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POTASSIUM PERMEABILITY ACTIVATED BY INTRACELLULAR CALCIUM ION CONCENTRATION IN THE PANCREATIC β-CELL

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SUMMARY

1. Membrane potentials and input resistance were measured in β-cells from mouse pancreatic islets of Langerhans in a study designed to assess the role of a K permeability specifically blocked by quinine or quinidine and activated by intracellular calcium ion concentration ($[Ca^{2+}]_i$-activated P_K).

2. Addition of 100 μM-quinine to the perifusion medium resulted in a 10–30 mV depolarization of the membrane and an increase in the input resistance of *ca.* $4 \cdot 10^7$ Ω.

3. In the absence of glucose, 100 μM-quinine induced electrical activity.

4. In the presence of glucose, 100 μM-quinine abolished the burst pattern of electrical activity and very much reduced the graded response of spike frequency normally seen with different concentrations of glucose.

5. Addition of mitochondrial inhibitors, KCN, NaN_3, DNP, CCCP, FCCP, to the perifusion medium containing glucose rapidly hyperpolarized the β-cell membrane, inducing a concomitant decrease in input resistance.

6. In the presence of glucose, these mitochondrial inhibitors reversibly blocked electrical activity; upon removal of the inhibitor, recovery of electrical activity followed a biphasic pattern.

7. The effects of mitochondrial inhibitors were partially reversed by 100 μM-quinine.

8. It is proposed that the membrane potential of the β-cell in the absence of glucose is predominantly controlled by the $[Ca^{2+}]_i$-activated P_K. It is further suggested that this permeability to K controls the level for glucose stimulation and leads to the generation of the burst pattern.

INTRODUCTION

Recent studies on the nature of the initial depolarization of the pancreatic β-cell membrane induced by glucose have indicated that this is due principally to a reduction in K permeability, P_K (Sehlin & Täljedal, 1975; Boschero, Kawazu, Duncan & Malaisse, 1977; Henquin, 1978; Atwater, Ribalet & Rojas, 1978). Tetraethylammonium, TEA, known to block P_K in many other tissues, was recently tested in

islets of Langerhans and found to be ineffective in inducing insulin release (Henquin, 1977) or electrical activity (Atwater, Ribalet & Rojas, 1979) unless glucose was present in the medium at concentrations above 2·8 mM.

Another P_K, which is gated by intracellular Ca ions, $[Ca^{2+}]_i$, and is insensitive to TEA has been described in some tissues (Gardos, 1958; Lew, 1970; Krnjević & Lisiewicz, 1972; Meech, 1974; Vassort, 1975; Ransom, Barker & Nelson, 1975; Lassen, Pape & Vestergaard-Bogind, 1976; for reviews see Meech, 1976, Lew & Ferreira, 1977).

The existence in the pancreatic β-cell of a K-permeability affected by Ca^{2+} was first postulated by Matthews (1975). He suggested that the Ca^{2+} influx during the spike potential might stimulate K-permeability and thus limit further Ca^{2+} influx. This $[Ca^{2+}]_i$-activated P_K was proposed to be present in the β-cell membrane by Atwater & Beigelman (1976) to explain the transient hyperpolarization observed upon re-introduction of Ca^{2+} and Mg^{2+} after the removal of these divalent cations. Armando-Hardy, Ellory, Ferreira, Fleminger & Lew (1975) showed that in red cells the Ca^{2+}-activated P_K is blocked specifically by quinine and quinidine. Atwater & Beigelman (1976) observed that the silent phases between bursts are blocked by quinidine and thus suggested that $[Ca^{2+}]_i$ plays a dual role in β-cell function, being both stimulatory, by activation of granule and membrane fusion, and inhibitory, by activation of potassium permeability. Quinine was found to augment insulin release from perifused rat islets in the absence of glucose by Henquin, Horemans, Nenquin, Verniers & Lambert (1975). Quinine was used in this study to block P_K in the absence of glucose. The effects of quinine upon the electrical properties of the β-cell membrane were found to be similar to those of glucose described before (Atwater *et al.* 1979).

It has been shown that external application of mitochondrial inhibitors and uncouplers induces intracellular liberation of Ca^{2+} in giant nerve cells (Rojas & Hidalgo, 1968; Blaustein & Hodgkin, 1969). Several reports, in a variety of tissues, have indicated that mitochondrial inhibitors induce hyperpolarization and an increase in P_K (Godfraind, Krnjević & Pumain, 1970; Grabowski, Lobsiger & Luttgău, 1972; Gorman & McReynolds, 1974; Meech, 1974). Recently, A. C. Boschero (personal communication) measured an increase in Rb efflux from pancreatic islets in the presence of 1 mM-KCN. In this study, several mitochondrial inhibitors and uncouplers were tested as a means of increasing intracellular ionized Ca and stimulating P_K.

