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Efflux of ^{86}Rb from rat and mouse pancreatic islets: the role of membrane depolarization

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1 The efflux of ^{86}Rb from rat or mouse perifused islets preloaded with the isotope has been used as an index of the potassium permeability of the islet β-cell membrane. Cellular transmembrane potentials were altered by changing $[K]_o$ or by direct electrical stimulation and the effects on potassium permeability examined.

2 Omission of KCl from the medium perifusing rat islets induced a biphasic change in ^{86}Rb efflux, a brief decline being superseded by a pronounced increase in efflux. Re-introduction of KCl, 4.7 mM, caused a further increase in ^{86}Rb efflux preceding a return to control values.

3 Increasing $[K]_o$ from 4.7 mM to 10 mM, 20 mM or 47 mM caused a phasic increase in ^{86}Rb efflux with the magnitude of both the peak and average rate of efflux being dependent upon the extent of the change in $[K]_o$.

4 The increase in ^{86}Rb efflux produced by $[K]_o$, 47 mM, was attenuated by Co^{2+}, 2.56 mM (51% inhibition) or quinine, 10 μM (47% inhibition), but efflux remained significantly ($P<0.001$) above control values.

5 Electrical stimulation of single microdissected mouse pancreatic islets by currents of 0.1 to 0.5 mA evoked a rapid, phasic increase in ^{86}Rb efflux. The magnitude of the response was unaffected by EGTA, 2 mM, or nupercaine, 100 μM.

6 These observations are discussed in relation to the mechanisms controlling the potassium permeability, membrane potential and insulin secretion of the pancreatic islet β-cell. It is concluded that β-cell depolarization by a raised $[K]_o$ increases potassium permeability and efflux by at least two mechanisms: (i) a calcium-dependent potassium efflux triggered by an increase in $[Ca]_i$ and (ii) an activation of voltage-sensitive potassium channels which occurs even when the calcium-dependent potassium permeability is blocked.

Introduction

The importance of islet cell electrical behaviour in the genesis of the insulin release signal is now well recognised. Following the demonstration (Dean & Matthews, 1968; 1970a) that agents stimulating the secretion of insulin, such as D-glucose, induce depolarization and electrical spiking activity in pancreatic β-cells, it was subsequently shown that the spikes are due to the gated entry of calcium through voltage-dependent channels, and can be inhibited by the addition of antagonists of calcium entry such as Mn^{2+} and D-600 (Dean & Matthews, 1970b; Matthews & Sakamoto, 1975) or Co^{2+} (Ribalet & Beigelman, 1980). These spikes are presumed to be secondary to the depolarization induced by D-glucose, since Sehlin & Täljedal (1974, 1975) observed that D-glucose markedly reduces the rate of loss of ^{42}K or ^{86}Rb from islets prelabelled with these isotopes, apparently by a decrease in the potassium permeability (P_K) of the islet-cell membrane. The resting transmembrane potential of the β-cell depends primarily on P_K and the potassium gradient across the membrane (Dean & Matthews, 1970b; Atwater *et al.*, 1978; Meissner *et al.*, 1978), and a decrease in islet membrane P_K will depolarize the β-cell membrane. The measurement of ^{86}Rb efflux from preloaded islets may be used to monitor changes in islet β-cell P_K because changes in ^{86}Rb

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efflux follow the same pattern as those of ^{42}K and β-cells are the predominant islet constituent (Boschero *et al.*, 1977; Malaisse *et al.*, 1978; Henquin, 1978a,b).

Although it is believed that D-glucose, by reducing the islet β-cell potassium permeability, generates the slow phase of depolarization leading to spike activity, the effect of depolarization itself on islet cell P_K has not been closely examined. Islet cell depolarization, induced by increasing the external potassium concentration, causes a phasic burst of insulin release (Gomez & Curry, 1973; Henquin & Lambert, 1974a), whilst total removal of external potassium causes a progressive attenuation of glucose-stimulated insulin release (Henquin & Lambert, 1974b). An incidental observation has been that increasing the extracellular potassium concentration, $[K]_o$, causes a phasic increase, and decreasing $[K]_o$ causes a reduction, in ^{86}Rb efflux from prelabelled islets (Boschero & Malaisse, 1979).

