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# Metabolic Control of the Potassium Permeability in Pancreatic Islet Cells

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The $K^+$ permeability of pancreatic islet cells was studied by monitoring the efflux of $^{86}Rb^+$ (used as tracer for $K^+$) from perifused rat islets and measuring the uptake of $^{42}K^+$. Glucose markedly and reversibly decreased $^{86}Rb^+$ efflux from islet cells and this effect was antagonized by inhibitors of the metabolic degradation of the sugar, i.e. mannoheptulose, iodoacetate, glucosamine and 2-deoxyglucose. Among glucose metabolites, glyceraldehyde reduced the $K^+$ permeability even more potently than did glucose itself; pyruvate and lactate alone exhibited only a small effect, but potentiated that of glucose. Other metabolized sugars, like mannose, glucosamine and *N*-acetylglucosamine, also decreased $^{86}Rb^+$ efflux from islet cells. Fructose was effective only in the presence of glucose. Non-metabolized sugars like galactose, 2-deoxyglucose and 3-*O*-methylglucose had no effect. The changes in $K^+$ permeability by agents known to modify the concentrations of nicotinamide nucleotides, glutathione or ATP in islet cells were also studied. Increasing NAD(P)H concentrations in islet cells by pentobarbital rapidly and reversibly reduced $^{86}Rb^+$ efflux; exogenous reduced glutathione produced a similar though weaker effect. By contrast, oxidizing nicotinamide nucleotides with phenazine methosulphate or Methylene Blue, or oxidizing glutathione by t-butyl hydroperoxide increased the $K^+$ permeability of islet cells. Uncoupling the oxidative phosphorylations with dicumarol also augmented $^{86}Rb^+$ efflux markedly. In the absence of glucose, but not in its presence, methylxanthines reduced $^{86}Rb^+$ efflux from the islets; such was not the case for cholera toxin or dibutyryl cyclic AMP. Glucose and glyceraldehyde had no effect on $^{42}K^+$ uptake after a short incubation (10 min), but augmented it after 60 min; the effect of glucose was suppressed by mannoheptulose and not mimicked by 3-*O*-methylglucose. The results clearly establish the importance of the metabolic degradation of glucose and other substrates for the control of the $K^+$ permeability in pancreatic islet cells and support the concept that a decrease in the $K^+$ permeability represents a major step of the B-cell response to physiological stimulation.

The exact mechanisms whereby glucose stimulates insulin release from pancreatic B-cells are not yet completely understood. Two theories have been advanced to explain how the sugar generates stimulating signals. The 'glucoreceptor' model postulates a direct interaction of the glucose molecule with a membrane or cytoplasmic receptor (Cerasi & Luft, 1970; Matschinsky *et al.*, 1971). On the other hand, the 'metabolic' model proposes that metabolites of glucose or cofactors of glucose metabolism initiate the secretory response (Grodsky *et al.*, 1963; Coore & Randle, 1964; Ashcroft & Randle, 1970). More recent experimental evidence (Malaisse *et al.*, 1976; Ashcroft, 1976; Zawalich *et al.*, 1978) strongly supports the latter of these models.

Glucose and many other insulin-releasing agents induce depolarization and electrical activity in pancreatic B-cells and a correlation exists between appearance of these electrical events and insulin secretion (Dean & Matthews, 1970; Meissner & Schmelz, 1974; Dean *et al.*, 1975; Meissner, 1976). The importance of glucose metabolism for induction of electrical activity in pancreatic B-cells has been established (Dean *et al.*, 1975), but the ionic events underlying these electrical changes remain unclear. The recent evidence (Henquin, 1978) that glucose rapidly and markedly reduces $K^+$ efflux from pancreatic islet cells supports the proposal (Sehlin & Täljedal, 1975) that the depolarization of B-cells by the sugar is mediated by a decrease in



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their $K^+$ permeability. The demonstration that pharmacological agents which potentiate the effect of glucose on $K^+$ permeability also potentiate its releasing effect, whereas those which augment $K^+$ permeability antagonize the insulinotropic effect of the sugar (Henquin, 1977; Henquin & Meissner, 1978; Henquin *et al.*, 1979) also favour this hypothesis.

The objective of the experiments reported here was to assess the importance of the metabolism of the islet cells for the control of their $K^+$ permeability and to search for a link between the changes in glucose metabolism and in membrane permeability to $K^+$. The modifications in the $K^+$ permeability of islet cells were evaluated by measuring the uptake of $^{42}K^+$ and by monitoring, in a perifusion system, the release of $^{86}Rb^+$ by isolated rat islets. The simultaneous measurement of $^{86}Rb^+$ and $^{42}K^+$ effluxes from islets preloaded with both isotopes has shown that $^{86}Rb^+$ is a valid and convenient substitute for $^{42}K^+$ to trace the qualitative changes in $K^+$ permeability in pancreatic islet cells (J.-C. Henquin, unpublished work).

## Experimental

### *Animals and solutions*

All experiments were made with islets isolated by collagenase digestion of the pancreas of fed male Wistar rats (275–325 g), killed 2.5 h after intraperitoneal injection of pilocarpine (20 mg/kg body wt.).

The medium utilized was a Krebs–Ringer bicarbonate buffer, pH 7.4, gassed with $O_2/CO_2$ (47:3), with the following ionic composition (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $KH_2PO_4$, 1.2; $NaHCO_3$, 25; supplemented with 0.5% (w/v) bovine serum albumin. Except for the sodium salts of pyruvate, lactate, succinate and glutamate, the test substances were added to the medium without iso-osmolar modification of NaCl content. When necessary, the pH of the medium was adjusted back to 7.4 after addition of the test substance. For uptake studies, 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] was added to the medium to prevent any change of pH in the small volumes of solutions used in these experiments.

