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The Stimulus–Secretion Coupling of Glucose-Induced Insulin Release

METABOLISM OF GLUCOSE IN K^+-DEPRIVED ISLETS

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1. When pancreatic islets were exposed to a K^+-free medium, the intracellular concentration of K^+ was decreased and that of Na^+ increased. 2. In the K^+-deprived islets, the utilization of [5-^{3}H]glucose, output of lactic acid and oxidation of [U-^{14}C]-glucose were decreased by about 30–40% below the control values found at normal extracellular K^+ concentration (5.0mM). However, the oxidation of [U-^{14}C]pyruvate was unaffected. 3. The omission of extracellular K^+ little affected the production of $^{14}CO_2$ from islets prelabelled with [U-^{14}C]palmitate and incubated in the absence of glucose, despite the fact that K^+ deprivation significantly increased the ATP concentration and ATP/ADP concentration ratio in the glucose-deprived islets. 4. At normal K^+ concentration, glucose increased the concentrations of phosphoenolpyruvate, NAD(P)H and ATP in the islets. In the glucose-stimulated islets, the concentration of phosphoenolpyruvate, but not that of either NAD(P)H or ATP, was higher in the absence than in the presence of extracellular K^+. In islet homogenates, the activity of pyruvate kinase (EC 2.7.1.40) was stimulated by K^+ (optimal activity at 100–150mM-K^+) and inhibited by Na^+ (except at very low K^+ concentrations). 5. K^+ could be replaced by NH_4^+, Rb^+, Cs^+ or Na^+ to maintain, at least to some extent, pyruvate kinase activity in islet homogenates. Addition of Rb^+ or Cs^+, but not NH_4^+, to K^+-deprived media also increased [U-^{14}C]glucose oxidation by intact islets. 6. The omission of K^+ did not cause any obvious anomaly in the apparent dependency of $^{45}Ca^{2+}$ net uptake on NAD(P)H concentration in the islets. 7. These data suggest that the coupling between metabolic and ionic events in the islet cells involves feedback mechanisms through which glucose oxidation may be modulated by cationic factors.

The hypothesis was recently advanced that the insulinotropic capacity of several nutrients depends on their ability to serve as a fuel in pancreatic islet cells (Malaisse *et al.*, 1979*b*). This view raises the question as to the existence and nature of feedback mechanisms through which the oxidation of nutrients would be regulated by the consumption of energy in islet cells. To evaluate the participation of a K^+-activated ATPase in such an energy expenditure, we have investigated to what extent the omission of extracellular K^+ may affect the metabolism of glucose in isolated pancreatic islets.

Materials and Methods

All experiments were performed with isolated islets removed from fed female albino rats (Lacy & Kostianovsky, 1967) and incubated in a bicarbonate-buffered medium (Malaisse *et al.*, 1970). The methods used for measuring the net uptake of $^{86}Rb^+$ (Malaisse *et al.*, 1978*a*), $^{22}Na^+$ (Kawazu *et al.*, 1978) or $^{45}Ca^{2+}$ (Malaisse-Lagae & Malaisse, 1971), the conversion of [5-^{3}H]glucose into ^3H_2O (Levy *et al.*, 1976), the output of lactic acid (Sener & Malaisse, 1976), the oxidation of [U-^{14}C]glucose (Malaisse *et al.*, 1974) or [U-^{14}C]pyruvate (Sener *et al.*, 1978), the production of $^{14}CO_2$ from islets prelabelled with [U-^{14}C]palmitate (Sener *et al.*, 1978) and the concentrations of ATP, ADP, AMP and NAD(P)H (Malaisse *et al.*, 1978*b*) in the islets were as previously described.

The measurement of phosphoenolpyruvate concentration was performed by the method of Sugden & Ashcroft (1977), with some modifications. Groups of 15 islets each were incubated for 60min in 40μl of incubation medium and then placed in liquid N_2. After addition of 10μl of 0.1M-HCl, the islets were homogenized by mechanical vibration

