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Potassium-Induced Insulin Release and Voltage Noise Measurements in Single Mouse Islets of Langerhans

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Summary. Insulin release and membrane potential fluctuations in response to increased extracellular potassium $[K^+]_o$ have been measured in single perifused islets of Langerhans from normal mice. An increase in $[K^+]_o$ from 5 mM to 50 mM induced a transient insulin release with a peak at about 1 min. The peak value was $[K^+]_o$-dependent but the half-time $t_{\frac{1}{2}}$ for the decline was constant at nearly 1 min. 2.5 mM cobalt completely inhibited the potassium-induced stimulation of insulin release. The insulin release elicited by 28 and 50 mM $[K^+]_o$ was similar in terms of peak, total release and half-time from maximum release. Stepwise increase in $[K^+]_o$ from 10 to 28 to 50 mM resulted in a normal response to 28 mM but no peak of release after the 28 to 50 mM increase. The results indicate good correlation between excess voltage noise, thought to reflect calcium channel activity, and insulin release evoked by changing extracellular potassium.

Key words β-cell · membrane potential · noise analysis · excess noise · voltage-dependent calcium channel · calcium channel inactivation · membrane depolarization · insulin release · mouse islet

Introduction

Stimulation of insulin release by potassium-induced depolarization of the β-cell membrane has been reported in a variety of pancreas and islet of Langerhans preparations; in fetal pancreas [34], in perfused rat pancreas [16, 19, 22], in rabbit [24, 33, 42] and rat [35] pancreas pieces, in isolated rat islets in both static incubation [33, 35] and perifusion [28, 30]. This stimulatory effect of raised extracellular potassium $[K^+]_o$ is transient and is seen in the absence of glucose [16, 19, 22, 28, 30] and in the presence of subthreshold [24, 28, 42] and suprathreshold [28, 30, 33–35] glucose concentrations.

In muscle cells, the depolarization resulting from raising $[K^+]_o$ causes an increase in the membrane permeability to calcium [11, 45]. In squid axons, the use of intracellular aequorin to monitor changes in the entry of calcium ions in response to prolonged electrically or potassium-induced depolarization demonstrated the existence of a voltage- and time-dependent calcium channel [8]. In adrenal medulla, catecholamine release can be stimulated by prolonged exposure to high extracellular potassium concentrations [9, 14]. Inactivation of a voltage-dependent calcium permeability was proposed as the most likely explanation for the transient release of catecholamines, with a peak at 1 min and a half-time for the decline from the peak of about 1.2 min.

Indirect evidence has suggested the existence of a voltage-sensitive calcium permeability in pancreatic β-cells [13, 37, 41]. Recently, analysis of the β-cell membrane potential fluctuations induced by an increase in $[K^+]_o$ has provided more direct proof of the presence of a voltage-dependent calcium channel [3, 5]. Characterization of this voltage-sensitive calcium channel by noise analysis requires membrane potential recordings from β-cells in single micro-dissected islets of Langerhans. The work described in this study attempts to correlate insulin release with calcium permeability changes induced by elevation of extracellular potassium in the same preparation, namely single micro-dissected islets from normal mice.

Glucose depolarizes the β-cell membrane [12, 13] and this depolarization may be associated with a decrease in the potassium permeability activated by intracellular free calcium [4]. It has been proposed that glucose may evoke insulin release by increasing calcium influx [17, 26, 29, 43]. In order to study the effects of potassium-induced depolarization independently of any contribution from glucose, glucose was omitted from the perifusion medium during all manipulations of $[K^+]_o$.

Materials and Methods

Perifusion Media

The standard perifusion solution used was a modified Krebs-Ringer's-bicarbonate (KRB) buffer (110mM NcCl, 25mM $NaHCO_3$,

0022-2631/82/0064-0033 $02.20

2.5 mM $CaCl_2$ and 1.1 mM $MgCl_2$) equilibrated with O_2/CO_2 (95%/5%) at 37 °C and containing 5 mg/cm^3 bovine serum albumin. When the potassium concentration was increased, osmolarity was maintained by a decrease in the sodium concentration, all other ions being kept constant. Cobalt was added as $CoCl_2$ to the perifusion solution only minutes before use to avoid the risk of precipitation. 11.2 mM glucose was present until stable electrical activity had been recorded. After this, all experimental protocols were carried out in the absence of glucose.

Experimental Procedure

The electrophysiological methods used here have been described previously [2, 6].

Ten to 14-week-old albino mice fed *ad libitum* were used in this study. A single partially-dissected islet from the tail region of the pancreas was pinned into the small chamber described in detail elsewhere [46] and perifused with modified KRB containing 11.2 mM glucose until recording of the normal burst pattern of electrical activity indicated β-cell impalement. The time of exposure to glucose varied from 5 to 30 min. Glucose was removed for 10 min before collection of samples (20 sec or 1 min) for insulin assay. The perifusion rate was 1.5 cm^3/min. Changes in membrane potential were recorded 2 sec after changing from 5 mM to 50 mM $[K^+]_o$ at the stopcock. Flame photometry analysis of the appearance of potassium in the effluent from the chamber after switching the solutions gave a settling time of 7 sec. Unfortunately, the electrical recording was not maintained throughout all experiments. In those experiments in which the recording of membrane potential was lost, to avoid the possibility of damaging the islet and causing nonspecific release of insulin, no attempt was made to impale another β-cell.

Insulin Assay

Samples were stored at −20 °C until assay. Immunoreactive insulin was measured on undiluted samples in duplicate by radioimmunoassay [25] using a dextran-coated charcoal separation [1] with mouse insulin standard, ^{125}I-labeled bovine insulin and guinea pig antiporcine insulin serum. None of the ionic modifications to the perifusion medium interfered in the insulin assay. The minimum detectable level of insulin was usually 16 pg/cm^3 (details in [46]).

Chemicals

Mouse insulin was obtained from the Novo Research Institute, Denmark, anti-insulin serum (guinea pig) from Wellcome Reagents Ltd., Dartford, Kent, England and ^{125}I-insulin (bovine) from the Radiochemical Centre, Amersham, England. Bovine serum albumin (Fraction V) was purchased from the Armour Pharmaceutical Company Ltd., Eastbourne, England. All other reagents were of analytical grade.

Analysis of Membrane Potential Fluctuations

The method for analyzing membrane potential fluctuations has been described in detail recently [3]. Two channels of a four-channel magnetic tape recorder (Store 4, Racal-Thermionic; band width from 0 to 1250 Hz) were used to record the membrane potential using a low noise differential amplifier; one channel at low gain and the other at high gain (typical 1000) and with flutter compensation. For computer manipulation of data the high gain record was digitized using a 10-bit analog to digital converter. The output from this channel was filtered, high pass to eliminate the DC shift accompanying a $[K^+]_o$-induced potential shift and low pass at 500 or 1000 Hz depending on the sampling frequency. The membrane potential records were sampled continuously and stored in data blocks of 3072 points, each block representing either 3.072 or 6.144 seconds.