To investigate further the relationship between islet cell transmembrane potential and islet-cell P_K we have now examined in detail the changes in ^{86}Rb efflux, and the action of pharmacological agents upon them, which result from systematic alterations of $[K]_o$. We have also developed a new perifusion system which allows the efflux of ^{86}Rb to be measured from a single isolated islet whilst permitting simultaneous electrical stimulation via surface electrodes. A brief preliminary account of some of this work has been given (Matthews & Shotton, 1983).

Methods

Multi-channel perifusion experiments

Islets were isolated from male Sprague-Dawley rats (180–300g) by a modification of the collagenase digestion procedure of Moskalewski (1965). Briefly, the rats were killed, exsanguinated, and the pancreas dissected free. Pancreases were chopped finely, rinsed in Krebs solution (composition given below), and digested at 37°C for between 6 and 12 min in a solution of 40 mg collagenase in 5 ml Krebs solution. The resulting suspension was washed five times, and the isolated islets harvested under a binocular microscope using a siliconized pasteur pipette. Groups of 50 islets were placed in each of 6 siliconized glass tubes and incubated for one hour in Krebs solution, 1 ml, containing a total activity of $^{86}RbCl$ of between 350 KBq and 1.8 MBq per tube. After incubation the islets were washed three times in Krebs solution before transfer to glass perfusion chambers, of approx. volume 0.25 ml, incorporating sintered glass filters to support the islets and perifused as described by Matthews & Shotton (1984).

Single-islet perifusion system

Islets were isolated by microdissection from a mouse pancreas pinned onto a block of black Silgard (Dow Corning) under a binocular microscope. Groups of 3 islets were incubated for 1 h in Krebs solution containing sufficient $^{86}RbCl$ to maintain the activity at between 1.4 and 4.4 MBq per tube. After incubation the islets were washed three times and transferred to a watch glass. Under a binocular microscope 1 islet was selected and attached by gentle suction (from a 5 ml syringe) to a blunt solution-filled micropipette of small tip size (approximately 100–200 μm diameter). The micropipette was mounted on a micromanipulator such that the islet was supported 2 mm above the surface of a plastic block. Krebs solution at room temperature (22°C) was pumped up through an orifice in the centre of the block to form a drop surrounding the islet. Excess fluid was drawn off above the islet through another micropipette with a bevelled tip connected to a second channel of the perifusion pump (Desaga PLG). In this manner a drop of fluid of fixed size could be maintained around the islet during continuous perifusion. Trains of biphasic pulses were applied to platinum wire electrodes from two stimulators (Grass Instruments SD5) driving constant current sources (Grass Instruments CCU1A). One platinum electrode was located within the suction micropipette itself and the other was placed in the drop of Krebs solution surrounding the islet.

Measurement of ^{86}Rb efflux

Efflux of rubidium was estimated by measuring the Cerenkov radiation emitted from samples of the perifusate in a standard scintillation spectrometer (Nuclear Enterprises model 8312). 7-Amino-1,3-naphthalene disulphonic acid (ANDA) 5 mM was added to increase the detection efficiency. After subtracting the background radiation the rate coefficient of rubidium efflux was calculated for each collection period as

$$\text{rate coefficient } (\text{min}^{-1}) = \frac{\Delta_x}{\Delta_t . x_t}$$

Where Δ_x is the amount of radioactivity collected in each collection interval, Δ_t is the length of the interval, and x_t is the total amount of rubidium released up until the start of the interval Δ_t.

Solutions used

All islets, whether isolated by the collagenase technique or by microdissection, were perifused in a Krebs-Henseleit medium of the following ionic composition (mmol l^{-1}): NaCl 118, $CaCl_2$ 2.56; KCl 4.7;

$MgCl_2$ 1.13, NaH_2PO_4 1.15, $NaHCO_3$ 25, supplemented with 2.8 mM D-glucose and, after islet isolation, by 0.5g $100ml^{-1}$ bovine serum albumin. Solutions were made freshly each day, and gassed with 95% O_2 5% CO_2 before and during each experiment, giving a final pH of 7.4. Except during perifusion of a single islet, which was performed at room temperature, all experiments were conducted at 37°C maintained by a thermostatically controlled water bath. The low concentration of 2.8mM D-glucose present throughout all experiments was selected to minimize any depolarizing or insulin releasing action of D-glucose itself.