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(Malaisse *et al.*, 1978*b*). The homogenate was mixed with 40 μl of a solution of 200 mM-triethanolamine/HCl, pH 7.4, containing KCl (200 mM), $MgSO_4$ (10 mM), EDTA (1 mM), glucose (2.2 mM) and ADP (0.01 mM). After the mixture had been heated for 5 min at 90°C, hexokinase [EC 2.7.1.1; 2.4 munits (nmol of substrate transformed/min) in 2 μl of the triethanolamine buffer] was added to the solution, which was maintained for 45 min at room temperature (23°C). The reaction was stopped by heating the solution for 5 min at 90°C, and a first sample (20 μl) removed for measurement of residual ATP. Pyruvate kinase [EC 2.7.1.40; 2.4 munits (nmol of substrate transformed/min) in 2 μl of the triethanolamine buffer] was then added to the remaining solution. After 20 min incubation at room temperature and 5 min heating at 90°C, the ATP concentration was again measured in samples (20 μl) of the solution. The method used for the measurement of ATP by the luciferase technique is detailed elsewhere (Malaisse *et al.*, 1978*b*). The concentration of phosphoenolpyruvate was judged from the paired difference in ATP concentration found before and after the final incubation with pyruvate kinase. Standard solutions of phosphoenolpyruvate containing 1–8 pmol/40 μl were prepared in the same medium as that used for incubation of the islets, except that ATP (200 pmol/40 μl) was also included in this medium. These standard solutions were treated in exactly the same manner as the samples containing the islets, and yielded a linear dose-related response (Fig. 1).

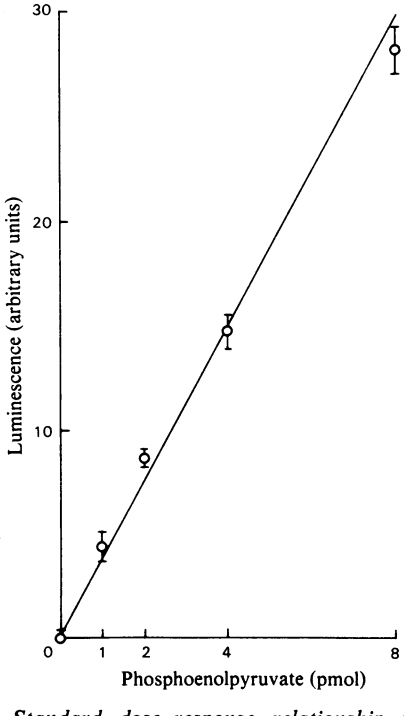

Fig. 1. *Standard dose–response relationship for the assay of phosphoenolpyruvate*
Experimental details are given in the text. Each point is the mean ± S.E.M. for 15 determinations collected in separate experiments.

The activity of pyruvate kinase (EC 2.7.1.40) was measured over 20 min incubation at 37°C in a solution (100 μl) of 50 mM-triethanolamine/HCl, pH 7.0, containing $MgCl_2$ (5 mM), EDTA (0.5 mM), albumin (1 mg/ml), ADP (2.5 mM), phosphoenolpyruvate (2.0 mM), NADH (0.75 mμ), dialysed lactate dehydrogenase [EC 1.1.1.27; 6 units (μmol of substrate transformed/min)/ml] and a sample (5 μl) of the islet homogenate. The latter contained 50 islets sonicated in 1.0 ml of a 12.5 mM-triethanolamine/HCl-buffered solution. The reaction was stopped by adding 300 μl of 1.0 M-HCl, and the NAD^+ was fluorescence developed and measured as described elsewhere (Malaisse *et al.*, 1979*a*). The rate of phosphoenolpyruvate conversion into pyruvate was proportional to both the concentration of tissue and length of incubation, determinations made with variable amounts of homogenate (5, 10 or 15 μl) over different times of incubation (10, 20 or 30 min) averaging 1.92 ± 0.13 nmol/min per islet ($n = 6$). The blank readings obtained in the absence of homogenate (0.18 ± 0.01, $n = 6$) were small relative to the values obtained with the standards (2.58 ± 0.13/nmol of NAD^+). The readings obtained with the standards were linear up to 40 nmol of NAD^+. The recovery of pyruvate averaged $102.1 \pm 1.6\%$ ($n = 5$).

All results are expressed as the means (± S.E.M.) together with the number of individual observations (n).

Results

Effect of extracellular K^+ on the intracellular concentration of K^+

When groups of 100 islets each were incubated for 60 min in K^+-depleted medium (1.0 ml) containing glucose (16.7 mM), the K^+ concentration of the incubation medium, as judged by flame photometry, increased from a control value of 10 ± 1 μM ($n = 3$) to 40 ± 1 μM ($n = 6$). This indicates that the islets released 295 ± 12 pmol of K^+/islet during incubation in the K^+-depleted medium. Their final content averaged 63 ± 5 pmol of K^+/islet ($n = 6$), representing $17.5 \pm 0.9\%$ of their calculated initial K^+ content. These values are in good agreement with radioisotopic measurements, indicating that, after 30 min preincubation at normal K^+ concentration (5 mM) and 90 min incubation in media (0.05 ml) containing glucose (16.7 mM) and $^{86}Rb^+$ (52 μM), with or without K^+ (5.0 mM), the $^{86}Rb^+$ content of the islets averaged 4.4 ± 0.4 and 7.4 ± 1.2 pmol/islet ($n = 6$ or 7)