Quinine was used to block the P_K activated by intracellular ionized calcium in the presence and absence of mitochondrial inhibitors and uncouplers. The results are discussed in the light of a $[Ca^{2+}]_i$-activated P_K involved in the control of membrane potential in the absence of glucose and of the burst pattern in the presence of stimulatory levels of glucose.

METHODS

Partly dissected mouse islets were continuously perifused with a modified Krebs solution: 110 mM-NaCl; 25 mM-$NaHCO_3$; 5 mM-KCl; 2·5 mM-$CaCl_2$; 1·1 mM-$MgCl_2$; equilibrated with 95 % O_2/5 % CO_2, pH 7·5, and temperature 37 °C. Details of the experimental procedure can be found in earlier publications from this laboratory (Atwater & Beigelman, 1976; Atwater *et al.* 1978), Micro-electrodes with a tip resistance of about 200 MΩ and filled with 2 mM-K citrate were used. The membrane potential was measured between two Ag/AgCl electrodes,

one in the K citrate and the other in the external solution. Current was injected through the same micro-electrode used to measure membrane potential as described previously (Matthews & Sakamoto, 1975; Atwater *et al.* 1978). Agents were added without correction for osmotic pressure.

Quinine was added as quinine sulphate. Carbonyl-cyanide, *p*-tri-fluoromethoxyphenylhydrazone (FCCP) was obtained from Boeringer Corporation (London) and carbonyl-cyanide, m-chlorophenylhydrazone (CCCP) was obtained from the Sigma (London) Chemical Company. 2,4-dinitrophenol (DNP) and *o*-dinitrobenzene were recrystallized from water and ethanol, respectively.

RESULTS

Inhibition of $[Ca^{2+}]_i$-*activated* P_K *by quinine*

β-cells were identified by the characteristic burst pattern of electrical activity induced by continuous perifusion with Krebs solution containing 11·1 mM-glucose (Atwater & Beigelman, 1976; Meissner, 1976). Fig. 1 shows membrane potential and resistance measurements during one experiment. In order to monitor the resistance changes during the course of the experiment, rectangular pulses of current were injected through the micro-electrode at regular intervals, as shown below each

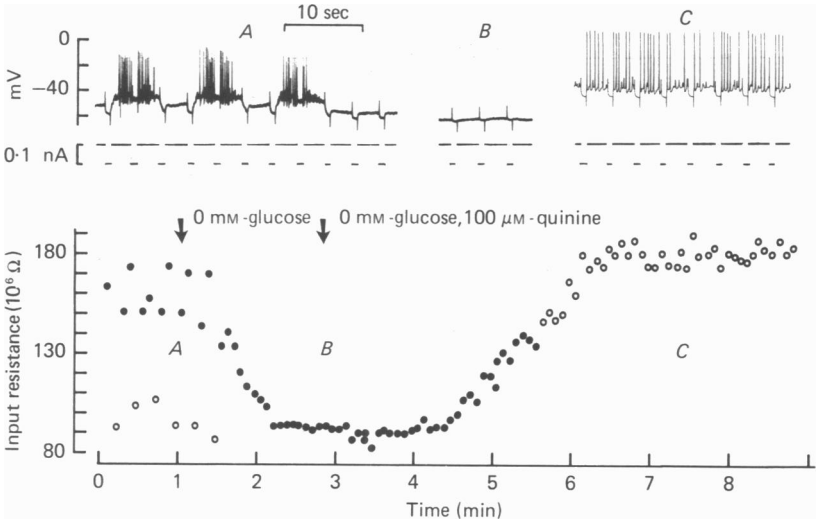

Fig. 1. Membrane potential and resistance changes induced by removal of glucose and addition of quinine. Experiment N.197. Upper part: portions of the membrane potential traces recorded continuously from a single β-cell in the presence of 11·1 mM-glucose (*A*), in the absence of glucose (*B*) and in the absence of glucose and presence of 100 μM-quinine (*C*). Traces show spike potentials in the presence of glucose and quinine. They also show voltage deflexions in the negative direction, due to the current injected through the micro-electrode. The size and duration of the current pulse (−0·1 nA) is shown below *A*, *B* and *C*; the pulse parameters were kept constant during the experiment. The same pulse with the micro-electrode in the solution outside the β-cell induced a positive deflexion of 4 mV. The lower portion of the figure represents a graph of the relative resistance changes during the experiment, as a function of time. Filled circles represent measurements during the silent phases, between bursts, and in the absence of glucose; open circles represent resistance measurements during the active phases. The rate of perifusion in this and the following experiments was 2·5 ml./min. The volume of the chamber was 0·3 cm^3 and the dead space less than 0·1 cm^3. Thus, the perifusion solution in the chamber was exchanged in about 10 sec from the time of switching solutions, indicated by the arrows over the graph.