All chemicals were of 'Analar' or comparable quality. ANDA was obtained from the Aldrich Chemical Co., collagenase from Boehringer Mannheim, ^{86}Rb from Amersham International and albumin (bovine serum, fraction V) from Sigma.

Statistical analysis

Results are expressed as the mean ± standard error (s.e.). Statistical significance was determined by application of Student's *t* test.

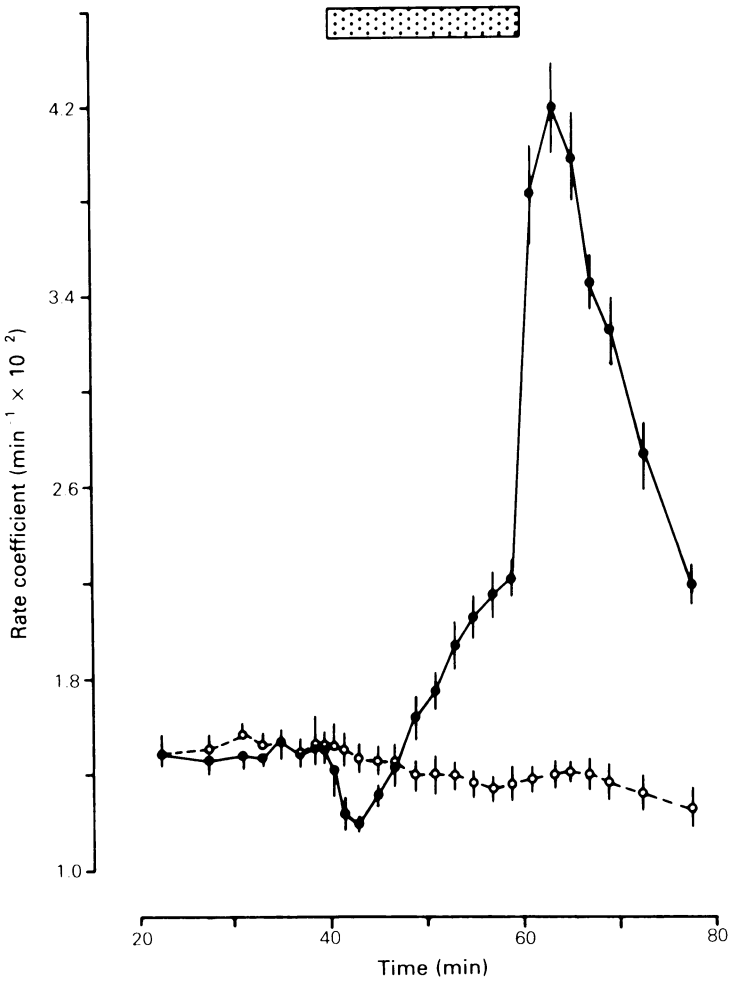

Figure 1 Effects of reducing $[K]_o$ from 4.7 mM on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. The plotted points are means ± s.e.mean. Control islets (open circles, $n = 4$) were perifused throughout with Krebs solution containing 2.8 mM D-glucose. During the period indicated by the shaded bar the test islets (filled circles, $n = 7$) were perifused with Krebs solution from which KCl had been omitted. Significant differences between control and test values existed from the 42nd min to the 45th min ($P < 0.05$) and from the 49th min to the end of the experiment ($P < 0.001$).

Results

Effects of changing the extracellular potassium concentration, $[K]_o$

Figure 1 illustrates the changes which occurred in ^{86}Rb efflux when groups of 50 islets were exposed for 20 min to Krebs solution without KCl. Upon removal of KCl a transient but significant ($P<0.001$) decrease in ^{86}Rb efflux occurred to a level 20% lower than the pre-stimulatory level. Efflux then increased rapidly to a peak more than 60% above the control rate. Readmission of KCl 4.7 mM evoked a further increase in efflux before the rate of loss of ^{86}Rb returned towards control values. At the end of the experiment efflux was still significantly higher than control levels.