The digitized data were analyzed in terms of the variance. The variance was calculated as

$$\sigma_v^2 = \frac{1}{N} \sum_{i=1}^{N} (V_i - \bar{V})^2$$

where $\bar{V}$ represents the mean value of the membrane potential.

Calculation of Results

Half-times $t_{\frac{1}{2}}$ (time for 0.5-fold decline in insulin release) were calculated assuming a single exponential decay. For each peak of insulin release and its decline, correction was made for the basal release by subtracting from all release values the mean basal release after each individual peak. For the 10-min stimulation with potassium, the correction value used was the mean release after the peak but before the return to 5 mM potassium. For this reason, the half-time for insulin release for the 10-min stimulation was calculated from the first 5 min of the stimulation. Where appropriate, results were expressed as mean ± the standard error of the mean (SEM).

Islet volume was estimated from the measurement of two diameters and assuming an ellipsoidal shape.

Results

Effect of $[K^+]_o$ on Membrane Potential

In a previous paper [3] it was shown that increasing $[K^+]_o$ from 5 to 50 mM induced a transient increase in membrane potential noise. Fig. 1 shows the time course of the changes in membrane potential when $[K^+]_o$ was suddenly increased from 5 to 50 mM. The recording was made in the experiment shown in Fig. 5 but is shown in Fig. 1 on an expanded time scale to demonstrate the 20-mV spikes. The spikes are similar in profile to those recorded during the burst activity elicited by suprathreshold glucose concentrations and to those induced by current injection [6, 12, 13]. Such large spikes are not always seen but the membrane potential fluctuations or excess noise are always larger after raising $[K^+]_o$ from 5 to 50 mM. The excess noise has been shown to be due to an increase in calcium channel activity of the membrane [3] by use of cobalt [3] and manganese [5] to block the voltage-sensitive calcium channel and by removal of extracellular calcium [3].

The time course of the transient increase in the variance produced by depolarizing the cell with 50 mM extracellular potassium is shown in Fig. 2. In this run the variance reached a peak about 3 sec after the introduction of 50 mM $[K^+]_o$ and then declined exponentially with a half-time of about 10 sec. This pattern of a rapid increase in membrane potential noise followed by an exponential decline is reproduc-

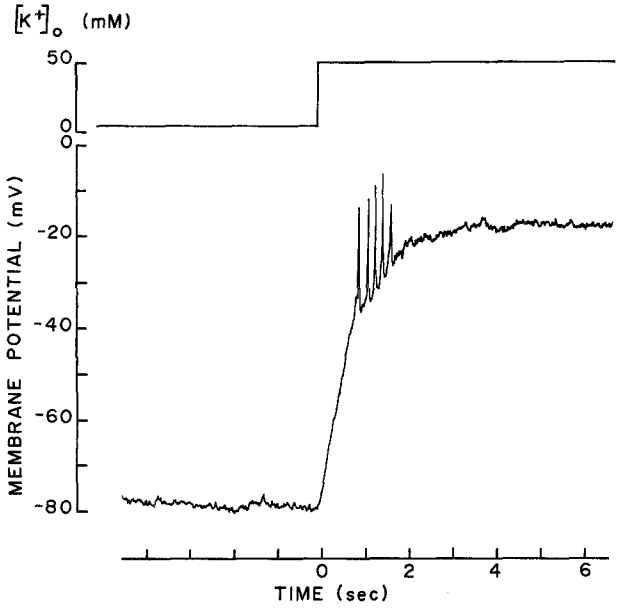

Fig. 1. Membrane potential fluctuations in response to an increase in $[K^+]_o$ from 5 to 50 mM. 50 mM potassium was introduced at time 0. The record was made in the experiment shown in Fig. 5 for the second peak and is shown here on an expanded time axis

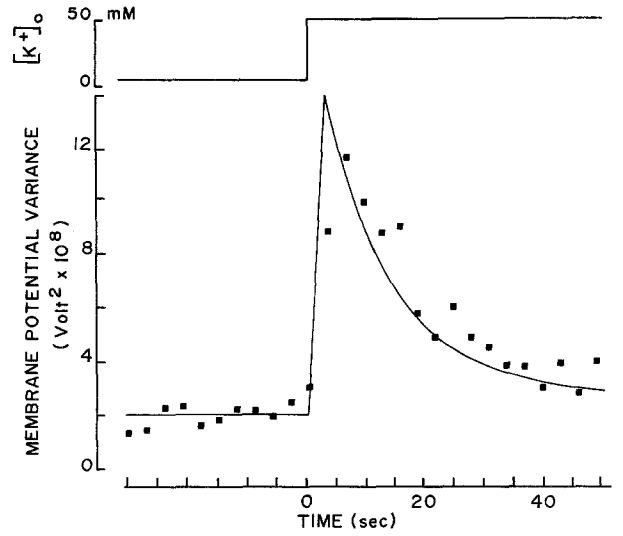

Fig. 2. Variance of voltage changes induced by a change in $[K^+]_o$ from 5 to 50 mM. The method for computation of the variance from the membrane potential record has been described previously (*see* ref. [3]). The line is a computer drawn best fit to a single exponential decay. Potassium was increased to 50 mM

ible in any islet for repeated 50 mM $[K^+]_o$-induced depolarizations, with 10 min at 5 mM potassium between challenges. The islet-to-islet variation in the half-time for the calcium channel inactivation measured by noise analysis [3] is large, the range being between 5 and 55 sec with most values falling between 10 and 20 sec.

Effect of Successive Stimulations with 50 mM $[K^+]_o$ on Insulin Release

Experiments were performed to test the reproducibility of insulin release to successive exposure to 50 mM potassium. Fig. 3 shows the results of one of three such experiments where three 3-min pulses of 50 mM $[K^+]_o$ were applied, 20 min apart. The potassium-induced depolarization produced a transient stimulation of insulin release with a return to basal levels within 5 to 10 min of repolarization. As in the other two identical experiments, the insulin released during the three 3-min periods of stimulation was relatively constant. The total release from the data in Fig. 3 was 2.27, 2.07 and 2.66 ng for the three consecutive challenges. These values for insulin release are higher than those measured in other experiments. This is almost certainly due to the fact that the islet was a large one with an estimated volume of 0.15 mm^3 compared with the more usual volume of 0.01–0.05 mm^3.