respectively. Taking into account the usual K^+ content of the islets (Malaisse *et al.*, 1978*a*), and the possible contamination by residual preincubation medium ($\leqslant 3\,\mu l$), the final K^+ content of the islets was estimated to be 315 ± 28 pmol/islet ($n = 6$) after incubation at normal K^+ concentration and $\leqslant 41 + 7$ pmol/islet ($n = 7$) after exposure to the K^+-depleted medium. The latter values were corrected for the λ_{Rb}/λ_K ratio, which is close to 0.7 in the islets (Boschero & Malaisse, 1979) as well as in other tissues (Stieve & Hartung, 1977). They confirm that the islets retain less than 20% of their normal K^+ content when exposed to a K^+-depleted incubation medium.

Over 90 min incubation, the omission of extracellular K^+ increased $^{22}Na^+$ net uptake by the islets from a control value (K^+ 5.0 mM) of 142 ± 17 to 332 ± 14 pmol/islet ($n = 6$) in the absence of glucose, and from 150 ± 21 to 412 ± 18 pmol/islet ($n = 10$) in the presence of glucose.

Effect of K^+ deprivation on glycolysis

Over 120 min incubation, the rate of [5-^{3}H]-glucose utilization, lactic acid production and [U-^{14}C]glucose oxidation were significantly diminished in K^+-deprived islets (Table 1). Relative to the mean control value found at normal K^+ concentration, the rates of glucose utilization, lactate output and glucose oxidation in the K^+-deprived islets averaged 59.0 ± 9.2, 67.0 ± 4.1 and 57.8 ± 3.9% respectively. None of these three values is significantly different from any other, suggesting an unaltered dependency of glucose oxidation on the rate of glycolysis. This view is supported by the failure of K^+ deprivation to affect significantly the oxidation of [U-^{14}C]-pyruvate.

Effect of K^+ deprivation on glucose oxidation

At normal K^+ concentration, the oxidation of [U-^{14}C]glucose increased as the glucose concentration was raised from 5.6 to 8.3 and 16.7 mM (Table 2).

Table 1. *Effects of K^+ deprivation on metabolic variables*

All data are expressed as pmol of substrate (glucose or pyruvate) metabolized/120 min per islet. Mean values ± S.E.M. are shown together with the numbers of individual observations in parentheses and the significance of differences due to the change in K^+ concentration (N.S., not significant).

Substrate	Metabolic parameter	Rate (pmol of substrate metabolized/120 min per islet)		
		With 5.0 mM-K^+	With no K^+	*P*
Glucose (16.7 mM)	[5-^{3}H]Glucose utilization	210.4 ± 17.7 (20)	124.1 ± 19.3 (20)	<0.005
Glucose (16.7 mM)	Lactate output	71.5 ± 6.5 (22)	47.9 ± 2.9 (21)	<0.001
Glucose (16.7 mM)	[U-^{14}C]Glucose oxidation	49.0 ± 3.5 (23)	28.3 ± 1.9 (23)	<0.001
Pyruvate (20.0 mM)	[U-^{14}C]Pyruvate oxidation	40.8 ± 4.1 (24)	36.6 ± 5.7 (24)	N.S.

Table 2. *Glucose oxidation by isolated islets*

The rate of glucose oxidation was measured, with two different tracers, at three glucose concentrations and over two lengths of time of incubation in the presence or absence of K^+, Rb^+, Cs^+ and NH_4^+. Control rates obtained in the presence of K^+ (5 mM) are expressed in absolute terms. Experimental findings are expressed as mean percentages ± S.E.M. of the mean control value found within the same experiment(s), and are shown together with the statistical significance of differences between them and control values (N.S., not significant).