segment of the voltage trace in the upper part of the figure. The graph in the lower part of Fig. 1 represents the calculated resistance changes as a function of time.

In the presence of 11·1 mM-glucose, the potential oscillates between a depolarized active state, during which time the input resistance is low, and a polarized silent phase, during which the input resistance is high (see *A* in the upper and lower parts of Fig. 1; open circles correspond to the active state and filled circles to the silent phases).

When glucose is removed from the perifusion solution, the membrane hyperpolarizes and the input resistance is decreased (see part *B* of Fig. 1; note that the deflexion in the voltage trace due to the pulse of current is practically nil, only the capacitative transients are still apparent).

Atwater *et al.* (1978) interpreted results like these to indicate an increased K permeability upon removal of glucose, since a decreased permeability to sodium or calcium, while an equally plausible explanation for the hyperpolarization, would be expected to lead to an increased membrane resistance.

In the experiment illustrated in Fig. 1, 100 μM-quinine was added to the perifusion solution when the membrane potential and resistance were stabilized after removal of glucose (second arrow in the lower part of Fig. 1). It can be seen that quinine, in the absence of glucose, depolarized the membrane, increased membrane resistance and, after three minutes, induced spike activity.

It should be noted that the membrane potential during quinine-induced electrical activity was depolarized with respect to the membrane potential during glucose-induced spike activity (compare part *A* to part *C* in Fig. 1). Membrane resistance, however, remained high during quinine-induced spike activity, comparable to the high membrane resistance measured towards the end of the silent phases (compare filled circles at beginning of lower trace to open circles at end in Fig. 1; also see Atwater *et al.* 1978). It follows that the drop in resistance during a normal glucose-induced burst (part *A* in Fig. 1) is not a prerequisite for the generation of spikes nor a direct consequence of them.

The experiment illustrated in Fig. 1 typifies the results found in seven cells from different islets and different mice: quinine induced depolarization and spikes in all cells tested. The spikes were different from glucose-induced spikes in that they were larger in amplitude and duration and did not occur in bursts. The time course of the frequency of quinine-induced spikes (not shown) did not follow a biphasic pattern, as do the glucose-induced spikes (Meissner & Atwater, 1976). The delay in the onset of electrical activity was variable, from 1·5 to 12 min.

Fig. 2 shows the voltage recordings from a cell during continuous perifusion with 0·1 mM-quinine. Glucose, absent from the perifusion medium during the first part of the trace, was added at the time indicated by the arrow above the record. It can be seen that glucose addition transiently inhibits spike activity, inducing a slight hyperpolarization.

In a previous paper (Atwater *et al.* 1978) some cells were reported to show a transient decrease in membrane resistance, accompanied by slight hyperpolarization, when switching from a glucose-free medium to one containing glucose; a transient increase in P_K was proposed to explain the observation. Recently, Malaisse, Boschero, Kawazu & Hutton (1978) have shown that there is a transient increase in Rb efflux

when switching from a glucose-free medium to one containing 16·6 mM-glucose. It would appear that the first effect of glucose on the β-cell membrane is to cause an increase in P_K which is quinine insensitive. It also seems that this transient effect is unmasked or even enhanced in the presence of quinine, possibly because, under these conditions, the membrane is depolarized.