In contrast, the effects of a stepwise increase in external potassium concentration, from 4.7 mM to 10, 20 or 47 mM, are illustrated in Figure 2. For all

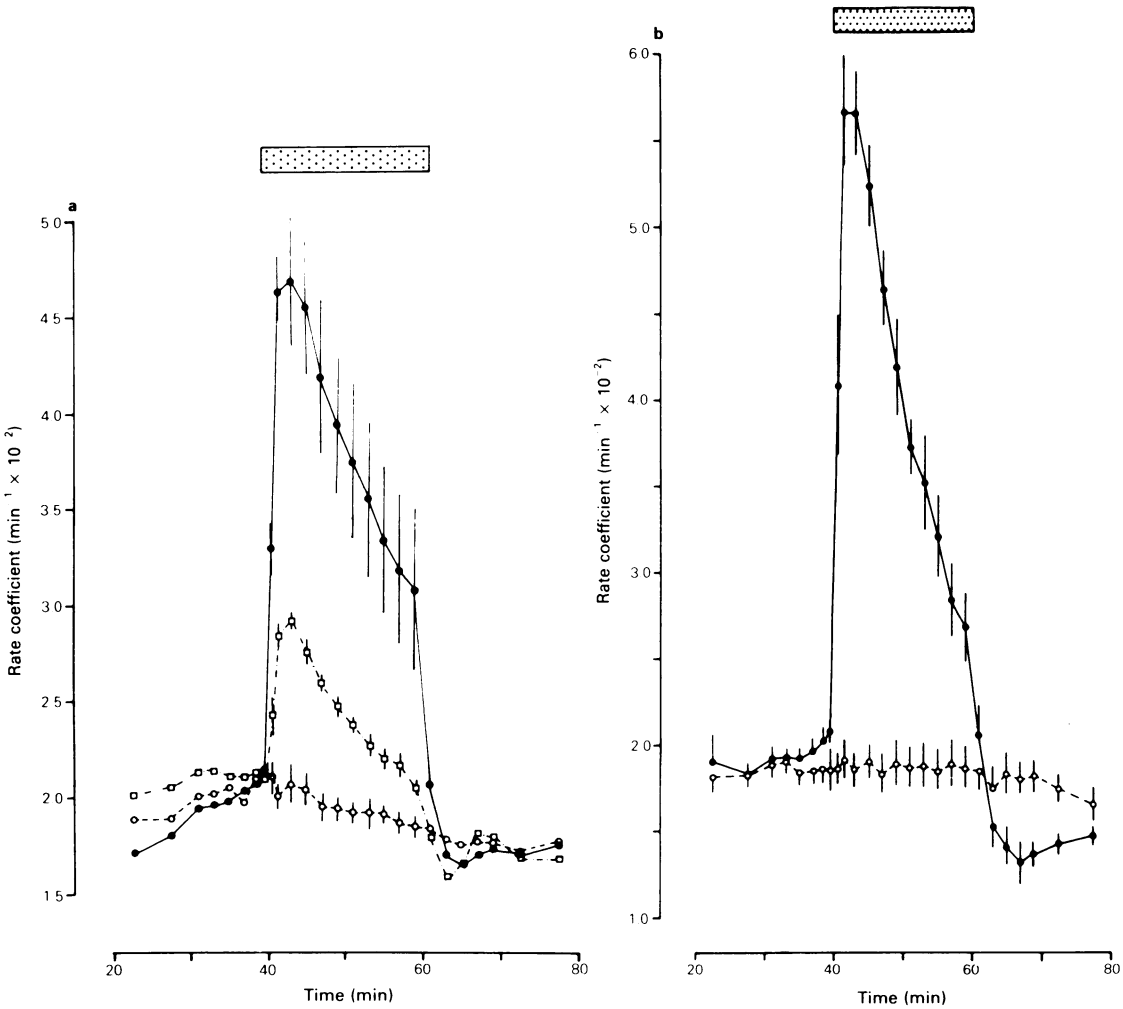

Figure 2 Effects of increasing $[K]_o$ from 4.7 mM to (a) 10 mM, 20 mM, or (b) 47 mM, on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. The plotted points are means ± s.e. mean. Control islets (open circles, $n=4$, (a), or $n=5$, (b)) were perifused throughout with Krebs solution containing 2.8 mM D-glucose. During the period indicated by the shaded bar the test islets were perifused with Krebs solution containing (a) KCl 10 mM (open squares, $n=8$) or KCl 20 mM (filled circles, $n=6$), or (b) KCl, 47 mM (filled circles, $n=7$). Significant differences ($P<0.05$) between control and test values existed in (a) from the 42nd min to the 45 min and ($P<0.001$) from the 49th min to the end of the stimulatory period and in (b) from the 41st to the 59th min ($P<0.01$) and from the 65th to the 72nd min ($P<0.05$). In (a) the standard error bars from 20 to 40 min and 60 to 80 min are omitted for clarity.

