curves was calculated. These differences represent the inhibitory effects of Na^+ deficiency on the glucose-induced secretory responses. In an analogous manner, the inhibitory effects of Na^+ deficiency on the glucose-induced metabolic responses were read off from the glucose-oxidation curves in Fig. 1 (a and b).

References

- Ashcroft, S. J. H., Hedeskov, C. J. & Randle, P. J. (1970) *Biochem. J.* **118**, 143–154
- Ashcroft, S. J. H., Bassett, J. M. & Randle, P. J. (1972) *Diabetes* **21 Suppl.** 2, 538–545
- Ashcroft, S. J. H., Weerasinghe, L. C. C. & Randle, P. J. (1973) *Biochem. J.* **132**, 223–231
- Bloom, G. D., Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J. & Täljedal, I.-B. (1972) *Biochem. J.* **129**, 241–254
- Dean, P. M. & Matthews, E. K. (1970) *J. Physiol. (London)* **210**, 265–275
- Grodsky, G. M. & Bennett, L. L. (1966) *Diabetes* **15**, 910–913
- Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B. & Smith, D. F. (1963) *Amer. J. Physiol.* **205**, 638–644
- Hales, C. N. & Milner, R. D. G. (1968a) *J. Physiol. (London)* **194**, 725–743
- Hales, C. N. & Milner, R. D. G. (1968b) *J. Physiol. (London)* **199**, 177–187
- Hellerström, C. (1964) *Acta Endocrinol. (Copenhagen)* **45**, 122–131
- Hellerström, C. & Gunnarsson, R. (1970) *Acta Diabetol. Lat. 7 Suppl.* **1**, 127–151
- Hellman, B., Idahl, L.-Å. & Danielsson, Å. (1969) *Diabetes* **18**, 509–516

- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1970) *Med. Exp.* **19**, 351–356
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1971a) *Biochim. Biophys. Acta* **241**, 147–154
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1971b) *Diabetologia* **7**, 256–265
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1971c) *Horm. Metab. Res.* **3**, 219–220
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1971d) *Amer. J. Physiol.* **221**, 1795–1801
- Hellman, B., Lernmark, Å., Sehlin, J. & Täljedal, I.-B. (1972) *Mol. Pharmacol.* **8**, 759–769
- Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J. & Täljedal, I.-B. (1973a) *Biochem. J.* **132**, 775–789
- Hellman, B., Sehlin, J. & Täljedal, I.-B. (1973b) *Eur. J. Physiol.* **340**, 51–58
- Idahl, L.-Å. (1971) *Hormones* **2**, 371–377
- Idahl, L.-Å. (1972) *Anal. Biochem.* **50**, 386–398
- Idahl, L.-Å. (1973) *Diabetologia* **9**, 403–412
- Landgraf, R., Kotler-Brajtburg, J. & Matschinsky, F. M. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 536–540
- Lernmark, Å. (1971) *Acta Diabetol. Lat.* **8**, 649–679
- Lernmark, Å. & Hellman, B. (1970) *Metab. Clin. Exp.* **19**, 614–618
- Malaisse, W. J., Malaisse-Lagae, F. & Brisson, G. (1971) *Horm. Metab. Res.* **3**, 65–70
- Malaisse, W. J., Pipeleers, D. G. & Mahy, M. (1973) *Diabetologia* **9**, 1–5
- Malaisse-Lagae, F. & Malaisse, W. J. (1971) *Endocrinology* **88**, 72–80
- Matschinsky, F. M. & Ellerman, J. (1973) *Biochem. Biophys. Res. Commun.* **50**, 193–199
- Matschinsky, F. M., Passonneau, J. V. & Lowry, O. H. (1968) *J. Histochem. Cytochem.* **16**, 29–39
- Milner, R. D. G. & Hales, C. N. (1969) *Biochem. J.* **113**, 473–479
- Milner, R. D. G. & Hales, C. N. (1970) in *The Structure and Metabolism of the Pancreatic Islets* (Falkmer, S., Hellman, B. & Täljedal, I.-B., eds.), pp. 489–493, Pergamon Press, Oxford
- Sehlin, J. (1973) *FEBS Lett.* **30**, 45–48
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964) *Manometric Techniques*, 4th edn., p. 132, Burgess Publishing Co., Minneapolis