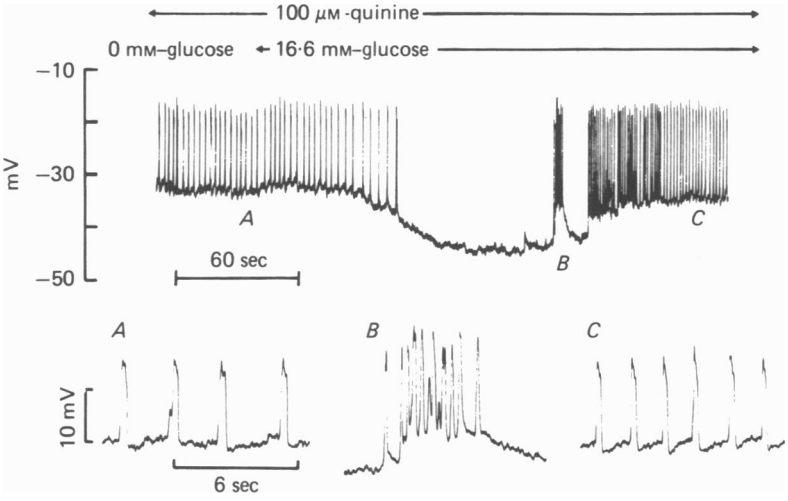

Fig. 2. Effects of glucose addition upon quinine-induced electrical activity. Upper part shows part of a continuous recording of the membrane potentials in the presence of 100 μM-quinine while switching from a glucose-free medium to one containing 16·6 mM-glucose. Lower parts show details of the spike characteristics with an expanded time scale; in the absence of glucose (*A*) two min after addition of 16·6 mM-glucose (*B*) and three min after addition of 16·6 mM-glucose (*C*). Experiment N145.

In the preceding paper we described an analogous experiment using TEA. No transient hyperpolarization was seen when switching from a glucose-free solution, or one containing 5·6 mM-glucose, to one containing 16·6 mM-glucose (Atwater *et al.* 1979). This may be an indication that the early transient increase in P_K is of a different nature to the Ca-activated P_K which is later inhibited.

When glucose is removed from the perifusion solution (records not shown), there is a transient enhancement of the spike frequency lasting about 2 min before returning to the slightly lower steady-state frequency.

In the steady state, glucose addition to the perifusion medium containing quinine only slightly increases the spike frequency. Fig. 3 shows spike frequency as a function of glucose before addition of quinine and during exposure to quinine (data taken from the same experiment as in Fig. 2). Steady-state spike frequency was reached about 5 min after switching solutions. In the presence of quinine the graded response of a single cell to glucose is almost eliminated (Meissner, 1976; Beigelman, Ribalet & Atwater, 1977).

The release of insulin from rat islets in 60 minute batch incubation experiments was shown to increase threefold between 5·6 and 16·0 mM-glucose in the presence of 100 μM-quinine (Henquin *et al.* 1975). While this may reflect a dissociation of spike frequency from insulin release, it should be mentioned that the experimental

conditions are not strictly comparable. The steady-state spike frequency presented in Fig. 3 was evaluated after 5–10 min; also electrical activity in mouse islets may be quite different from that in rat islets (Pace & Price, 1972). It is of interest that Meissner & Schmidt (1976), recording electrical potentials in islet cells from diabetic mice of the strain C57 BL/KsJ-db/db, observed a similar insensitivity to glucose from those cells (in the absence of quinine). In fact, Fig. 3 shows a striking similarity to their fig. 3 which contrasts spike frequency as a function of glucose in β-cells rom normal and diabetic mice. If the $[Ca^{2+}]_i$-activated P_K is in any way altered in

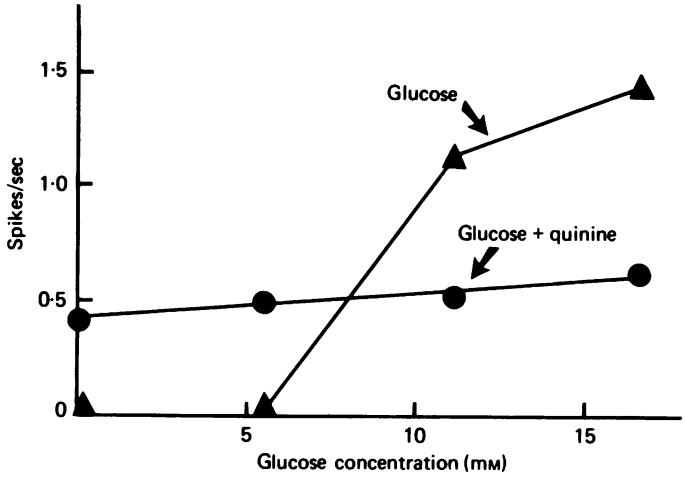

Fig. 3. Spike frequency dose–response to glucose in the same cell before and during treatment with 100 μM-quinine. Average spike frequency was calculated by counting the total number of spikes occurring during several minutes and dividing by the total time. In the presence of quinine, the steady-state spike frequency in this cell was reached within 3 min. Same experiment as in Fig. 2 (N145).

diabetic mice, this could explain the lack of sensitivity to glucose and the shortened or non-existent silent phases in the electrical activity recorded in islet cells from diabetic mice.