Tracer	Concn. of K^+ (mM)	Concn. of Rb^+ (mM)	Concn. of Cs^+ (mM)	Concn. of NH_4^+ (mM)	Time (min)	Glucose oxidation (fmol/min per islet)	Glucose oxidation (% of control)	*P*
[U-^{14}C]Glucose (5.6 mM)	5	—	—	—	120	105 ± 9 (11)		
[U-^{14}C]Glucose (5.6 mM)	—	—	—	—	120		63.5 ± 8.9 (12)	<0.010
[U-^{14}C]Glucose (8.3 mM)	5	—	—	—	120	208 ± 12 (30)		
[U-^{14}C]Glucose (8.3 mM)	—	—	—	—	120		71.2 ± 4.8 (22)	<0.001
[U-^{14}C]Glucose (16.7 mM)	5	—	—	—	30	393 ± 36 (24)		
[U-^{14}C]Glucose (16.7 mM)	—	—	—	—	30		74.7 ± 2.8 (7)	<0.005
[U-^{14}C]Glucose (16.7 mM)	5	—	—	—	120	408 ± 32 (48)		
[U-^{14}C]Glucose (16.7 mM)	—	—	—	—	120		61.3 ± 3.6 (16)	<0.001
[U-^{14}C]Glucose (16.7 mM)	—	5	—	—	120		90.5 ± 7.3 (16)	N.S.
[U-^{14}C]Glucose (16.7 mM)	—	—	5	—	120		90.8 ± 5.9 (16)	N.S.
[U-^{14}C]Glucose (16.7 mM)	—	—	—	5	120		26.3 ± 2.7 (8)	<0.001
[U-^{14}C]Glucose (16.7 mM)	—	—	—	50	120		25.0 ± 2.0 (8)	<0.001
[1-^{14}C]Glucose (16.7 mM)	5	—	—	—	120	353 ± 31 (14)		
[1-^{14}C]Glucose (16.7 mM)	—	—	—	—	120		60.9 ± 4.7 (15)	<0.001

Over 120 min incubation, the oxidation of [U-^{14}C]-glucose was diminished in K^+-deprived media. On pooling of the results obtained at three glucose concentrations (5.6, 8.3 and 16.7 mM), the degree of inhibition averaged 33.8 ± 3.1% ($n = 50$). An inhibition of glucose oxidation was observed within the first 30 min of exposure to the K^+-depleted medium. At 16.7 mM-glucose, the oxidations of [U-^{14}C]- and [1-^{14}C]-glucose were affected to the same extent, suggesting that there was no major anomaly in the rate of glucose metabolism through the pentose phosphate shunt relative to other oxidative pathways.

Effect of K^+ deprivation on the oxidation of endogenous substrates

When islets are prelabelled with [U-^{14}C]palmitate and then incubated in the absence of glucose, the rate of $^{14}CO_2$ production is illustrative of the oxidation of endogenous substrates (Malaisse *et al.*, 1979*a*; Sener *et al.*, 1978). The omission of K^+ had little effect on the production of $^{14}CO_2$ from the prelabelled islets (Table 3), a significant fall in $^{14}CO_2$ output being observed in only one out of four separate experiments.

Effect of K^+ deprivation on the concentrations of adenine and nicotinamide nucleotides

When islets are incubated in the absence of glucose, the ATP concentration falls below its initial value (Ashcroft *et al.*, 1973; Malaisse *et al.*, 1976). The ATP concentration and ATP/ADP concentration ratio were decreased to a smaller extent ($P < 0.05$ or less) when the glucose-deprived islets were incubated in the absence of K^+ (Table 4). When the incubation medium contained glucose, the concentrations of adenine nucleotides were not significantly different in the presence and in the absence of K^+. Both in the presence and in the absence of glucose the omission of K^+ tended to decrease the concentration of reduced nicotinamide nucleotides; however, this effect failed to achieve statistical significance in the absence of glucose. The glucose-induced increment in NAD(P)H concentration was evident both at normal K^+ concentration and in the absence of extracellular K^+ ($P < 0.02$).

Effect of K^+ deprivation on the concentration of phosphoenolpyruvate

At normal K^+ concentration an increase in glucose concentration from 1.7 to 16.7 mM slightly but

Table 3. *Output of $^{14}CO_2$ from islets prelabelled with [U-^{14}C]palmitate*

The outputs of $^{14}CO_2$ were measured over a 120 min incubation period in the absence of glucose, and are expressed as mean percentages ± S.E.M. of the final radioactivity content of the islets. The latter content averaged 4064 ± 206 c.p.m./islet ($n = 77$), corresponding to 2.31 ± 0.12 pmol of [U-^{14}C]palmitate with the same specific radioactivity as that of the preincubation medium. The ratio $^{14}CO_2$ output/^{14}C content was also normalized relative to the mean control value (first row) found within the same experiment. Such a control value corresponded to a mean $^{14}CO_2$ output of 255.1 ± 16.2 c.p.m./120 min per islet.

Concn. of K^+ (mM)	Concn. of antimycin A (mM)	$^{14}CO_2$ output/^{14}C content of the islets (%)	(normalized)	(n)
5	—	7.10 ± 0.43	100.0 ± 3.3	27
—	—	6.53 ± 0.51	94.3 ± 5.1	31
5	0.01	2.11 ± 0.16	31.0 ± 1.8	19

Table 4. *Effects of glucose and K^+ on the concentrations of adenine and reduced nicotinamide nucleotides in isolated islets*

All measurements were performed after 30 min incubation under the conditions indicated at the top of each column. Results are expressed as mean values ± S.E.M. for the numbers of observations given in parentheses.