Perifusion of the islet with 11.2 mM glucose followed by 12 min in the absence of glucose had no potentiating effect on the stimulation of insulin release elicited by 50 mM $[K^+]_o$, since the first peak of insulin release was no greater than subsequent peaks. This observation was confirmed in all experiments performed in this study, where the time of exposure to glucose varied from 5 to 30 min depending on the time taken to obtain a stable electrical recording. A possible explanation for the constancy of insulin release to repeated potassium stimulations is that 11.2 mM glucose produces a long-lasting potentiation. If this were the case, the effect would have to last at least 90 min since a 3-min stimulation with 50 mM $[K^+]_o$ produced an insulin release profile similar to the first 3-min stimulation.

The insulin release evoked by 50 mM $[K^+]_o$-induced depolarization shows a rapid rise followed by an exponential decline. In the experiment shown in Fig. 3, the time taken to reach peak release was little different for the three potassium pulses; between 80 and 100 sec for the first two peaks and between 60 and 80 sec for the third peak. In most experiments the potassium-stimulated maximum release was at about 1 min after exposure to potassium but occasionally the maximum was reached after 2–3 min. The changes in membrane potential are faster, reaching the fully depolarized level after about 7 sec, but occasionally taking 20 sec (no correction has been made for the delay between stopcock and outflow from the chamber). Therefore, the time difference between membrane depolarization and insulin release was about 40 sec.

Half-times for the decline in insulin release from

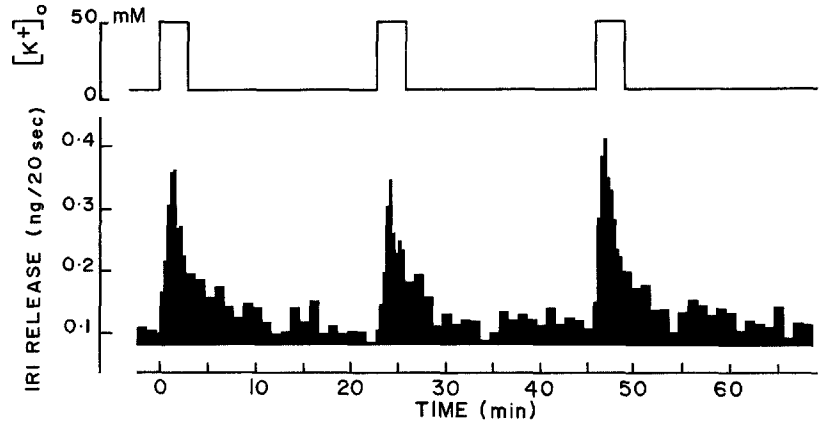

Fig. 3. The effect on insulin release of successive 3-min stimulations with 50 mM $[K^+]_o$. Insulin release is expressed as ng/20 sec. The first increase in potassium was made at time 0, 12 min after the removal of 11.2 mM glucose. There was a 20-min period at 5 mM $[K^+]_o$ between changes. The islet volume was 0.15 mm^3

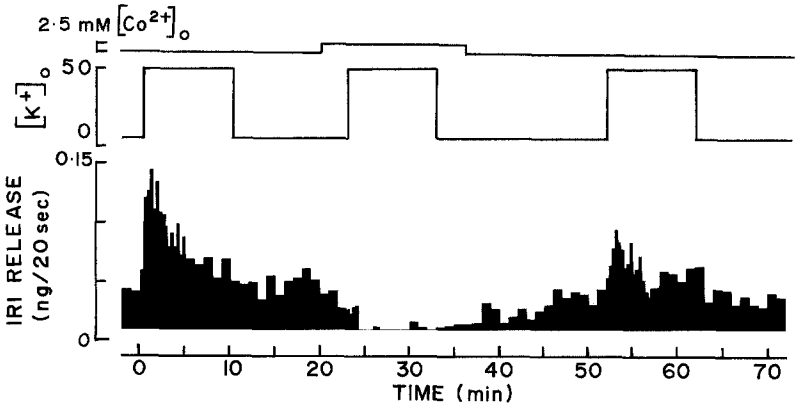

Fig. 4. 2.5 mM cobalt inhibition of 50 mM $[K^+]_o$-induced insulin release. Insulin release was below assay limit of 16 pg/cm^3 in some samples. Islet volume was 0.032 mm^3

the peak can be calculated assuming a single exponential decay. From the data in Fig. 3, the half-times for the successive 50 mM $[K^+]_o$ stimulations were 48, 41 and 61 sec. There was no consistent pattern of increase or decrease in the half-times for the three peak decays in any experiment. The mean $t_{\frac{1}{2}}$ from 16 experiments in which the islet was exposed to 50 mM $[K^+]_o$ for 3 min was 50 ± 5 sec.

Effect of Cobalt on $[K^+]_o$-Stimulated Insulin Release

The addition of 2 mM Co^{2+} has been shown to induce a 2–3 mV hyperpolarization of the β-cell membrane [3]. This hyperpolarization was presumed to be due to a sealing action of cobalt. The resting potential change induced by 50 mM potassium in the presence of cobalt is the same size (Nernst-type) but takes the membrane potential to different levels (2–3 mV more negative) [3].

Cobalt is a specific blocker of the voltage-dependent calcium permeability channel [7, 23] and it abolishes the excess voltage noise produced by potassium-induced depolarization of the β-cell membrane [3]. Glucose- and potassium-stimulated insulin release, as well as basal release, are inhibited by cobalt in perifused isolated rat islets [29]. Fig. 4 shows the results of an experiment to test the effect of 2.5 mM cobalt on the stimulation of insulin release produced by 10-min exposure to 50 mM $[K^+]_o$. Before the addition of cobalt, the transient peak of insulin release fell to a level above basal and stayed at this raised level until the $[K^+]_o$ was reduced to 5 mM. 2.5 mM $CoCl_2$ not only completely inhibited the stimulatory effect of 50 mM $[K^+]_o$ on insulin release, but also reduced the basal release. The insulin concentration in many of the samples collected in the presence of cobalt was below the minimum detectable limit of the immunoassay used. Removal of cobalt restored basal release but the response to a 10-min increase in $[K^+]_o$ to 50 mM 17 min after removal of cobalt was attenuated. The peak of release was smaller and total release for the 10-min stimulation after cobalt treatment was 1.80 ng compared with 2.48 ng for the response before cobalt addition. It is unlikely that this reduced response is due to a general diminution with time of the ability of the islet to respond to 50 mM $[K^+]_o$ since, in experiments similar to that illustrated in Fig. 3, equivalent stimulations were seen at the beginning and end of 90-min experiments. Exposure of the islet to 2.5 mM $CoCl_2$ for 16 min appeared to have a persistent adverse effect on $[K^+]_o$-induced insulin release.