### *Measurement of $^{86}Rb^+$ efflux*

The perifusion system utilized to monitor the efflux of $^{86}Rb^+$ from preloaded islets has been described previously (Henquin, 1977, 1978). After isolation, groups of 60–90 islets were incubated at 37°C for 2.5 h in 0.5 ml of medium containing 3 mM-glucose and 0.25 mM-$^{86}$RbCl (sp. radioactivity 450–680 mCi/mmol). They were then washed three times at room temperature with 5 ml of non-radioactive medium and transferred to the perifusion chambers. The flow rate of the system was 1.1 ml/min, the dead time 1 min and the temperature 37°C. $^{86}Rb^+$ in the effluent fractions, collected at 2 min intervals, and remaining in the islets at the end of the experiment was counted by the Cerenkov radiation (Henquin, 1978). When the test substance was coloured, the control collections were quenched with an appropriate amount of the substance, in order to count all samples with the same efficiency. For each collection interval, the fractional efflux of $^{86}Rb^+$ ($^{86}Rb^+$ released during time interval/$^{86}Rb^+$ remaining in tissue during that time interval) was calculated.

### *Measurement of $^{42}K^+$ uptake*

$^{42}K^+$ uptake by islet cells was measured in relation to that of [6,6′-$^3$H]sucrose as marker of the extracellular space. The incubation medium, the composition of which is given in the legend to Table 2, was layered on silicone oil (Versilube F50). After 10 or 60 min at 37°C, the islets (batches of seven) were separated from the radioactive medium by centrifugation through the oil. The radioactivity entrapped in the pellet was counted by liquid scintillation immediately after the experiment and 1 week later, when $^{42}K^+$ had decayed to such an extent (99.99%) that it no longer affected the recording of $^3$H counts. Other details of the method have been published previously (Henquin & Lambert, 1975).

### *Reagents*

The sources of the different reagents were as follows: D-glyceraldehyde and glycerol from Koch–Light Laboratories (Colnbrook, Bucks., U.K.); sodium glutamate and Hepes from BDH (Poole, Dorset, U.K.); glyceric acid, sodium succinate, phenazine methosulphate, t-butyl hydroperoxide and 3-isobutyl-1-methylxanthine from Aldrich Europe (Beerse, Belgium); pentobarbital and sodium L-lactate from Serva (Heidelberg, Germany); glucosamine and *N*-acetylglucosamine from Calbiochem (San Diego, CA, U.S.A.); mannoheptulose, 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, nicotinamide and sodium iodoacetate from Sigma Chemical Co. (St Louis, MO, U.S.A.), collagenase, dibutyryl cyclic AMP, reduced and oxidized glutathione, NADH and NADPH from Boehringer (Mannheim, Germany); Versilube F50 from General Electric Silicones Europe (Bergen op Zoom, Netherlands). Cholera toxin (lot 0673) was purified (Finkelstein & Lo Spalluto, 1970) and provided by Dr. R. A. Finkelstein, University of Texas, Southwestern Medical School, Dallas, TX, U.S.A. All other reagents were obtained from Merck A.-G. (Darmstadt, Germany). High-purity water (Milli-Q system; Millipore, Bedford, MA, U.S.A.) was used throughout. $^{86}$RbCl and [6,6′-$^3$H]sucrose were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.) and

$^{42}KCl$ from the Institut national des Radioéléments (Fleurus, Belgium).

*Presentation of results*

All results are presented as means ± S.D. and the statistical significance of differences between experimental groups was assessed by Student's *t* test for unpaired data.

## Results

*Effect of glucose on $^{86}Rb^+$ efflux*

In the absence of glucose, the rate of $^{86}Rb^+$ efflux from perifused islets declined slowly and regularly (Fig. 1*a*, dotted line). Addition of 10 mM-glucose to the medium resulted in a rapid and marked decrease in the efflux rate, which then remained fairly stable. The effect of the sugar was completely reversible on return to a glucose-free medium (Fig. 1*a*).

To investigate whether glucose must be metabolized by islet cells to reduce their $K^+$ permeability, the effect of the sugar on $^{86}Rb^+$ efflux was tested in the presence of different metabolic inhibitors. Mannoheptulose, iodoacetate and glucosamine (Ashcroft *et al.*, 1972; Zawalich *et al.*, 1978) as well as 2-deoxyglucose inhibit glucose metabolism and insulin release by pancreatic islets. There exists some controversy about the effects of 2-deoxyglucose on B-cell function. We have observed that, in islets incubated for 60 min in the presence of 16 mM-glucose, 10 mM-2-deoxyglucose inhibits insulin release by 60% and glucose utilization by 35%. As illustrated in Fig. 1(*b*), when added together with mannoheptulose or iodoacetate, glucose reduced $^{86}Rb^+$ efflux only transiently. The inhibitory effect of mannoheptulose was rapid and completely reversible after withdrawal of the drug, whereas that of iodoacetate appeared later and was not reversible. In another series of experiments shown in Fig. 2, the islets were first perifused in the presence of 7 mM-glucose alone; this resulted in a low stable rate of $^{86}Rb^+$ efflux. Addition of glucosamine produced a rapid but transient increase in the rate of efflux, and withdrawal of the substance was not followed by significant effects. By contrast, 2-deoxyglucose steadily and reversibly increased the rate of $^{86}Rb^+$ efflux (Fig. 2).