	Concentration or concentration ratio			
Glucose ...	—	—	16.7 mM	16.7 mM
K^+ ...	5 mM	—	5 mM	—
ATP + ADP + AMP (pmol/islet)	10.8 ± 0.5 (28)	9.9 ± 0.8 (7)	11.2 ± 0.5 (32)	13.9 ± 1.1 (10)
ATP (% of ATP + ADP + AMP)	50.4 ± 2.3 (28)	60.1 ± 1.8 (7)	68.0 ± 1.3 (32)	67.3 ± 2.4 (10)
ADP (% of ATP + ADP + AMP)	26.0 ± 1.3 (28)	18.0 ± 1.4 (7)	17.5 ± 0.8 (32)	16.8 ± 0.9 (10)
AMP (% of ATP + ADP + AMP)	23.6 ± 1.9 (28)	21.9 ± 1.0 (7)	14.5 ± 1.1 (32)	15.9 ± 2.5 (10)
[ATP]/[ADP] ratio	2.15 ± 0.19 (26)	3.46 ± 0.34 (7)	4.23 ± 0.28 (32)	4.20 ± 0.30 (10)
[ATP + 0.5 ADP]/[ATP + ADP + AMP] ratio	0.633 ± 0.020 (28)	0.700 ± 0.017 (7)	0.760 ± 0.010 (32)	0.757 ± 0.023 (10)
NADH + NADPH (fmol/islet)	284 ± 37 (34)	230 ± 22 (18)	406 ± 28 (32)	323 ± 25 (30)

significantly ($P<0.05$) augmented the concentration of phosphoenolpyruvate in the islets from 216 ± 27 ($n=27$) to 298 ± 24 ($n=28$) fmol/islet. At the high glucose concentration a further increase ($P<0.01$) in phosphoenolpyruvate concentration to 399 ± 27 fmol/islet ($n=27$) was observed when K^+ was omitted from the incubation medium.

Effect of K^+ on the activity of pyruvate kinase

At pH 7.0 and in the presence of 100 mM-K^+ and 5 mM-Na^+ the activity of pyruvate kinase averaged 2.63 ± 0.64 pmol/min per islet. The activity of the enzyme was maximal at pH 6.5 (Table 5) and at 100–150 mM-K^+, a dose-related fall in activity being observed at lower K^+ concentrations (Fig. 2*a*). Except at low K^+ concentration (0–10 mM), Na^+ causes a dose-related inhibition of pyruvate kinase activity (Fig. 2*b* and Table 5).

Replacement of K^+ by other univalent cations

The activity of pyruvate kinase was maintained at its normal value when K^+ was replaced by Rb^+ or NH_4^+ (Table 5). However, Cs^+ exerted a smaller stimulant effect than did K^+. When intact islets were incubated in a medium in which K^+ was replaced by either Rb^+ or Cs^+, the oxidation of [U-^{14}C]glucose was close to its control value found in the presence of K^+ (Table 2). In contrast, replacement of K^+ by

Table 5. *Effects of pH, K^+, Rb^+, Cs^+, NH_4^+ and Na^+ on the activity of pyruvate kinase*

All results are expressed relative to the mean control value found with the same homogenate at pH 7.0 in the presence of 100 mM-K^+ and 5 mM-Na^+, and are given as mean values ± S.E.M. for the numbers of determinations given in parentheses or as individual values.

Concn. of K^+ (mM)	Concn. of Rb^+ (mM)	Concn. of Cs^+ (mM)	Concn. of NH_4^+ (mM)	pH	Pyruvate kinase activity (% of control) With 5.0 mM-Na^+	With 55.0 mM-Na^+
100	—	—	—	6.0	81.6–88.2 (2)	53.1–74.3 (2)
100	—	—	—	6.5	124.7–141.1 (2)	105.0–109.8 (2)
100	—	—	—	7.0	100.0 (5)	69.7 ± 2.8 (5)
100	—	—	—	7.5	99.1–101.1 (2)	67.0–86.6 (2)
—	100	—	—	7.0	89.1–107.1 (2)	65.2–69.8 (2)
—	—	100	—	7.0	29.6–49.9 (2)	17.8–35.2 (2)
—	—	—	100	7.0	100.8 (1)	
—	—	—	—	7.0	1.8 ± 1.2 (3)	11.5 (1)