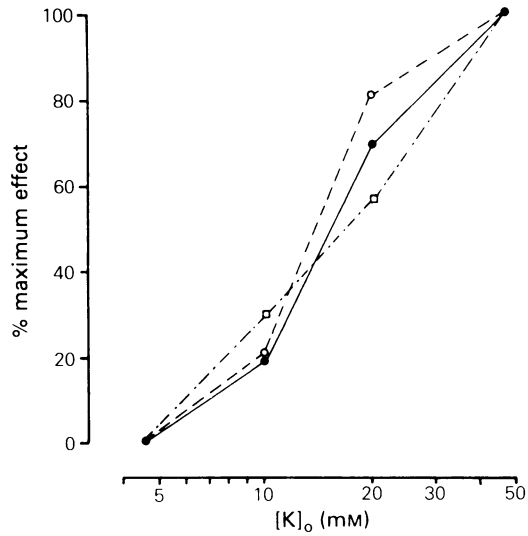

Figure 3 Relationship between $[K]_o$ and the peak (filled circles) and mean (open circles) increase in ^{86}Rb efflux, and the change in membrane potential (open squares). The % maximum effect is calculated as the change produced by the given concentration of KCl × 100 divided by the change produced by KCl 47 mM. The mean rate of ^{86}Rb efflux during the test period was found by dividing the area under the efflux/time curve during the test period by the duration of the test period. Membrane potential data are from Meissner *et al.*, (1978) and ranged from −63 mV in KCl 4.7 mM to −18 mV in KCl 47 mM.