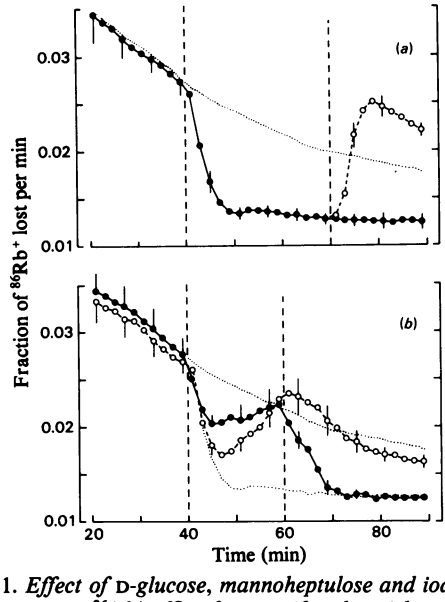


Fig. 1. *Effect of* D-*glucose, mannoheptulose and iodoacetate on $^{86}Rb^+$ efflux from perifused rat islets*

(*a*) The experiment was started in the absence of glucose, which was added at a concentration of 10 mM at 40 min and maintained until the end (●) or withdrawn at 70 min (○). The dotted line shows control experiments in the absence of glucose throughout. Values are means ± S.D. for six experiments until 70 min and for three experiments thereafter. (*b*) Glucose (10 mM) was added at 40 min, together with either 10 mM-mannoheptulose (●) or 1 mM-iodoacetate (○). Both inhibitors were withdrawn at 60 min. The upper dotted line shows the results of control experiments without glucose and inhibitors throughout; the lower dotted line shows the effect of 10 mM-glucose alone. Values are means ± S.D. for four experiments. The data are expressed as the fractional efflux rate, i.e. the fraction: $^{86}Rb^+$ lost per min/$^{86}Rb^+$ present in the tissue at the time of measurement. Displayed in this manner, efflux at a constant rate from a single compartment appears as a horizontal line.

*Effect of glucose metabolites on $^{86}Rb^+$ efflux*

A second approach to assess the importance of glucose metabolism for the reduction of $K^+$ permeability in islet cells was to determine whether metabolites of the hexose or agents 'feeding' into glyco-

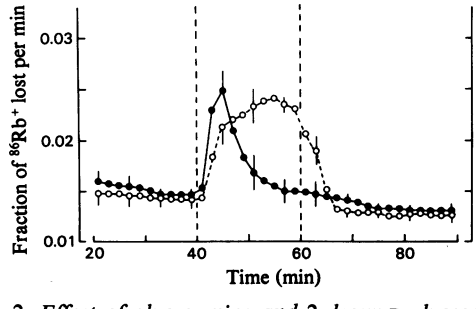


Fig. 2. *Effect of glucosamine and 2-deoxy-*D-*glucose on $^{86}Rb^+$ efflux from perifused rat islets*

The concentration of glucose was 7 mM throughout; glucosamine (●) and 2-deoxy-D-glucose (○) were added at a concentration of 15 mM between 40 and 60 min. Values are means ± S.D. for three experiments.

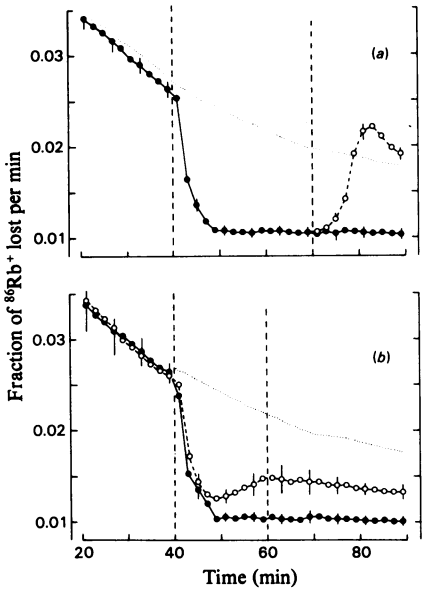


Fig. 3. *Effect of* D-*glyceraldehyde, mannoheptulose and iodoacetate on* $^{86}Rb^+$ *efflux from perifused rat islets*
(*a*) Glyceraldehyde (10 mM) was added at 40 min until 70 (○) or 90 min (●). The dotted line shows control experiments in the absence of glyceraldehyde throughout. Values are means ± S.D. for six experiments until 70 min and for three experiments thereafter. (*b*) Glyceraldehyde was added at 40 min, together with either 10 mM-mannoheptulose (●) or 1 mM-iodoacetate (○). Both inhibitors were withdrawn at 60 min. The dotted line shows results of control experiments in the absence of glyceraldehyde and inhibitors throughout. Values are means ± S.D. for four experiments.