The effects of quinine were not completely reversible in that the membrane potential during the silent phases between bursts and after removal of glucose (for example, see Fig. 6, last part) tended to be less polarized. This result is in agreement with the earlier observation that recovery was only partial after treatment with quinidine (Atwater & Beigelman, 1976). While quinine and quinidine specifically block the $[Ca^{2+}]_i$-activated P_K in red cells, Meech (1974) reported no such effect of the drug on snail neurones. Since membrane resistance was increased by quinine, it seems unlikely that the effects reported here were due to an increased sodium permeability. Henquin *et al.* (1975) also ruled out the possibility that quinine non-specifically augmented β-cell membrane leakage by experiments carried out at low temperature.

Stimulation of P_K by increased $[Ca^{2+}]_i$

Mitochondrial uncouplers, DNP, CCCP and FCCP and electron transfer inhibitors, NaN_3 and KCN, are known to cause release of Ca^{2+} from isolated mitochondria (see review by Bygrave, 1978). They have also been shown to induce a marked

release of Ca^{2+} intracellularly (Rojas & Hidalgo, 1968; Blaustein & Hodgkin, 1969). Figs. 4, 5, 6 and 7 show results of experiments using these inhibitors in order to test the effects of an increase in $[Ca^{2+}]_i$ upon P_K in the pancreatic β-cell.

Membrane potentials recorded during an experiment in which 0·25 mM-DNP was added to the perifusing solution in the presence of 11·1 mM-glucose are shown in Fig. 4. As can be seen, electrical activity ceases in the presence of DNP and the membrane hyperpolarizes. The inhibition is reversed upon removal of DNP, the bursts of electrical activity following a biphasic pattern essentially identical to that recorded from cells during removal and reintroduction of glucose (Meissner & Atwater, 1976).

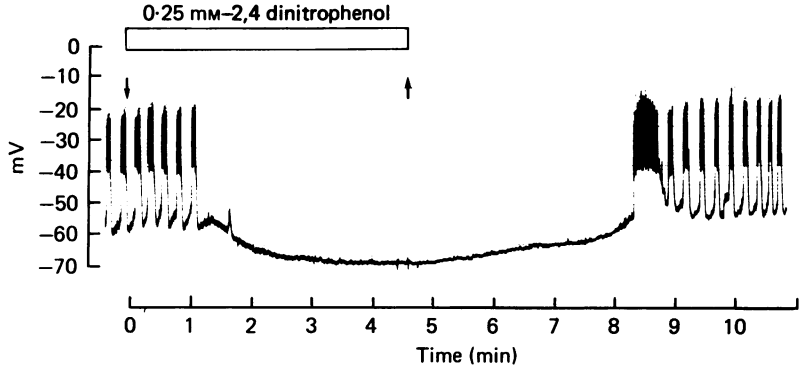

Fig. 4. Effects of DNP on membrane potentials in the presence of 11·1 mM-glucose. DNP was added to the perifusion solution at the first arrow and removed at the second arrow. Experiment N122.

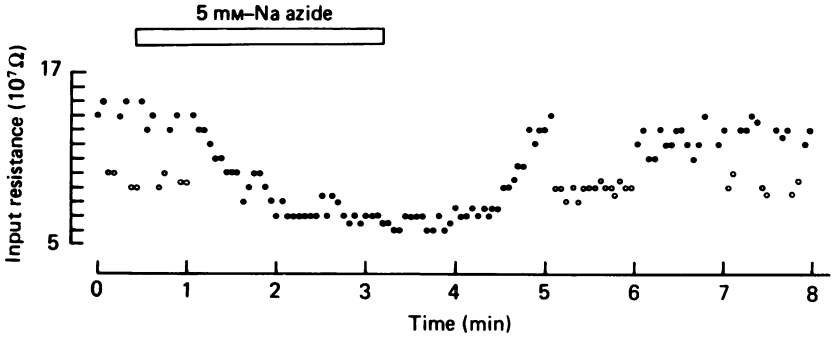

Fig. 5. Effects of azide on relative resistance measurements in the presence of 11·1 mM-glucose. Input resistance calculated as in Fig. 1. Filled circles represent measurements during the silent phases and during azide-induced inhibition of electrical activity; open circles represent measurements made during the active phases. Experiment N122c. The islet was perifused with Krebs solution plus 5mM-azide for the length of time indicated above.