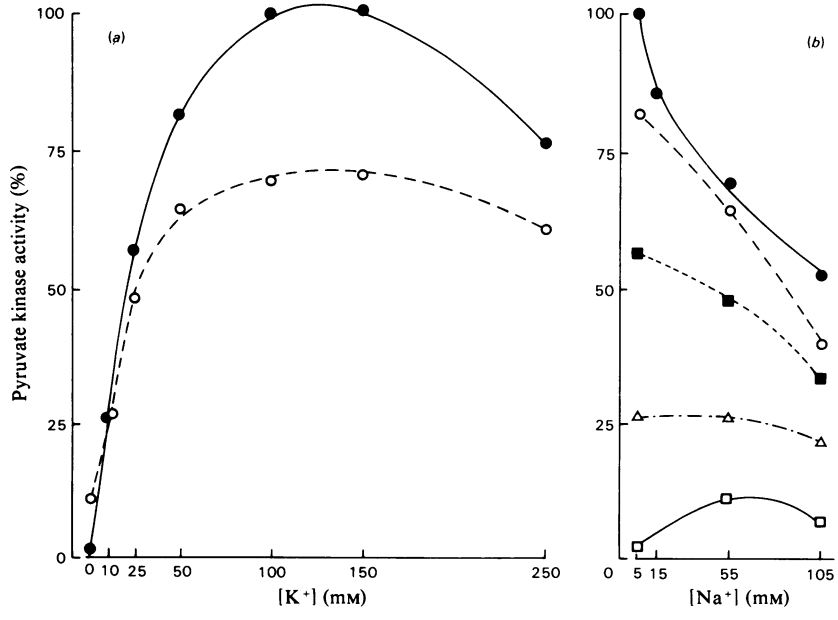

Fig. 2. *Effects of K^+ (a) and Na^+ (b) on the activity of pyruvate kinase in islet homogenates*

Experimental details are given in the text. (*a*) ●, 5 mM-Na^+; ○, 55 mM-Na^+. (*b*) □, 0 mM-K^+; △, 10 mM-K^+; ■, 25 mM-K^+; ○, 50 mM-K^+; ●, 100 mM-K^+. The mode of expression of results in the same as that used in Table 5.

NH_4^+ provoked a dramatic inhibition of glucose oxidation (Table 2).

Relationship between $^{45}Ca^{2+}$ net uptake and metabolic parameters

There was a significant correlation ($P < 0.05$) between the net uptake of $^{45}Ca^{2+}$ by the islets and the concentration of NAD(P)H (Fig. 3). No correlation was found between $^{45}Ca^{2+}$ net uptake and the concentration of either ATP or phosphoenolpyruvate in the islets.

Discussion

Cation concentrations in K^+-deprived islets

In the islets exposed to K^+-depleted media, the intracellular concentration of K^+ was decreased to less than 20% of its normal value. Since the omission of extracellular K^+ is known to decrease the fractional outflow rate of K^+ from the islets (Boschero & Malaisse, 1979), the decreased K^+ content is obviously the consequence of a decrease in K^+ influx.

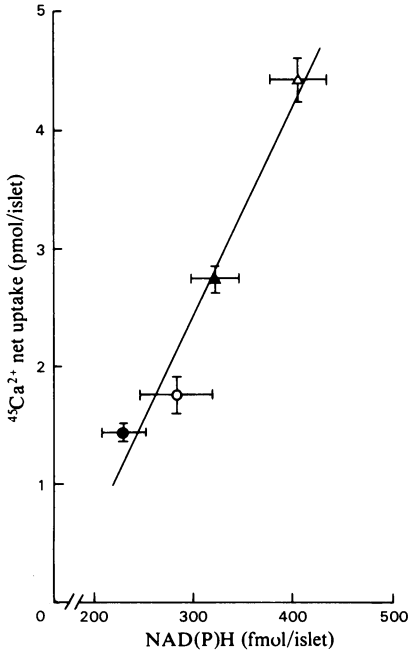

Fig. 3. *Correlation between the net uptake of $^{45}Ca^{2+}$ and the concentration of reduced nicotinamide nucleotides in islets incubated in the absence* (○ *and* ●) *or presence* (△ *and* ▲) *of glucose* (16.7 mM) *with* (○ *and* △) *or without* (● *and* ▲) *K^+* (5.0 mM)

Experimental details are given in the text. Mean values ± S.E.M. for $^{45}Ca^{2+}$ net uptake refer to 12–55 individual observations. The data for NAD(P)H are taken from Table 4.