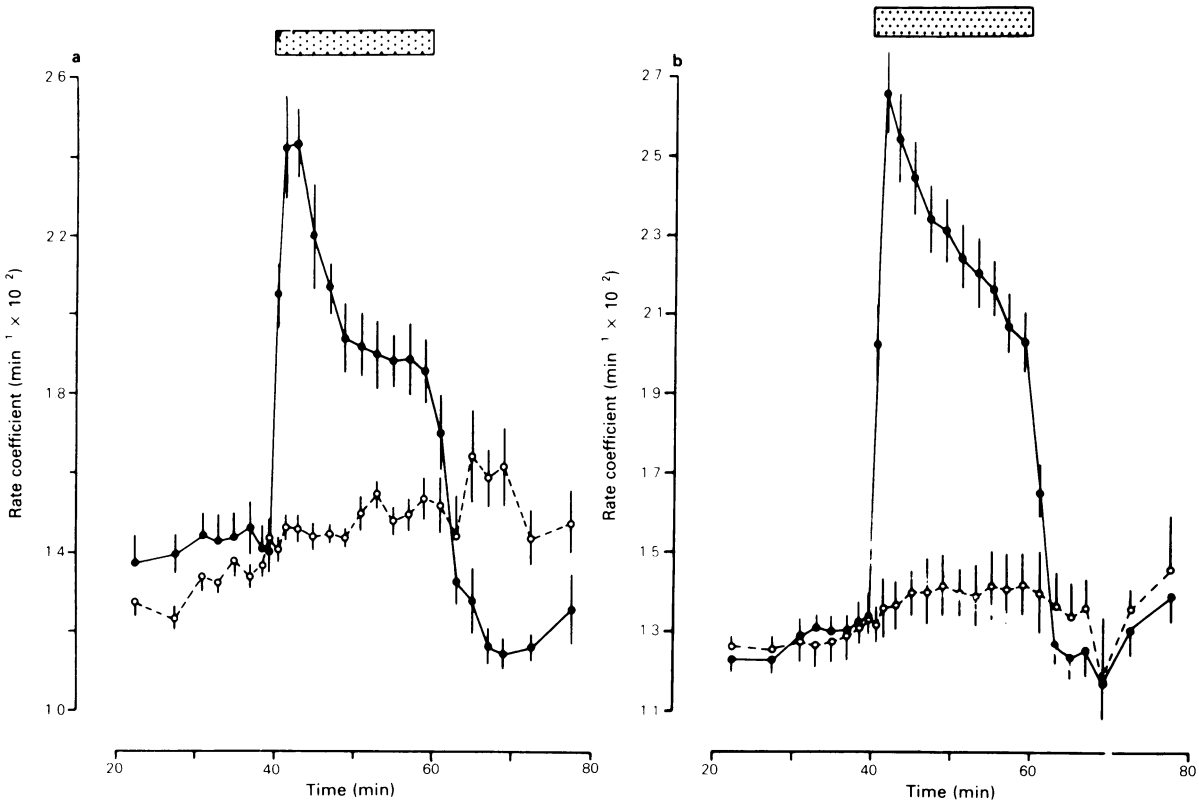

Figure 4 Effects of $[K]_o$ 47 mM in the presence of (a) cobalt 2.56 mM or (b) quinine 10^{-5}M on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. The plotted points are means ± s.e. mean. In (a), both control (open circles, $n = 5$) and test islets (filled circles, $n = 7$) were perifused throughout with Krebs solution in which $CaCl_2$ was replaced by $CoCl_2$ 2.56 mM and NaH_2PO_4 was omitted to prevent the precipitation of cobalt salts. In (b), both control (open circles, $n = 5$) and test islets (filled circles, $n = 11$) were perifused throughout with Krebs solution containing quinine 10^{-5}M. During the period indicated by the shaded bar the test solution contained KCl 47 mM. Significant differences ($P < 0.005$) between control and test values existed in (a) from the 41st to the 57th min and from the 67th to the 73rd min, and in (b) from the 41st to the 59th min ($P < 0.001$).

three increased potassium concentrations a phasic increase in the rate of rubidium efflux was seen, an initial marked increase in efflux being followed by an exponential decline. Upon restoration of $[K]_o$ to the normal concentration of 4.7 mM, efflux returned rapidly to control values, with a statistically significant ($P<0.001$) undershoot occuring after removal of KCl 47 mM.

The peak and average rates of rubidium efflux during the stimulatory period were linearly related to log $[K]_o$ (Figure 3), and the magnitude of each rate showed a similar dependency upon the membrane potential measured at increasing external concentrations of potassium by Meissner *et al.*, (1978).

The involvement of a calcium-dependent potassium permeability in the generation of these effects is implicated by the pronounced attenuation of the response to KCl 47 mM in the presence of cobalt, 2.56 mM, an inhibitor of voltage-dependent calcium entry in β-cells (Ribalet & Beigelman, 1981) which decreased efflux by 51% (Figure 4a) and by the effect of quinine, 10 μM, a compound that inhibits the calcium-dependent activation of potassium permeabilities in many tissues including pancreatic islets (Atwater *et al.*, 1979) and which decreased ^{86}Rb efflux by 47%. Both agents also decreased the basal level of ^{86}Rb efflux but it should be noted that, although cobalt and quinine attenuated the response to depolarization with KCl 47 mM, stimulated efflux remained significantly above basal values ($P<0.001$).

Electrical stimulation of isolated pancreatic islets

The above findings demonstrate that the rate of ^{86}Rb efflux, and hence the islet β-cell P_K, is profoundly influenced by manipulation of the islet cell transmembrane potential through alteration of the external potassium concentration. Direct electrical manipulation of the cell membrane potential would obviously offer some advantage over indirect procedures involving the alteration of the extracellular potassium concentration. A perifusion system was therefore designed which permitted direct electrical stimulation of a single perifused islet simultaneously with the measurement of ^{86}Rb efflux.