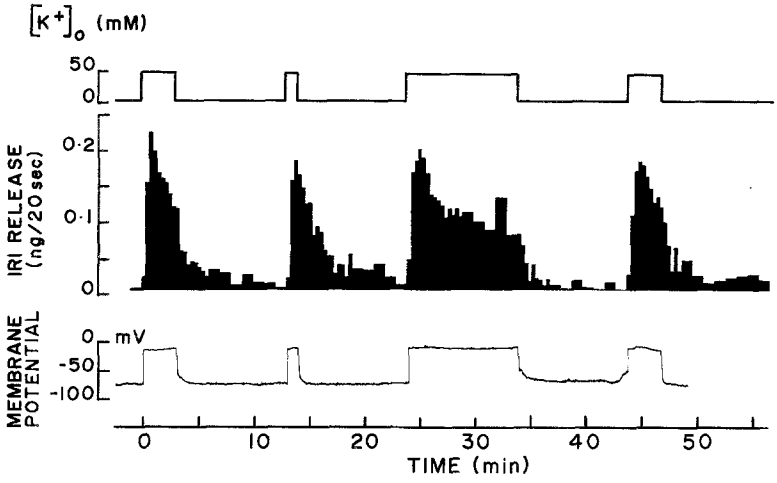

Fig. 5. Effect of 3-, 1-, 10- and 3-min stimulation with 50 mM $[K^+]_o$ on insulin release and membrane potential in the same islet. The solid bars represent exposure to 50 mM potassium. Resting membrane potential in 5 mM $[K^+]_o$ was −76 mV. The period between changes to 50 mM was 10 min. Islet size was 0.014 mm^3

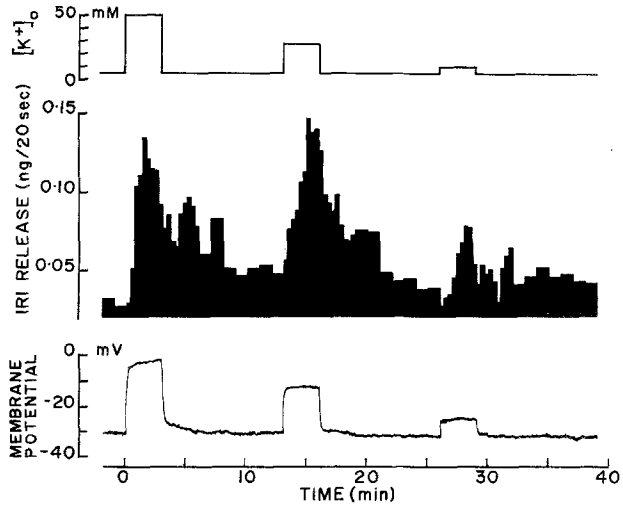

Fig. 6. Effect of exposure for 3 min to 10, 28 and 50 mM $[K^+]_o$ on insulin release and membrane potential. Resting membrane potential in 5 mM $[K^+]_o$ was −32 mV. Assay limit was 16 pg/cm^3. Islet size 0.015 mm^3

Effect of Membrane Potential on the Time Course of Insulin Release

The data in Fig. 3 demonstrate that constant decay of the insulin release can be used to characterize the process of insulin release from the β-cells of an islet. Two experimental procedures were used to study the effects of membrane potential on insulin release. In the first procedure, the $[K^+]_o$ was raised from 5 to 50 mM and the islet was perifused with this elevated potassium concentration for various times. A typical experiment of this type is illustrated in Fig. 5. In the second procedure, the $[K^+]_o$ was first increased from 5 to 50 mM, then from 5 to 28 and from 5 to 10 mM but the exposure time to high potassium concentrations was kept constant at 3 min. A typical experiment is shown in Fig. 6.

Other experiments (*not shown*) indicated that reduction of the time at 5 mM potassium between switches to 50 mM $[K^+]_o$ from 20 to 10 min had no effect on insulin release. The size and time course of release was unaffected by reducing the repolarization time and basal release was reached in 10 min.

The resting membrane potential recorded in the cell in Fig. 5 was −75 mV and the 50 mM $[K^+]_o$-induced depolarization was 60 mV (maximum Nernst change equals 61 mV) to a level of −15 mV. This depolarization was maintained as long as the $[K^+]_o$ was at 50 mM. The time course of the change in membrane potential differed between the depolarization and repolarization. The time taken for full depolarization after switching the stopcock was about 7 sec whereas the repolarization took about 60 sec, but repolarization to −45 mV took only 6 sec. The repolarization has two phases, a rapid first phase followed by a slower second phase, as seen in muscle [31] and nerve [44]. This slow recovery of the resting potential or tail effect (*see* membrane potential records in Figs. 5 and 6) may be associated with slow readjustments in the anion concentration, particularly chloride [31].

The basal insulin release was low in the islet shown in Fig. 5. The peak release was constant for the four stimulations and the time taken for peak release to be reached was similar; between 40 and 60 sec for the first two peaks and between 60 and 80 sec for the last two peaks. As was seen in Fig. 4, when the membrane potential was maintained at the depolarized level for 10 min, the initial rapid rise in insulin release declined to a level above basal and returned to basal only after repolarization caused by 5 mM potassium. This observation confirms data obtained in perfused rat pancreas [16] and isolated rat islets [30]. The time course of the decline of insulin release over the control basal release can be described in terms of two time constants. The first can be mea-

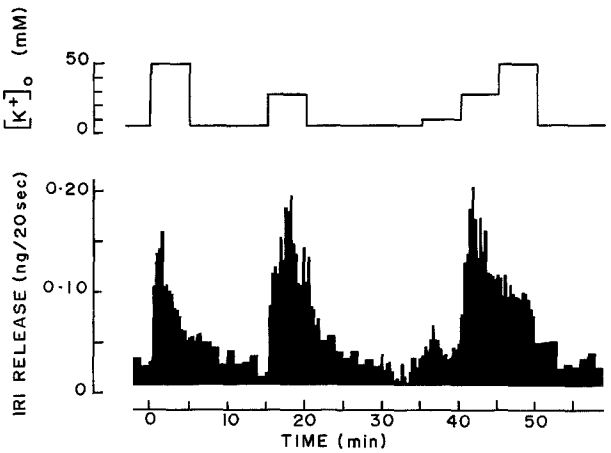

Fig. 7. Effect of stepwise changes in $[K^+]_o$ on insulin release. The effect of 5 to 50 and 5 to 28 mM $[K^+]_o$ steps were first tested. The duration of each step was 5 min. Islet size 0.027 mm^3

sured during the application of 50 mM potassium. The second time constant can be measured only during the return to 5 mM $[K^+]_o$ after long exposure to 50 mM $[K^+]_o$. For short time exposures to 50 mM potassium, the time constant calculated may have contributions from both the time constants mentioned above.