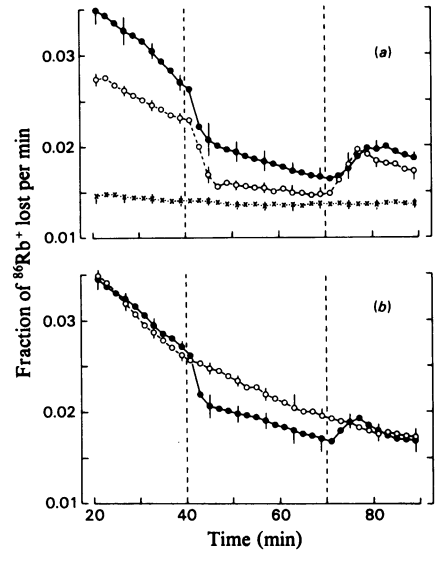


Fig. 4. *Effect of pyruvate, glyceric acid and glycerol on* $^{86}Rb^+$ *efflux from perifused rat islets*
(*a*) Pyruvate (15 mM) was added from 40 min until 70 min to a medium containing 0 (●), 3 (○) or 10 (×) mM-glucose. Values are means ± S.D. for three experiments. (*b*) Glyceric acid (15 mM) (●) or 15 mM-glycerol (○) was added from 40 min until 70 min to a medium containing no glucose. Values are means ± S.D. for three experiments.

lysis at the triose phosphate step could mimic its effects on $^{86}Rb^+$ efflux. D-Glyceraldehyde reversibly decreased and stabilized the efflux rate (Fig. 3*a*). At this concentration of 10 mM, the triose was slightly more potent than glucose itself: the fractional efflux rates at 60 min were $0.0135 \pm 0.0008$ and $0.0107 \pm 0.0005$ in the presence of glucose and glyceraldehyde respectively ($P < 0.001$, $n = 6$). A similar observation has been made as regards the insulin-releasing and fuel properties of the two agents. Iodoacetate, but not mannoheptulose (Zawalich *et al.*, 1978), inhibits the insulinotropic property of glyceraldehyde and the metabolism of the triose by the islets. As shown in Fig. 3(*b*), the reduction of $^{86}Rb^+$ efflux by glyceraldehyde was unaffected by mannoheptulose and partially impaired by iodoacetate. Yet, removal of this latter inhibitor was not followed by recovery of a complete effect of the triose. In addition to being phosphorylated, D-glyceraldehyde could theoretically be first reduced to glycerol or oxidized to glyceric acid (Bentler & Guinto, 1974). The rate of $^{86}Rb^+$ efflux from islet cells was unaffected by glycerol, but slightly and reversibly reduced by glyceric acid (Fig. 4*b*). Fig. 4(*a*) shows that pyruvate slightly and reversibly reduced $^{86}Rb^+$ efflux in absence of glucose and that its effect was stronger at 3 mM-glucose, but no longer detectable when the efflux rate was already markedly decreased by 10 mM-glucose.

### *Effect of other sugars and metabolites on* $^{86}Rb^+$ *efflux*

The ability of several sugars and metabolites to modify $^{86}Rb^+$ efflux from pancreatic islets was studied in experiments similar to those shown previously. The results are summarized in Table 1. Galactose, 3-*O*-methylglucose, 2-deoxyglucose and fructose were without significant effect in the absence of glucose, whereas mannose, glucosamine and *N*-acetylglucosamine potently reduced $^{86}Rb^+$ efflux. The effect of these three latter agents followed a time course (results not shown) similar to that of the effect of glucose and was completely reversible (Table 1). L-Lactate exhibited the same effects as those already described for pyruvate: it reduced $^{86}Rb^+$ efflux slightly in the absence of glucose and

Table 1. *Effect of various agents on the rate of $^{86}Rb^+$ efflux from perifused rat islets*

After loading with $^{86}Rb^+$ as described in the Experimental section, islets were perifused for 90 min in the absence (*a*) or in the presence of 3 mM-glucose (*b*). Test substances were added to the perifusion medium for 30 min (between 40 and 70 min). The efflux rate of $^{86}Rb^+$ is expressed as the percentage of label leaving the tissue/min. In each individual experiment the effect of the substance was calculated by the mean efflux rate between 58 and 62 min (18–22 min after addition of the substance). The reversibility of the effect is given as the highest efflux rate recorded after withdrawal of the substance; these values were obtained at different times. When a substance was without effect, the rate of $^{86}Rb^+$ efflux decreased regularly and no value is given for the 'after stimulation' period. Results are means ± S.D. for three experiments except when otherwise indicated in parentheses.

| | Efflux of $^{86}Rb^+$ (%/min) | |
|---|---|---|
| Test substance (mM) | During stimulation | After stimulation |
| (*a*) Glucose (0) | 2.19 ± 0.13 (6) | |
| Galactose (15) | 2.11 ± 0.10 | |
| 3-*O*-Methylglucose (15) | 2.18 ± 0.11 | |
| 2-Deoxyglucose (15) | 2.16 ± 0.10 | |
| Fructose (15) | 2.15 ± 0.05 | |
| Mannose (15) | 1.42 ± 0.08* | 2.80 ± 0.11 |
| Glucosamine (15) | 1.44 ± 0.07* | 2.47 ± 0.16 |
| *N*-Acetylglucosamine (15) | 1.52 ± 0.02* | 3.06 ± 0.20 |
| Pyruvate (15) | 1.79 ± 0.10* | 2.01 ± 0.05 |
| Lactate (15) | 1.85 ± 0.06* | 2.08 ± 0.02 |
| Glutamate (15) | 2.08 ± 0.03 | |
| Succinate (15) | 2.23 ± 0.09 | |
| NADH (2.5) | 2.09 ± 0.05 | |
| NADPH (1) | 2.08 (2) | |
| Cholera toxin (2 μg/ml) | 2.15 ± 0.12 | |
| 3-Isobutyl-1-methylxanthine (0.1) | 2.09 ± 0.07 | |
| 3-Isobutyl-1-methylxanthine (1) | 1.66 ± 0.03* (4) | 2.14 ± 0.04 |
| Theophylline (10) | 1.65 ± 0.13* | 2.28 ± 0.23 |
| Dibutyryl cyclic AMP (1) | 2.08 ± 0.01 | |
| (*b*) Glucose (3) | 1.93 ± 0.08 (6) | |
| Pyruvate (15) | 1.52 ± 0.08* | 1.97 ± 0.10 |
| Lactate (15) | 1.61 ± 0.08* | 2.05 ± 0.11 |
| Fructose (15) | 1.62 ± 0.04* | 1.98 ± 0.09 |