The absence of extracellular K^+ is likely to cause inhibition of the $Na^+ + K^+$-activated ATPase thought to normally mediate, in part at least, the uphill inflow of K^+ and outflow of Na^+ across the plasma membrane of the islet cells. In good agreement with the latter concept, the intracellular concentration of Na^+ was dramatically increased in the K^+-deprived islets. In such islets the loss of intracellular K^+ appeared to be matched by the increase in intracellular Na^+. In islets exposed to glucose (16.7 mM), the total concentration of these cations averaged 445 ± 29 and 475 ± 19 pmol/islet in the presence and absence of extracellular K^+ respectively.

The net uptake of $^{22}Na^+$ is little affected (Sehlin & Täljedal, 1974*a*) or slightly diminished (Kawazu *et al.*, 1978) by glucose when the islets are incubated at normal extracellular K^+ concentration. When K^+ was omitted from the incubation medium, the net uptake of $^{22}Na^+$ was higher ($P < 0.01$) in the presence (412 ± 18 pmol/islet) than absence (332 ± 14 pmol/islet) of glucose. A comparable situation was previously found in islets incubated at normal extracellular K^+ concentration in the presence of ouabain (Kawazu *et al.*, 1978). The export of Na^+ being inhibited in either the absence of K^+ or the presence of ouabain, the glucose-induced increment in $^{22}Na^+$ net uptake may reflect a stimulant action of the sugar on the inflow of Na^+ into the islet cells (Kawazu *et al.*, 1978).

Metabolic changes due to K^+ deprivation in the absence of glucose

The absence of extracellular K^+ exerted several effects on the metabolism of the islets. In the K^+-deprived islets and in the absence of glucose, the metabolic situation was compatible with the existence of a diminished energy expenditure, leading to less rapid or less severe falls in ATP concentration and ATP/ADP concentration ratio than those usually seen when islets are incubated in the absence of glucose at normal K^+ concentration (Ashcroft *et al.*, 1973; Malaisse *et al.*, 1976).

A minor decrease in the utilization of endogenous nutrients might occur in the K^+- and glucose-deprived islets (Table 3). This view is supported by the fact that the removal of extracellular K^+ diminishes by $10.7 \pm 1.5\%$ the rate of O_2 consumption in islets not exposed to glucose (Hutton & Malaisse, 1980). The control rate of O_2 consumption in the absence of glucose averaging 8.5 ± 1.5 pmol of O_2/min per islet, the fall in O_2 uptake attributable to the absence of K^+ amounts to 0.91 ± 0.20 pmol of O_2/min per islet, corresponding to an estimated loss in ATP generation of 5.5 ± 1.2 pmol/min per islet. This last value is not vastly different ($P > 0.3$) from the energy thought to be normally required for the appropriate function of

the ATPase mediating K^+ inflow in islet cells. Under steady-state conditions and at normal K^+ extracellular concentration (5.0mM), the inflow–outflow rate of K^+ (as judged from the net uptake and fractional outflow rate of $^{42}K^+$) averages, in the absence of glucose, 14.2 ± 1.6pmol/min per islet (Boschero *et al.*, 1977). Assuming a 3:2:1 stoicheiometry for $Na^+/K^+/ATP$ active transport of univalent cations by the plasma-membrane-associated ATPase (Garrahan & Glynn, 1967), the inflow rate of K^+ would correspond to an energy expenditure of 7.1 ± 0.8pmol of ATP/min per islet, a value indeed close to the above-mentioned 5.5 ± 1.2pmol of ATP/min.

In the absence of glucose, the concentration of reduced nicotinamide nucleotides and the net uptake of $^{45}Ca^{2+}$ were slightly decreased in the absence of extracellular K^+, such effects failing to achieve statistical significance.

Metabolic changes due to K^+ deprivation in the presence of glucose

The metabolic changes evoked by K^+ deprivation were different in the presence of glucose from those in the absence of glucose. When glucose was present in the incubation medium, no significant change in the concentration of adenine nucleotides could be detected (Table 4).

The absence of extracellular K^+ decreased glucose oxidation, as already noted by Henquin & Lambert (1974). The oxidation of glucose was much more affected than that of endogenous substrates. These findings are again in good agreement with the data on O_2 uptake. In the presence of glucose (16.7mM) and at normal K^+ concentration (5.0mM) the rate of O_2 consumption is increased by 3.0 ± 0.2pmol of O_2/min per islet above the basal value, and the removal of extracellular K^+ causes a $28.8 \pm 5.0\%$ inhibition of O_2 consumption (Hutton & Malaisse, 1980). The fall in O_2 consumption due to K^+ deprivation is thus higher in the presence of glucose (3.31 ± 0.75pmol of O_2/min per islet) than in its absence (0.91 ± 0.20pmol of O_2/min per islet). The major part of this difference may well be attributed to the observed decrease in oxidation rate of exogenous glucose.