For the experiment shown in Fig. 5, the half-times calculated after raising $[K^+]_o$ to 50 mM were 60, 62, 79 and 75 sec for the 3-, 1-, 10- and 3-min exposures, respectively. The average half-times for four experiments identical to that shown in Fig. 5 were 45 ± 5 sec for the first 3-min pulse, 43 ± 8 sec for the 1-min pulse, 78 ± 7 sec for the 10-min pulse and 49 ± 10 for the last 3-min pulse. In two experiments using the same time protocol but 28 mM potassium instead of 50 mM, similar results were obtained (*data not shown*); the peak and total insulin release were similar with 28 and 50 mM $[K^+]_o$. The mean half-times were 51, 41, 75 and 42 sec for the 3-, 1-, 10- and 3-min challenges with 28 mM potassium. There was no measurable difference between the responses to 28 and 50 mM $[K^+]_o$ with respect to half-times of the transient insulin release. The results for the 3- and 1-min challenges indicate that the time constant is independent of the time of exposure.

The data in Fig. 5 indicate that the repolarization after the 10-min exposure to 50 mM potassium resulted in a rapid fall in insulin release to basal levels. This repolarization-induced reduction in insulin release represents the second time constant mentioned above and may contribute to the half-time of the decline from peak release in the 3- and 1-min but not the 10-min challenge to high potassium. The mean half-time of the repolarization-induced fall in insulin release is 66 ± 10 sec ($n=4$). This value is close to the half-times calculated for the 3- and 1-min stimulations with potassium and probably contributes to these half-times. The time constant for the 10-min 50 mM potassium challenges of 78 sec represents only the inactivation of the insulin release process.

Analysis of the membrane potential fluctuations in response to the 50 mM potassium pulses in Fig. 5 indicated time constants $t_{\frac{1}{2}}$ for the calcium channel inactivation of between 3 and 10 sec.

The effect on insulin release and membrane potential of raising $[K^+]_o$ from 5 to 50, 28 and 10 mM for the same time of 3 min, with 10 min at 5 mM potassium in between, is shown in Fig. 6. It has been shown previously [3, 6, 40] that the membrane potential of the β-cell can be controlled by adjusting the extracellular potassium concentration. The results shown in the lower part of Fig. 6 confirm these previous observations. In this experiment, the resting potential was -32 mV. The change in membrane potential caused by the different $[K^+]_o$ was small; 27, 20 and 7 mV for 50, 28 and 10 mM potassium. The absolute value for the resting membrane potential varies in different experiments, perhaps as a reflection of differences in the size of the leakage pathway around the microelectrode.

In Fig. 6, at all potassium concentrations, the peak of insulin release occurred later than is usually seen; 100–120 sec for the 50 mM and 120–140 sec for the 28 and 10 mM $[K^+]_o$ stimulations. The total insulin released for the 3-min periods at 50, 28 and 10 mM potassium was 0.87, 1.01 and 0.47 ng, respectively. For three identical experiments the mean total releases were 1.04 ± 0.11, 1.12 ± 0.07 and 0.37 ± 0.08 ng.

The half-times of the decline from peak for the 3-min challenges with 50 and 28 mM $[K^+]_o$ are similar; 50 ± 5 sec ($n=16$) and 53 ± 11 sec ($n=5$), respectively. Calculation of half-times for the 10 mM $[K^+]_o$ is difficult due to the small difference between the release values (because of the small peaks) and therefore the increased error implicit in the line drawn. The peak of insulin release in response to 10 mM $[K^+]_o$ has a half-time of 28 ± 7 sec ($n=3$) a value smaller than those for 28 and 50 mM potassium. However, the errors are large in the 10 mM half-time calculation so many more experiments would be needed to prove a significant difference.

Effect of Stepwise Changes in $[K^+]_o$

In experiments designed to examine the excess noise or membrane potential fluctuations produced by stepwise doubling of the potassium concentration from 7 mM via 14 and 28 mM to 56 mM, it was found that the largest fluctuations were produced by the 14- to 28-mM step and there was no increase in noise between 28 and 56 mM [3].

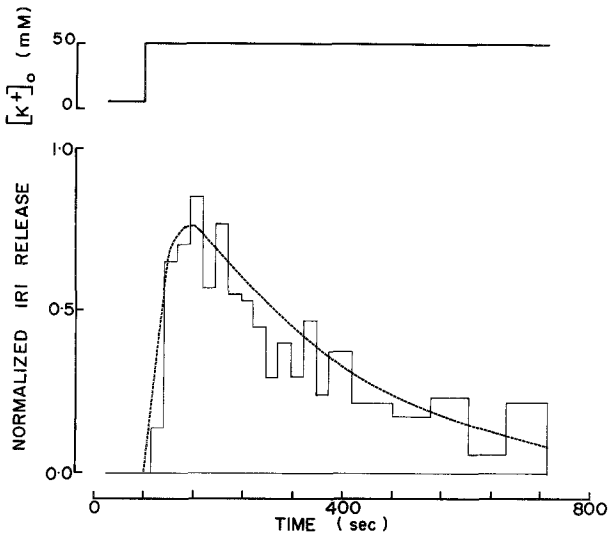

Fig. 8. Computer 'best fit' to the data from the 10-min stimulation with 50 mM $[K^+]_o$ in Fig. 5. The curve was calculated using Eq. (1) and the data normalized

Fig. 7 shows the effect on insulin release of a similar protocol of stepwise increases in potassium from 10 to 28 to 50 mM. The characteristics of the release pattern of this islet were first determined by changing the $[K^+]_o$ from 5 to 50 mM and from 5 to 28 mM for 5 min each. In this islet the response to the 5-min stimulation with 28 mM potassium was greater than the response to 50 mM; the total release for the 5-min period was 1.91 and 1.38 ng for the 28 and 50 mM $[K^+]_o$. The step from 5 to 10 mM potassium produced a small transient increase in insulin release. The step from 10 to 28 mM produced a similar response to the 5- to 28-mM step (total release slightly higher at 2.26 ng for the 5-min period). However, the step from 28 to 50 mM potassium prevented the fast decay in insulin release seen with a step increase in $[K^+]_o$ from 5 to 50 mM. The 28 to 50 mM potassium step elicited no peak of release although the release was maintained at a level above basal until the potassium was reduced to 5 mM. The total insulin output for the 50 mM $[K^+]_o$ after 28 mM was very similar to the output for the 5 to 50 mM stimulation; 1.49 and 1.38 ng, respectively.

Apparatus and Islet Diffusional Time Constants

The results of experiments on the exchange dynamics of the experimental chamber suggest that it took less than 7 sec after switching from 5 to 50 mM $[K^+]_o$ at the stopcock to achieve 50 mM $[K^+]_o$ around the islet. As the time constant to achieve 90% of the depolarization induced by elevation of potassium is less than 2 sec (*see* Figs. 5 and 6) it may be assumed that the calcium channels would be activated without delay. In all our measurements of insulin release it took at least 40 sec, and sometimes 2 min, for peak value to be reached. If a raised intracellular calcium concentration resulting from increased calcium influx is the trigger for insulin release in response to potassium-induced depolarization, a delay between the maxima of calcium channel activation and insulin release would be predicted. The delay of at least 40 sec between calcium channel activation and insulin release may be due to the inherent delay in the insulin release process from entry of calcium across the cell membrane to exocytosis of insulin from the cell or may be due to diffusional delay of insulin from the islet intercellular space.