* Significantly different ($P < 0.005$) from appropriate controls without glucose or with glucose (3 mM).

potentiated the effect of 3 mM-glucose (Table 1), but was without effect in the presence of 10 mM-glucose (results not shown). Glutamate and succinate did not significantly modify the efflux rate of $^{86}Rb^+$. Although ineffective in the absence of glucose, fructose clearly and reversibly decreased the rate of $^{86}Rb^+$ efflux in the presence of 3 mM-glucose (Table 1), following a time course similar to that of the effect of pyruvate.

### *Mechanism of the effect of glucose on $^{86}Rb^+$ efflux*

The following experiments were designed to elucidate the metabolic events that could account for the observed changes in $K^+$ permeability. Attention was focused on reduced nicotinamide nucleotides and cyclic AMP, the concentrations of which rapidly increase in response to various insulinotropic agents (Panten *et al.*, 1973; Grill & Cerasi, 1974; Charles *et al.*, 1975).

NADH and NADPH added to a glucose-free medium (Table 1) and their precursor, nicotinamide, in the presence of 3 mM-glucose (Fig. 5*a*) were without effect on $^{86}Rb^+$ efflux. Pentobarbital (3 mM), which markedly increases NAD(P)H concentrations in islet cells (Panten *et al.*, 1973), rapidly and reversibly reduced $^{86}Rb^+$ efflux (Fig. 5*a*). The opposite effect was observed with phenazine methosulphate (5 μM) and Methylene Blue (10 μM), two substances that, at these concentrations, oxidize nicotinamide nucleotides without changing ATP concentrations and glucose metabolism through glycolysis (Ashcroft *et al.*, 1972; Ammon *et al.*, 1973). Both produced a progressive and slowly reversible increase in the rate of $^{86}Rb^+$ efflux when added to a medium containing 7 mM-glucose (Fig. 5*b*).

One possible function of NADPH being to keep glutathione in a reduced state (Flohé *et al.*, 1974), the effects of this tripeptide on $K^+$ permeability were tested. Addition of reduced glutathione to a glucose-

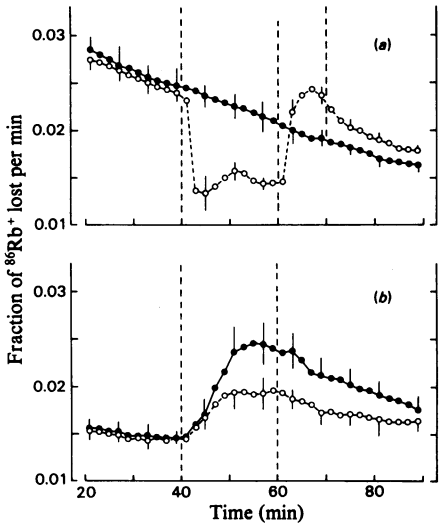


Fig. 5. *Effect of nicotinamide, pentobarbital, phenazine methosulphate and Methylene Blue on $^{86}Rb^+$ efflux from perifused rat islets*

(*a*) Nicotinamide (15 mM) (●) from 40 min until 70 min or 3 mM-pentobarbital (○) from 40 min until 60 min was added to a medium containing 3 mM-glucose. Values are means ± S.D. for three experiments. (*b*) 5 μM-Phenazine methosulphate (●) or 10 μM-Methylene Blue (○) was added for the period 40 to 60 min to a medium containing 7 mM-glucose. Values are means ± S.D. for four experiments.

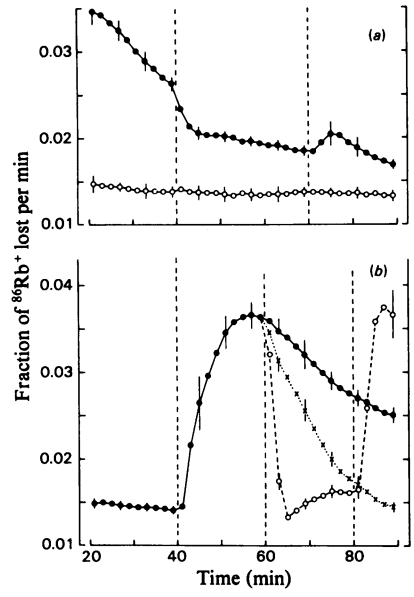


Fig. 6. *Effect of glutathione and t-butyl hydroperoxide on $^{86}Rb^+$ efflux from perifused rat islets*

(*a*) Reduced glutathione (10 mM) (●) or 5 mM oxidized glutathione (○) was added from 40 min until 70 min to a medium containing 0 or 7 mM-glucose respectively. Values are means ± S.D. for three experiments. (*b*) t-Butyl hydroperoxide (0.2 mM) was added for the period 40 to 90 min (●, ○) to a medium containing 7 mM-glucose; in one series of experiments (○) 3 mM-pentobarbital was also added between 60 and 80 min. In a further series of experiments (×), t-butyl hydroperoxide was added only from 40 until 60 min. Values are means ± S.D. for nine experiments until 60 min and for three experiments thereafter.