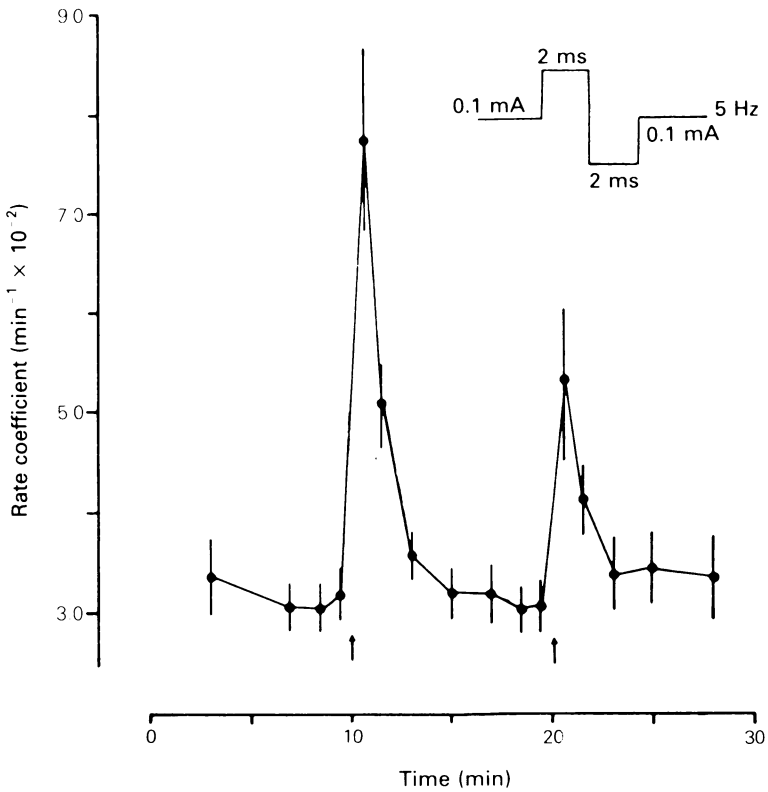

Figure 5 Effect of electrical stimulation on the coefficient of ^{86}Rb efflux from single mouse islets isolated by dissection. The plotted points are means ± s.e.mean of the efflux rates from 5 individual islets. Arrows indicate start of two 10 s periods of electrical stimulation. Pulse parameters are shown by the inset.

Single mouse islets were perifused and set up for stimulation by trains of biphasic pulses passed between two electrodes (see Methods). Biphasic pulses were selected to prevent electrolysis occurring at the electrode tips. The period of an individual pulse was usually 2 ms, and current strengths of between 0.1 and 0.5 mA were employed. A 10 s period of stimulation with a current strength of 0.1 mA produced a marked and reversible increase in rubidium efflux of 140% (Figure 5). A further 10 s period of stimulation 10 min after the first resulted in a smaller increase in efflux, of approximately 76%. Omission of extracellular calcium and the addition of EGTA 2 mM did not significantly reduce the magnitude of the response to such electrical stimulation nor did the local anaesthetic nupercaine (100 μM).

Discussion

Measurement of rubidium efflux from a variety of cell types has been used widely as an indicator of the permeability of the cell membrane to potassium but the cellular transmembrane potential is rarely considered in the interpretation of such experiments. An increase in $[K]_o$ is known to depolarize islet cells (Dean & Matthews, 1970b; Atwater *et al.*, 1978; Meissner *et al.*, 1978): it also causes a net uptake of calcium (Malaisse-Lagae & Malaisse, 1971), as well as evoking insulin release (Grodsky & Bennett, 1966). Our experiments demonstrate that potassium-induced depolarization also causes a phasic increase in rubidium efflux from preloaded islet cells. There are several possible mechanisms which may contribute to this effect. First, depolarization may directly increase potassium (and therefore rubidium) net efflux by altering the electrochemical gradient driving the ion flux. Alternatively, depolarization may gate voltage-dependent potassium channels or, by causing the entry of calcium, activate a calcium dependent P_K. Other possibilities include direct effects on islet cell metabolism and alteration of the islet Na/K-ATPase activity. This study establishes that the entry of calcium through voltage-dependent channels is a major factor increasing ^{86}Rb efflux by acting through a quinine-sensitive calcium-activated P_K, since in the presence of either cobalt or quinine the response is considerably attenuated. A calcium-activated potassium channel in islet cells was first postulated to account for the cyclic behaviour of the electrical events associated with glucose stimulation of insulin release (Matthews, 1975) and subsequently confirmed experimentally (Atwater & Beigelman, 1976). In erythrocytes the increase in P_K stimulated by calcium entry is inhibited by quinine (Armando-Hardy *et al.*, 1975). When applied to islets quinine potentiates the insulinotropic action of glucose (Henquin *et al.*, 1975) and depolarizes the cell whilst increasing the input resistence (Atwater *et al.*, 1979). Henquin (1979) demonstrated that the calcium ionophore A23187 stimulated an increase in rubidium efflux that was abolished in the absence of external calcium or the presence of quinine. As is evident from the experiments described here, a calcium-activated potassium efflux must be an important component in the interaction between islet cell membrane potential and potassium permeability, and recent patch-clamp experiments have confirmed the calcium-dependent K-permeability to be of particularly large magnitude in islet cells (Marty & Neher, 1982).