Analysis of the experiment in Fig. 5 to determine the time constants for the rise to peak of insulin release and the inactivation constant can be performed using,

$$IRI(t)=IRI(o)[1-e^{-t/\tau_D}]\,e^{-t/\tau_R}+IRI(\infty). \quad (1)$$

where τ_D represents a time constant for the diffusion of insulin out of the islet, τ_R represents a time constant associated with the release process and $IRI(O)$ and $IRI(\infty)$ are the initial and steady levels of insulin release, respectively. Fig. 8 shows the best fit from the data; $\tau_D=30$ sec and $\tau_R=245$ sec (τ_R as calculated from Fig. 5 data, using the last 5 min of the 10-min stimulation with potassium to set the final level of release and using only the first 5 min of the stimulation for the calculation of the time constant with no adjustment for the diffusional time constant, was $79/0.69=115$ sec). In the case of radial diffusion, we have

$$\frac{r^2}{4D^{37°}_{H_2O}\tau_D}=1$$

where r represents the radius of the islet. Taking r as 125×10^{-4} cm and $D^{37°}_{H_2O}$ as 12×10^{-7} cm^2 sec^{-1}, τ_D equals 22 sec. There is good agreement between τ_D as calculated from radial diffusion and τ_D calculated from the insulin release data from Fig. 5. However, in experiments where the time to peak is slower, then both diffusion and the release process may contribute to the delay between depolarization and insulin release.

Calcium Entry during the $[K^+]_o$-Induced Depolarization

From a graph of the variance $\sigma_v^2(t)$ (*see* Fig. 2) as a function of the potential difference $\mu_v(t)$ (derived from the membrane potential records made in the absence and in the presence of Ca^{2+}-channel blocker) the size of the unit event $\delta V(0)$ is obtained [3].

In one experiment (using 2 mM Co^{2+} as a Ca^{2+}-channel blocker) $\delta V(O)$ was estimated as 53.4×10^{-6} volt [3]. In two other experiments, where the excess membrane potential noise was suppressed by lowering the external calcium to 0.1 mM, the average value $\delta V(O)$ is 103.2×10^{-6} V. Thus, the average size of the unit event is 78.3×10^{-6} V. The calcium entry per unit event may be calculated as

$$\frac{1}{F}\int_o^\tau i_{Ca}\,dt = \frac{\gamma_{Ca}}{F}\int_o^\tau (V - V_{Ca})\,dt \tag{2}$$

where γ_{Ca} is the conductance per channel, V_{Ca} is the equilibrium potential for Ca^{2+}, τ is the duration of the open state of the channel and F is the Faraday's number. From Eq. (13) in reference [3] one also gets

$$\frac{1}{F}\int_o^\tau i_{Ca}\,dt = \frac{\delta V(O)\,\tau}{2F R_m(1-p)} \tag{3}$$

where $R_m = 85 \times 10^6\ \Omega$ [6] and $\tau = 25 \times 10^{-3}$ sec [3]. Whence, the calcium entry per channel amounts to 11.5×10^{-20} moles. The total entry during the $[K^+]_o$-induced depolarization may be estimated from the total number of events N during the potassium challenge. This is calculated by integration of the function relating the frequency of the events to $\mu_v(t)$. Thus,

$$N = \int_o^T \bar{\omega}(t)\,dt = \frac{1}{\delta V(O)\,\tau}\int_o^T \mu_v(t)\,dt \tag{4}$$

where T is the duration of $[K^+]_o$ pulse [3]. As $\mu_v(t)$ is proportional to $\sigma_v^2(t)$, then the value of $\int_o^T \mu_v(t)\,dt$ equals the product of $\mu_v(O)$ times the time constant of inactivation of $\sigma_v^2(t)$ (*see* Fig. 2). Taking $\mu_v(O)$ as 45×10^{-3} V and the time constant as 15 sec, N is calculated as 3.4×10^5. The total calcium entry per β-cell during the $[K^+]_o$ stimulation is calculated as 39×10^{-15} moles or 2×10^{10} Ca^{2+}.

Discussion

The transient stimulation of insulin release elicited by raising extracellular potassium in the absence of glucose reported here from single micro-dissected mouse islets confirms results in perfused pancreas [16, 19, 22] and in perifused rat islets [28, 30]. The similarity of potassium-induced insulin release, muscle contraction [11], and catecholamine release from adrenal medulla [9, 14] was noted by Henquin and Lambert [28]. It was suggested that in these processes, the depolarization produced by raising extracellular potassium activates a calcium permeability in the cell membrane with consequent rise in intracellular calcium, as was measured using aequorin in squid axons [8]. The hypothesis that increasing $[K^+]_o$ stimulates calcium influx is supported by studies on $^{45}Ca^{2+}$ uptake in rat [36] and obese hyperglycemic mouse [27] islets. Increased net uptake of ^{45}calcium was observed over 90-min incubation [36] and over 5 and 120 min [27].

Insulin Release and Calcium Channel Activity

In the study described here, the increased insulin release and calcium channel activity resulting from potassium-induced depolarization of the β-cell have been studied in the same preparation, namely single microdissected islets from normal mice. The data are consistent with the proposal that the transient insulin release elicited by raising $[K^+]_o$ is due to a transient rise of calcium entry into the islet cells.

As reported previously [29] in rat islets, cobalt, a specific blocker of voltage-dependent calcium permeability [7, 23], inhibits the potassium-induced insulin release in mouse islets (*see* Fig. 4). It also abolishes the increase in excess noise of the membrane potential after depolarization by high potassium [3] as does manganese [5] which blocks the same calcium permeability. Removal of calcium from the perifusing medium completely inhibits the potassium-stimulated insulin release in rat islets [28] and the depolarization-induced excess voltage noise in single mouse islets [3]. These observations lend strong support to the suggestion that increased calcium influx is responsible for the rise in insulin secretion produced by elevated extracellular potassium.

The insulin release pattern is very similar to the release of catecholamines from perfused adrenal glands in response to prolonged potassium stimulation [9, 14]. Catecholamine and insulin release show a peak at about 1 min then a fall with a half-time of 70 and 78 sec, respectively. It is unlikely that the fall in insulin release is due to exhaustion of a 'labile' pool since there was no attenuation of the response to 50 mM $[K^+]_o$ with repeated stimulations (*see* Fig. 3).

Although in general terms the profile of increase in calcium channel activity measured by noise analysis (Fig. 2) and insulin release (Figs. 3–7) are similar, i.e. a peak followed by inactivation, the transient increase in calcium channel activity is faster than that of insulin release.