At this point, it should be emphasized that the decrease in oxidation rate, as observed with glucose in the K^+-depleted media, is not an universal phenomenon. For instance, the oxidation of either pyruvate (Table 1) or 4-methyl-2-oxopentanoate (Hutton *et al.*, 1980), which is a very potent insulinotropic nutrient, is not significantly diminished in the presence of K^+-depleted media. This dissociated behaviour led us to consider that the decrease in glucose oxidation attributable to the absence of K^+ could be due, in part at least, to a specific defect in the metabolism of glucose. Because of its well-established sensitivity to K^+ and Na^+ (Seubert & Schoner, 1971), the enzyme pyruvate kinase was considered as a likely candidate for such a site-specific inhibition. The findings illustrated in Fig. 2 and Table 5, which concern the influence of K^+ and Na^+ on the activity of pyruvate kinase, and our measurements of phosphoenolpyruvate in the islets are compatible with the view that the reaction catalysed by pyruvate kinase was indeed inhibited in the K^+-deprived islets. Incidentally, the effect of glucose itself to increase the concentration of phosphoenolpyruvate in the islets confirms a previous report (Sugden & Ashcroft, 1977), although the absolute concentration of the substrate was definitely lower in the present than in the former study.

The ions Rb^+ and Cs^+ were able to replace, in part at least, K^+ in stimulating the activity of pyruvate kinase, and restored glucose oxidation at close-to-normal values. These univalent cations can also replace K^+ in stimulating the activity of the $Na^+ + K^+$-dependent ATPase (Skou, 1965). Any difference encountered in the effect of each cation (K^+, Rb^+ or Cs^+) on the activity of pyruvate kinase in islet homogenates and glucose oxidation in intact islets respectively could be due to the known differences in the fractional outflow rate of these different cations (Boschero & Malaisse, 1979; Stieve & Hartung, 1977), and hence their steady-state concentrations in intact cells. The ion NH_4^+ was able to replace K^+ in the reaction catalysed by pyruvate kinase, but inhibited glucose oxidation when added to K^+-free media. We have not explored the mechanism of such a dissociated response.

The inhibition of glucose metabolism due to K^+ deprivation may well be responsible for a decreased generation of reduced nicotinamide nucleotides. In turn, the resulting fall in NAD(P)H concentration could account for a decreased net uptake of $^{45}Ca^{2+}$, since these two parameters are generally linked by a relationship of apparent proportionality (Malaisse *et al.*, 1979*b*).

Previous reports have emphasized the view that glucose does not appear to exert any obvious effect on the inflow rate of K^+ into the islet cells (Boschero *et al.*, 1977; Malaisse *et al.*, 1978*a*; Sehlin & Täljedal, 1974*b*). It now appears that the overall oxidation of nutrients and consumption of O_2 are more severely affected by K^+ deprivation in the presence than in the absence of glucose. Taken together, these findings suggest that K^+ deprivation, in addition to inhibiting the K^+-activated ATPase, also suppressed some glucose-dependent energy-consuming process(es). Several functional changes occur in the islets deprived of K^+, including the already mentioned decrease in glucose-stimulated $^{45}Ca^{2+}$ net uptake, a close-to-total inhibition of glucose-stimulated proinsulin biosynthesis (Sener *et al.*, 1979) and a delayed diminution in the glucose-

stimulated insulin release (Henquin & Lambert, 1974). Some of these functional alterations may well contribute to the postulated decrease in ATP utilization evoked by K^+ deprivation.

In conclusion, in the islet cells the coupling between metabolic and cationic events, which, in our opinion, represents an essential step in the glucose-stimulated secretory sequence, is susceptible to feedback regulatory mechanisms through which a primary change in cationic movements results in an alteration of glucose metabolism. Other examples of such a situation can be found in previous reports on the influence of Na^+ or Ca^{2+} deprivation on islet-cell metabolism (Hellman *et al.*, 1974; Malaisse *et al.*, 1978*c*). In the K^+-deprived islets, the inhibition of glucose oxidation appears to be associated with an inhibition of pyruvate kinase and a decrease in energy expenditure. Since glucose itself increases K^+ concentration (Malaisse *et al.*, 1978*a*) and tends to decrease Na^+ concentration (Kawazu *et al.*, 1978) in the islet cells, modulation of pyruvate kinase activity by univalent cations could play a role in the stimulus–secretion of glucose-induced insulin release at normal extracellular concentrations of Na^+ and K^+.

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