The phasic nature of the increase in rubidium efflux following potassium-induced depolarization is indicative of the existence of an inactivation process. Voltage-dependent calcium channels of the squid axon inactivate in a time-dependent manner following stimulation (Baker *et al.*, 1973); that such a phenomenon might occur in islet cells is supported by the finding that potassium-induced depolarization causes a transient burst of electrical activity followed by electrical silence (Matthews & Sakamoto, 1975; Dawson *et al.*, 1982) and by the fact that hyperpolarizing current can temporarily restore the active phase. High $[K]_o$ also increases the membrane potential noise that is believed to arise from calcium channel activation (Dawson *et al.*, 1981; 1982), but only transiently. The increase in membrane potential noise decays with a time constant of about 10 s, whereas the half-time for the exponential decay in rubidium efflux measured here was much longer, some 13 min. This longer period may represent the half-time of the different processes that sequester and expel calcium in order to return $[Ca]_i$ to normal values, such as the sodium-calcium counter-transport system (Hales & Milner, 1868), or the direct activation of a Ca-dependent ATPase (Formby *et al.*, 1976). A calcium-dependent P_K, although of large magnitude (see above), is evidently not solely responsible for the increase in rubidium efflux since some efflux elicited by increasing $[K]_o$ still persists in the presence of either quinine or cobalt. At least a part of this remaining response must be due to the change in electrochemical gradient by depolarization and to the activation of voltage-dependent potassium channels (see Matthews, 1984).

It is unlikely that the increase in rubidium efflux after removal of external potassium is a voltage-dependent phenomenon, since the hyperpolarization produced by potassium removal (Atwater *et al.*, 1978; Meissner *et al.*, 1978; Henquin & Meissner, 1981) would be expected to lower rubidium efflux by reducing the elctrochemical gradient driving efflux. Moreover hyperpolarization would, by progressively closing the voltage-dependent potassium channels, reduce their contribution to the overall potassium

permeability of the cell membrane. That Ca^{2+} voltage-dependent K^+-channels show some activity in the resting state (i.e., at about −60 mV) is indicated by the decrease in rubidium efflux which follows the addition of tetraethylammonium (Henquin, 1977; Carpinelli & Malaisse, 1980; Matthews & Shotton, 1984). Inhibition of this 'resting' permeability by the hyperpolarization following potassium removal would explain the transient decrease in rubidium efflux observed in our experiments (Figure 1). The subsequent slow increase in ^{86}Rb efflux seen in Figure 1 is attributable most readily to an increase in $[Ca]_i$ which activates a calcium-dependent potassium permeability (see Herchuelz & Malaisse, 1980). The increase in $[Ca]_i$ is secondary to an increase in $[Na]_i$ which would occur because the Na-pump is inhibited by removal of external potassium. Since calcium extrusion is mediated by Na-Ca counter transport (Donatsch *et al.*, 1977) a rise in $[Na]_i$ would lead to a corresponding increase in $[Ca]_i$ and hence activation of P_{K-Ca}.

Direct electrical stimulation produced in single mouse islets a change in ribidium efflux similar to that obtained in rat islets by an increase in $[K]_o$ but, in addition to the mechanisms proposed for the increase in rubidium efflux following potassium-induced depolarization, electrical stimulation may also affect islet cell ionic permeabilities by the dissociation of calcium bound to the inner membrane surface as has been suggested to occur in mouse fibroblasts (Okada *et al.*, 1982). This would explain the inability of calcium removal or a local anaesthetic to affect the response. The current strengths used in our experiments are far below (i.e., more than two orders of magnitude less) those required to produce dielectric breakdown of cell membranes in pancreatic islets (Pace *et al.*, 1980) and more comparable to those shown by microelectrode studies to depolarize the β-cell membrane (Cook *et al.*, 1981). Although the precise nature of the membrane changes induced by direct electrical stimulation in our experiments must await a more extensive investigation it should be pointed out that these experiments demonstrate for the first time the feasibility of measuring ^{86}Rb efflux from single islets, a technique which has a number of advantages over the usual methods for studying islet β-cell pharmacology.

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