The insulin release measured from a single islet is the total output from all the cells in the islet whereas the membrane potential is recorded from a single β-cell. Cell-to-cell coupling [39] has been reported to decrease in the absence of glucose [15, 38]; therefore it is possible that the islet behaves less as a functional

syncitium in the absence of glucose than during stimulation with high glucose concentrations. All the experiments described here were performed in the absence of glucose. The half-time measured for the inactivation of the voltage-dependent calcium channel (5–20 sec) characterizes a single cell whereas the insulin release half-time (approximately 75 sec) is the mean characteristic half-time for all the cells in the islet.

The flow rate of 1.5 cm^3/min and the need for duplicate samples for insulin assay impose a minimum sampling time in these experiments of 20 sec. This 20-sec collection period limits the time resolution. The resolution is also dependent on the size of the peak of insulin release. Thus, with 5 samples, a peak of 0.15 ng above basal has a minimum half-time of 13 sec and for a peak of 0.08 ng above basal the minimum half-time resolvable is 16 sec. It is clear that the value of about 75 sec for the insulin release 'inactivation' half-time is not at the limits set by the methodology.

Although the voltage variance decreases during $[K^+]_o$-induced depolarization (*see* Fig. 1), suggesting inactivation of the calcium channel activity, the inactivation is not complete (*see* Fig. 1, the residual variance). The continued secretion of insulin above basal rate seen when $[K^+]_o$ is raised for 10 min (Figs. 4 and 5) is probably due to this residual calcium channel activity. This maintained secretion of insulin to prolonged exposure to potassium was reported in perfused rat pancreas [16] and in perifused rat islets [30] but not by Henquin and Lambert [28]. The fall to basal insulin release after the change from 50 to 5 mM potassium is rapid with a half-time of approximately 65 sec. The time constant calculated for the fall in insulin release after a stepwise increase in glucose to 33 mM was reported to be larger than this; half-time was 8.8 min [46].

Repeated exposure of an islet to high potassium elicits similar transient increases in calcium channel activity ([3] and *unpublished observations*). Within one experiment the insulin release in response to successive challenges with 50 mM $[K^+]_o$ was constant, in terms of peak release and half-time from the peak (Fig. 3). In rat islets it has been reported that the insulin release in response to a second stimulation with 24 mM $[K^+]_o$, in the presence of 2.8 mM glucose, 10 min after the first, was not significantly different [28]. However, in the same study, exposure to 16.7 mM glucose for 5 min followed 10 min later by 24 mM potassium produced a potentiation of the response to potassium. A priming effect of glucose to subsequent stimulation with glucose has been reported in perfused rat pancreas [21] and rat islets [20, 28] but not in mouse pancreas [10]. In the experiments performed here, 11.2 mM glucose, for up to 30 min, 12 min before exposure to increased potassium had no potentiating effect.

The dependence of insulin release on voltage or potassium concentration was shown in Fig. 6. The lack of significant difference between the 50 and 28 mM $[K^+]_o$ stimulations confirms data from static incubation experiments with mouse islets [3]. In the 10-min static incubation 10 mM potassium did not produce a significant stimulation of insulin release, a result that is not surprising in view of the small transient effect seen in the single perifused islet (Fig. 6). An increase of insulin release with increasing $[K^+]_o$ was reported in perfused rat pancreas [16, 19]; an approximate doubling was seen between 18.3 and 53.3 mM potassium [16] and a sigmoid relationship with a plateau at about 25 mM was reported for the insulin release between minutes 2 and 6 of the stimulation with up to 30 mM potassium [19].

The voltage dependence of the calcium channel activity in β-cells has been reported previously [3]. The threshold voltage varies slightly; e.g., the data in Fig. 1 indicate a threshold of -38 mV equivalent to a potassium concentration of 26 mM from the Goldman-Hodgkin-Katz equation [18, 32].

The inactivation of the insulin release does not seem to be affected by changing the membrane potential at different times after the initial stimulation (Fig. 5). The effect of changing membrane potential within two half-lives of the calcium channel inactivation has not been studied; a very rapid change in potassium of about 15 sec would be required.

Fig. 7 shows data on insulin release in response to a stepwise increase in $[K^+]_o$ from 5 to 10 to 28 to 50 mM. There was no peak of insulin release after the 28 to 50 mM step but the level of release was maintained above basal until potassium was restored to 5 mM. Raising extracellular potassium from 14 to 28 mM elicited a large increase in excess noise presumably due to calcium channel activity, whereas the subsequent step from 28 to 56 mM produced no increase in noise [3]. Thus, there is good correlation between calcium channel activity and insulin release in response to a stepwise increase in potassium.

The experimental results presented here suggest a causal relationship between the transient increase in the voltage-dependent calcium permeability of the β-cell membrane and the transient stimulation of insulin release elicited by potassium-induced depolarization.