free medium produced a small reversible decrease in $^{86}Rb^+$ efflux (Fig. 6*a*), an effect not augmented by 3 mM-glucose (result not shown). Both in the absence of glucose (result not shown) and at 7 mM-glucose (Fig. 6*a*) oxidized glutathione was without effect. Since glutathione poorly permeates cell membranes (Flohé *et al.*, 1974), t-butyl hydroperoxide was used to oxidize intracellular reduced glutathione (Flohé *et al.*, 1974; Ammon *et al.*, 1978). Its addition to a medium containing 7 mM-glucose caused a prompt and important increase in $^{86}Rb^+$ efflux (Fig. 6*b*). This effect was reversible when the peroxide was removed. In addition, pentobarbital immediately and reversibly counteracted the increase in efflux produced by t-butyl hydroperoxide (Fig. 6*b*).

It should be underlined, however, that high concentrations of pentobarbital (10 mM and higher) no longer decreased, but considerably increased, the efflux of $^{86}Rb^+$ from perifused islets (results not shown), probably because of the prevailing shortage of ATP. This is supported by the increase in $K^+$ permeability observed with mitochondrial uncouplers. For instance, 0.1 mM-dicumarol in the presence of 7 mM-glucose augmented the rate of $^{86}Rb^+$ efflux from $0.0158 \pm 0.0010$ to $0.0341 \pm 0.0019$ ($n = 3$) after 20 min.

To evaluate a possible role of cyclic AMP, dibutyryl cyclic AMP was added to the medium or the concentrations of the endogenous nucleotide were modified (Montague & Howell, 1975) by stimulating the adenylate cyclase with cholera toxin, by inhibiting the phosphodiesterase with methylxanthines and by activating the phosphodiesterase with imidazole. In the absence of glucose, cholera toxin (Table 1) and dibutyryl cyclic AMP (Fig. 7*a*) did not significantly affect $^{86}Rb^+$ efflux. On the contrary, a high concentration of theophylline (10 mM) reversibly reduced $^{86}Rb^+$ efflux after an initial short-lived increase (Fig. 7*a*); a similar effect was observed with 1 mM-3-isobutyl-1-methylxanthine (Table 1). Surprisingly, when the rate of $^{86}Rb^+$ efflux was decreased by 7 mM-glucose, 2 mM-theophylline (Fig. 7*b*) and 0.1 mM-3-isobutyl-1-methylxanthine (result not shown), concentrations which markedly potentiate the insulin-releasing effect of the sugar, produced a transient increase in $^{86}Rb^+$ loss; such an

effect was not seen with cholera toxin. Imidazole also promptly, but more steadily, augmented the efflux rate in the presence of 7 mM-glucose (Fig. 7*b*).

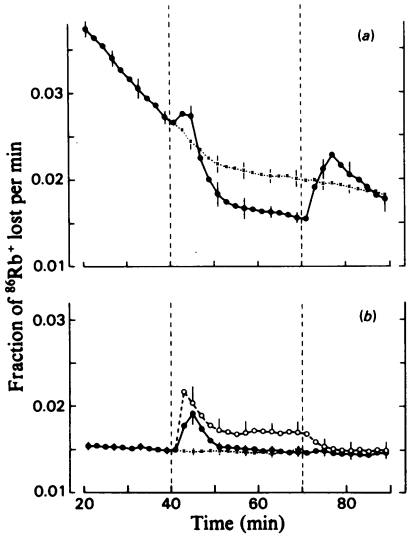


Fig. 7. *Effect of modifiers of cyclic AMP concentrations on $^{86}Rb^+$ efflux from perifused rat islets*

(*a*) 10 mM-Theophylline (●) or 1 mM-dibutyryl cyclic AMP (×) was added between 40 and 70 min to a medium containing no glucose. Values are means ± S.D. for six experiments until 40 min and for three experiments thereafter. (*b*) Theophylline (2 mM) (●), 10 mM-imidazole (○) or 2 μg of cholera toxin/ml (×) were added between 40 and 70 min to a medium containing 7 mM-glucose. Values are means ± S.D. for nine experiments until 40 min and for three experiments thereafter.

*Effect of glucose and other agents on $^{42}K^+$ uptake by islet cells*

The uptake of $^{42}K^+$ by islet cells was measured after 10 min of incubation to evaluate the influx of the cation and after 60 min to estimate the net uptake under conditions approaching equilibrium. Ouabain (0.1 mM) inhibited $K^+$ uptake by 20 and 50% after 10 and 60 min of incubation respectively (Table 2). Glucose, glyceraldehyde, pyruvate and 3-isobutyl-1-methylxanthine had no effect after 10 min. After 60 min, 10 mM-glucose increased $K^+$ uptake by about 50%; this effect was blocked by mannoheptulose and was mimicked by glyceraldehyde but not by 3-*O*-methylglucose. Pyruvate and 3-isobutyl-1-methylxanthine also increased $K^+$ uptake after 60 min, but less markedly than glucose (Table 2).

## Discussion

The present study conclusively establishes the importance of the metabolic degradation of glucose and other substrate molecules for the control of $K^+$ permeability in pancreatic islet cells. Although the present flux measurements were made in the heterogeneous population of the whole islets, their confrontation with insulin release and electrical recordings made in B-cells is instructive.