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References

1. Albano, J.D.M., Ekins, R.P., Maritz, G., Turner, R.C. 1972. A sensitive, precise radioimmunoassay of serum insulin relying on a charcoal separation of bound and free hormone moieties. *Acta Endocrinol. (Copenhagen)* **70**:487–509
2. Atwater, I., Beigelman, P.M. 1976. Dynamic characteristics of electrical activity in pancreatic β-cells. I. Effects of calcium and magnesium removal. *J. Physiol (Paris)* **72**:769–786
3. Atwater, I., Dawson, C.M., Eddlestone, G.T., Rojas E. 1981. Voltage noise measurements across the pancreatic β-cell membrane: Calcium channel characteristics. *J. Physiol. (London)* **314**:195–212
4. Atwater, I., Dawson, C.M., Ribalet, B., Rojas, E. 1979. Potassium permeability activated by intracellular calcium ion concentration in the pancreatic β-cell. *J. Physiol. (London)* **288**:578–588
5. Atwater, I., Eddlestone, G.T., Ribalet, B., Rojas, E. 1979. Calcium channel voltage noise across the β-cell membrane in islet of Langerhans of the mouse. *J. Physiol. (London)* **291**:69–70P
6. Atwater, I., Ribalet, B., Rojas, E. 1978. Cyclic changes in potential and resistance of the β-cell membrane induced by glucose in islets of Langerhans from mouse. *J. Physiol. (London)* **278**:117–139
7. Baker, P.F. 1972. Transport and metabolism of calcium ions in nerve. *Prog. Biophys. Mol. Biol.* **24**:177–223
8. Baker, P.F., Meves, H., Ridgway, E.B. 1973. Calcium entry in response to maintained depolarization of squid axons. *J. Physiol. (London)* **231**:527–548
9. Baker, P.F., Rink, T.J. 1975. Catecholamine release from bovine adrenal medulla in response to maintained depolarization. *J. Physiol. (London)* **253**:593–620
10. Berglund, O. 1980. The response of the mouse pancreas to repeated stimulations of insulin release. *Diabetologia* **19**:257 (*Abstr.* 36)
11. Bianchi, C.P., Shanes, A.M. 1959. Calcium influx in skeletal muscle at rest, during activity, and during potassium contracture. *J. Gen. Physiol.* **42**:803–815
12. Dean, P.M., Matthews, E.K. 1970. Glucose-induced electrical activity in pancreatic islet cells. *J. Physiol. (London)* **210**:225–264
13. Dean, P.M., Matthews, E.K. 1970. Electrical activity in pancreatic islet cells. Effect of ions. *J. Physiol. (London)* **210**:265–275
14. Douglas, W.W., Rubin, R.P. 1961. The role of calcium in the secretory response of the adrenal medulla to acetylcholine. *J. Physiol. (London)* **159**:40–57
15. Eddlestone, G.T., Rojas, E. 1980. Electrical coupling between β-cells in the mouse islet of Langerhans. *Diabetologia* **19**:272 (*Abstr.* 115)
16. Epstein, G., Fanska, R., Grodsky, G.M. 1978. The effect of potassium and valinomycin on insulin and glucagon secretion in the perfused rat pancreas. *Endocrinology* **103**:2207–2215
17. Frankel, B.J., Imagawa, W.T., O'Connor, M.D.L., Lundquist, I., Kromhout, J.A., Fanska, R.E., Grodsky, G.M. 1978. Glucose-stimulated ^{45}calcium efflux from isolated rat pancreatic islets. *J. Clin. Invest.* **62**:525–531
18. Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27**:37–60
19. Gomez, M., Curry, D.L. 1973. Potassium stimulation of insulin release by the perfused rat pancreas. *Endocrinology* **93**:1126–1134
20. Grill, V., Adamson, U., Cerasi, E. 1978. Immediate and time-dependent effects of glucose on insulin release from rat pancreatic tissue. Evidence for different mechanisms of action. *J. Clin. Invest.* **61**:1034–1043
21. Grodsky, G.M. 1972. A threshold distribution hypothesis for packet storage of insulin and its mathematical modelling. *J. Clin. Invest.* **51**:2047–2059
22. Grodsky, G.M., Bennett, L.L. 1966. Cation requirements for insulin secretion in the isolated perfused pancreas. *Diabetes* **15**:910–913
23. Hagiwara, S., Takahashi, K. 1967. Surface density of calcium ions and calcium spikes in the barnacle muscle fibre membrane. *J. Gen. Physiol.* **50**:583–601
24. Hales, C.N., Milner, R.D.G. 1968. The role of sodium and potassium in insulin secretion from rabbit pancreas. *J. Physiol. (London)* **194**:725–743
25. Hales, C.N., Randle, P.J. 1963. Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* **88**:137–146
26. Hellman, B., Sehlin, J., Täljedal, I.-B. 1976. Effects of glucose on $^{45}Ca^{2+}$ uptake by pancreatic islets as studied with the lanthanum method. *J. Physiol. (London)* **254**:639–656
27. Hellman, B., Sehlin, J., Täljedal, I.-B. 1978. Effects of Na^+, K^+ and Mg^{2+} on $^{45}Ca^{2+}$ uptake by pancreatic islets. *Pfluegers Arch.* **378**:93–97
28. Henquin, J.-C., Lambert, A.E. 1974. Cationic environment and dynamics of insulin release. II. Effect of a high concentration of potassium. *Diabetes* **23**:933–942
29. Henquin, J.-C., Lambert, A.E. 1975. Cobalt inhibition of insulin secretion and calcium uptake by isolated rat islets. *Am. J. Physiol.* **228**:1669–1677
30. Herchuelz, A., Thonnart, N., Sener, A., Malaisse, W.J. 1980. Regulation of calcium fluxes in pancreatic islets: The role of membrane depolarization. *Endocrinology* **107**:491–497
31. Hodgkin, A.L., Horowicz, P. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (London)* **148**:127–160
32. Hodgkin, A.L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108**:37–77
33. Howell, S.L., Taylor, K.W. 1968. Potassium ions and the secretion of insulin by islets of Langerhans incubated *in vitro*. *Biochem. J.* **108**:17–24
34. Lambert, A.E., Jeanrenaud, B., Junod, A., Renold, A.E. 1969. Organ culture of fetal rat pancreas. II. Insulin release induced by amino and organic acids, by hormonal peptides, by cationic alterations of the medium and by other agents. *Biochim. Biophys. Acta* **174**:540–553
35. Malaisse, W.J., Malaisse-Lagae, F., Brisson, G. 1971. The stimulus-secretion coupling of glucose-induced insulin release. II. Interaction of alkali and alkaline earth cations. *Horm. Metab. Res.* **3**:65–70
36. Malaisse-Lagae, F., Malaisse, W.J. 1971. The stimulus-secretion coupling of glucose-induced insulin release. III. Uptake of ^{45}calcium by isolated islets of Langerhans. *Endocrinology* **88**:72–80
37. Matthews, E.K., Sakamoto, Y. 1975. Electrical characteristics of pancreatic islet cells. *J. Physiol. (London)* **246**:421–437
38. Meda, P., Perrelet, A., Orci, L. 1979. Increase of gap junctions between pancreatic β-cells during stimulation of insulin secretion. *J. Cell Biol.* **82**:441–448
39. Meissner, H.P. 1976. Electrophysiological evidence for coupling between β-cells of pancreatic islets. *Nature (London)* **262**:502–504
40. Meissner, H.P., Henquin, J.-C., Preissler, M. 1978. Potassium dependence of the membrane potential of pancreatic β-cells. *FEBS Lett.* **94**:87–89
41. Meissner, P.M., Schmelz, H. 1974. Membrane potential of beta-cells in pancreatic islets. *Pfluegers Arch.* **351**:195–206

42. Milner, R.D.G., Hales, C.N. 1968. Cations and the secretion of insulin. *Biochim. Biophys. Acta* **150**:165–167
43. Naber, S.P., MacDaniel, M.L., Lacy, P.E. 1977. Effect of glucose on acute uptake and efflux of calcium-45 in isolated rat islets. *Endocrinology* **101**:686–693
44. Rojas, E., Taylor, R.E., Atwater, I., Bezanilla, F. 1969. Analysis of the effects of calcium or magnesium on voltage-clamp current in perfused squid axons bathed in solutions of high potassium. *J. Gen. Physiol.* **54**:532–552
45. Sandow, A. 1965. Excitation-contraction coupling in skeletal muscle. *Pharmacol. Rev.* **17**:265–320
46. Scott, A.M., Atwater, I., Rojas, E. 1981. A method for the simultaneous measurement of insulin release and β-cell membrane potential in single mouse islets. *Diabetologia (in press)*

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