*Characteristics of the membrane change*

After short incubation (10 min), glucose did not affect $K^+$ uptake by islet cells, whereas ouabain reduced it. This is in agreement with the previous conclusion, based on $^{86}Rb^+$ uptake studies (Sehlin & Täljedal, 1974), that glucose does not markedly change the activity of the $Na^+/K^+$ pump. As previously reported (Howell & Taylor, 1968; Malaisse *et al.*, 1978*a*), glucose increased $^{42}K^+$ uptake after

Table 2. *Effect of various agents on $K^+$ uptake by rat islet cells*

After 30 min of preincubation in the absence of glucose, batches of seven islets were transferred into 100 μl of incubation medium containing the indicated substances, 6 mM-$^{42}$KCl (sp. radioactivity 4–8 mCi/mmol) and 0.25 mM-[6,6′-$^3$H]sucrose (sp. radioactivity 0.1 Ci/mmol). The medium was layered on silicone oil and, after 10 or 60 min of incubation at 37°C, the tissue was separated from the radioactive solution by centrifugation through the oil. Values are means ± S.D. for the numbers of batches of islets given in parentheses. Significance levels versus appropriate controls with no glucose present are indicated as follows: *, $P < 0.001$; †, $P < 0.01$; ‡, $P < 0.05$.

| Test substance (mM) | $K^+$ uptake (pmol/islet) 10 min | 60 min |
|---|---|---|
| Glucose (0) | 204 ± 29 (18) | 356 ± 53 (20) |
| Glucose (10) | 202 ± 22 (14) | 529 ± 56 (16)* |
| Glucose (10) + mannoheptulose (10) | — | 390 ± 57 (10) |
| Glyceraldehyde (10) | 195 ± 18 (11) | 519 ± 76 (11)* |
| Pyruvate (15) | 211 ± 15 (10) | 423 ± 57 (10)† |
| 3-*O*-Methylglucose (15) | — | 369 ± 42 (10) |
| 3-Isobutyl-1-methylxanthine (1) | 221 ± 24 (10) | 412 ± 63 (10)‡ |
| Ouabain (0.1) | 165 ± 20 (12)* | 174 ± 28 (11)* |

longer incubation (60 min). Mannoheptulose prevented this increase as well as the decrease in $^{86}Rb^+$ efflux normally produced by glucose. The ability of other agents to augment $^{42}K^+$ uptake after 60 min also correlated with their ability to decrease the rate of $^{86}Rb^+$ efflux. It seems therefore that the primary effect of glucose and the other substances is to decrease the passive permeability of the membrane to $K^+$ ions. This results in a secondary increase in the size of the $K^+$ pool, which could be important in the long-term regulation of the B-cell function. Thus, it has been reported that omission of extracellular $K^+$ causes a delayed inhibition of glucose metabolism and insulin release by isolated islets (Henquin & Lambert, 1974).

*Correlations with electrical effects in B-cells*

Not all agents tested in this report have been examined for their electrical effects in B-cells. It has been shown (Dean *et al.*, 1975), however, that, besides glucose, mannose and glyceraldehyde produce depolarization and electrical activity, whereas galactose, 3-*O*-methylglucose, 2-deoxyglucose, glycerol and fructose alone are ineffective. Mannoheptulose, iodoacetate and mitochondrial uncouplers suppress glucose-induced electrical activity and hyperpolarize B-cells (Dean *et al.*, 1975). It is thus striking that the substances which reduce $K^+$ permeability depolarize B-cells, that those which increase $K^+$ permeability hyperpolarize B-cells and that those without effect on $^{86}Rb^+$ efflux do not change the membrane potential. These correlations reinforce the previous evidence (Henquin, 1977, 1978; Henquin & Meissner, 1978; Henquin *et al.*, 1979) that the stimulus-induced decrease in $K^+$ permeability mediates the depolarization of pancreatic B-cells.

*Correlations with insulin release and islet metabolism*

There is now general agreement that only metabolized sugars stimulate insulin release, provided their metabolic degradation is not impaired. This report shows that all metabolized sugars tested decrease the $K^+$ permeability of islet cells, that inhibitors of their metabolism antagonize this effect and that non-metabolized sugars are without effect. The possibility that the changes in $K^+$ efflux simply reflect changes in secretion is ruled out by several lines of evidence: the decrease in $K^+$ efflux produced by glucose persists when insulin release is blocked by $Ca^{2+}$ omission (Henquin, 1978) or addition of cobalt (J.-C. Henquin, unpublished work); low non-insulinotropic concentrations of the sugar markedly change $K^+$ efflux (Henquin, 1978); 3 mM-pentobarbital decreases $K^+$ permeability (Fig. 5*a*), while inhibiting insulin release (Panten *et al.*, 1973).

The effects of glucosamine are noteworthy. Glucosamine is metabolized by the islets and stimulates insulin release (Ashcroft *et al.*, 1973), but it also inhibits glucose metabolism and glucose-stimulated release (Ashcroft *et al.*, 1972). A dual effect is also found in these experiments: in the presence of a stimulating concentration of glucose, glucosamine increases $^{86}Rb^+$ efflux, but this effect is transient, probably because glucosamine itself reduces the $K^+$ permeability, as seen in the absence of glucose. The effects of pyruvate, lactate and fructose also deserve a comment. They potentiate the insulinotropic action of glucose, but are not or are very weak, initiators of insulin secretion (Ashcroft *et al.*, 1973; Sener *et al.*, 1978). The evidence that the three agents augment the effect of glucose on $K^+$ permeability, while having no or only a weak effect alone, is again in agreement with the above findings.

*Role of reduced nicotinamide nucleotides*

Despite intensive investigation, the precise links between the metabolism of glucose or other stimuli and the release of insulin have not been unequivocally identified. Various factors have been proposed to play the role of messenger.

Several authors (Watkins *et al.*, 1971; Ammon *et al.*, 1973; Malaisse *et al.*, 1978*b*) have emphasized the possible role of reduced nicotinamide nucleotides, the concentrations of which rapidly increase in islet cells stimulated with glucose (Panten *et al.*, 1973). The present data show that a reduced redox state of islet cells is attended by a decrease in $K^+$ permeability, whereas a shift to a more oxidized state increases $K^+$ permeability. There are several mechanisms by which reduced nicotinamide nucleotides could modulate the membrane permeability to $K^+$. They might directly affect the gating mechanism of the $K^+$ channels. Alternatively, as originally proposed by Hellman *et al.* (1974*b*), the membrane cationic permeability could be controlled by a thiol–disulphide system, kept in a reduced state by reduced glutathione. Thus, islet cells contain high concentrations of glutathione (Havu, 1969) and a high activity of glutathione reductase (Berne, 1975). The slight decrease in $^{86}Rb^+$ efflux by exogenous reduced glutathione, as well as the increase observed with t-butyl hydroperoxide, could support this view. However, even if reduced glutathione is specifically oxidized by this peroxide (Flohé *et al.*, 1974; Ammon *et al.*, 1978), NADPH depletion will likely ensue. In addition, the correction, by pentobarbital, of the increase in $^{86}Rb^+$ efflux produced by t-butyl hydroperoxide can result from the restoration of high NAD(P)H concentrations or the secondary reduction of oxidized glutathione by NADPH.

*Role of cyclic AMP*

Glucose stimulation of the islets increases their content of cyclic AMP (Grill & Cerasi, 1974;

Charles *et al.*, 1975). Furthermore, there exists a parallelism between the effects of various sugars on insulin release and islet cyclic AMP levels (Grill & Cerasi, 1976). In the light of the preceding discussion, it is thus not surprising that similar correlations can be drawn between the changes in $K^+$ permeability and in cyclic AMP content of the islet cells. However, the differences appear more important. Unlike the decrease in $K^+$ efflux (Henquin, 1978), the increase in cyclic AMP (Charles *et al.*, 1975; Zawalich *et al.*, 1975) in response to glucose requires the presence of extracellular $Ca^{2+}$. The concentrations of cyclic AMP do not change below 6mM-glucose (Grill & Cerasi, 1974), whereas the major reduction in $K^+$ efflux occurs between 3 and 6mM-glucose (Henquin, 1978). The effect of glucose on $K^+$ permeability cannot be produced by dibutyryl cyclic AMP nor by large increases in cellular cyclic AMP concentrations (Hellman *et al.*, 1974*a*) with cholera toxin or 0.1mM-3-isobutyl-1-methylxanthine. In the presence of glucose, both the stimulation and the inhibition of the phosphodiesterase slightly increase $^{86}Rb^+$ efflux. Finally, it cannot be excluded that the decrease in $K^+$ permeability produced by high concentrations of methylxanthines is a direct effect of the drugs on the membrane. Previous reports have already suggested that certain effects of methylxanthines in the islets are not mediated by cyclic AMP (McDaniel *et al.*, 1977; Sugden & Ashcroft, 1978). Taken as a whole, these considerations tend to rule out that the physiological changes in $K^+$ permeability of islet cells are secondary to variations in the concentrations of cyclic AMP.

*Role of ATP*

Inhibition of oxidative phosphorylations in the islets suppresses stimulation of insulin release by glucose and other agents (Coore & Randle, 1964). The major increase in ATP concentrations in islet cells (Hellman *et al.*, 1969; Ashcroft *et al.*, 1973), as well as the major decrease in $K^+$ efflux (Henquin, 1978), occurs when the glucose concentration is raised from 0 to 5mM. A decrease in cellular ATP, as produced by high concentrations of pentobarbital or by dicumarol increases $^{86}Rb^+$ efflux, an effect which may explain the hyperpolarization observed in similar conditions (Dean & Matthews, 1970). However, there are so many energy-requiring processes involved in the B-cell response to stimulation that it cannot be inferred, from the present data that ATP exerts any direct control (e.g. phosphorylation of membrane proteins) on $K^+$ permeability. Indeed, it has recently been observed that islet cells possess a $Ca^{2+}$-sensitive $K^+$-permeability system and that this system is activated by liberation of $Ca^{2+}$ from cellular stores (Henquin, 1979).

*Conclusions*

The $K^+$ permeability of pancreatic islet cells appears tightly controlled by their metabolic activity. Particularly, stimulation by glucose and other insulin-releasing substrates leads to a decrease in $K^+$ permeability. This effect requires the metabolic degradation of the stimulus and seems to result, at least in part, from an increase in the concentrations of reduced nicotinamide nucleotides and possibly also of reduced glutathione. A direct role for ATP in the control of the $K^+$ permeability cannot be inferred, but cyclic AMP does not seem to be involved. If one accepts that the present studies satisfactorily reflect the permeability changes in B-cells and link them to electrophysiological recordings and measurements of insulin release, there remains little doubt that the control of $K^+$ permeability of the B-cell membrane represents a fundamental step in the physiological stimulus–secretion coupling. This step follows the metabolic recognition of the secretagogue, underlies the depolarization of the membrane and precedes (causes) the remodelling of the transmembrane $Ca^{2+}$ fluxes.

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