Several other mitochondrial inhibitors and uncouplers were tested in the presence of 11·1 mM-glucose: KCN (1 mM), NaN_3 (5 mM), CCCP (100 μM) FCCP (1 μM). These inhibitors had the same effects as DNP. Each inhibitor was tested several times in individual cells and in cells of at least two islets from different mice. When tested in the same cell 5 mM-NaN_3 blocked electrical activity in 30 sec, 1 mM-KCN in 45 sec and 0·25 mM-DNP in 60 sec; there was complete recovery between each

test period. However, the recovery time depended on the length of time that the islet had been exposed to the inhibitor. Recovery time also appeared to be dependent upon the level of glucose present. This is illustrated in Fig. 7 and will be discussed later.

During most of the experiments, pulses of current were injected at regular intervals in order to monitor changes in input resistance, as in the experiment described by Fig. 1. The resistance changes during application of 5 mM-NaN_3 in the presence of 11·1 mM-glucose are presented in Fig. 5. Filled circles represent measurements taken during the silent phases between bursts and during the inhibition of activity by NaN_3 and consequent hyperpolarization. Open circles represent measurements taken during electrical activity. The results obtained with the other inhibitors were essentially the same as those shown in Fig. 5 using NaN_3.

Some reports indicate that mitochondrial inhibitors may have a direct action on the cell membrane (Liberman & Topaly, 1968). Since DNP, CCCP and FCCP are weak acids which also induce a substantial increase in conductivity of black lipid films, the conducting ions being the weak acids (Lea & Croghan, 1969), one has to establish that the observed effect is not at the level of the β-cell membrane. Benzoic acid is reported to have a similar effect on lipid bilayers as the mitochondrial inhibitors, but, as it is an impermeant anion, it is not likely to affect mitochondrial membranes when added to the perifusing medium. In several experiments, 1, 2 and 4 mM-benzoic acid was added to the perifusion medium containing 11·1 mM-glucose, with no effects observed. Croghan & Lea (1972) showed that membranes could be made permeable to benzoate by addition of *o*-dinitrobenzene (see also Kubel, 1974). In four experiments 0·5 and 1 mM-*o*-dinitrobenzene was observed to have no inhibitory effect on electrical activity in the presence of glucose. However, when 1 mM-benzoic acid was added to the perifusion medium together with 0·5 mM-*o*-dinitrobenzene, the membrane was hyperpolarized and electrical activity was blocked (as in the presence of the previously tested mitochondrial inhibitors). Thus, it seems unlikely that the inhibitors tested induced the large hyperpolarization seen in Fig. 4 by their effect upon membrane properties but rather by their effect upon mitochondrial function. The decrease in membrane resistance during exposure to mitochondrial inhibitors suggests that the hyperpolarization of the membrane, and resultant inhibition of electrical activity, is due to an increase in potassium permeability. Tetraethylammonium, TEA, specifically blocks P_K in nerve (Armstrong & Binstock, 1965; also see preceding paper by Atwater *et al.* 1979). However, 20 mM-TEA when added to the perifusion medium did not counteract the effects of mitochondrial inhibitors (results not shown).

Quinine, a specific blocking agent of Ca-activated P_K, blocked or greatly reduced the effects of mitochondrial inhibitors. Fig. 6 shows the voltage records from an experiment in which 1 μM-FCCP, the most potent mitochondrial inhibitor used in this study, was added in the presence of 11·1 mM-glucose. When the burst pattern had recovered after removal of FCCP, quinine was added to the perifusion medium. After 9 min, when the silent phases between bursts had been blocked, 1 μM-FCCP was again added, this time in the presence of glucose and quinine. No hyperpolarization was observed and spike activity continued for about 6 min, as compared to 15 sec in the absence of quinine. This experiment suggests that the potassium per-

meability which is increased in the presence of mitochondrial inhibitors is blocked by quinine.

Henquin (1978) observed that the glucose-induced reduction in $^{42}K^+$ efflux was maximal at 20 mM-glucose. Membrane potential dependence upon $[K^+]_o$ has also been shown to be minimal at 22·2 mM-glucose (Atwater *et al.* 1978). High concentrations of glucose block the silent phases between bursts and generate continuous spike activity (Dean & Matthews, 1970; Meissner & Schmelz, 1974; Beigelman *et al.* 1977). Thus, perifusion with glucose above 20 mM appears effectively to minimize P_K. In one experiment, 5 mM-NaN_3 was added to the perifusion solution first in the

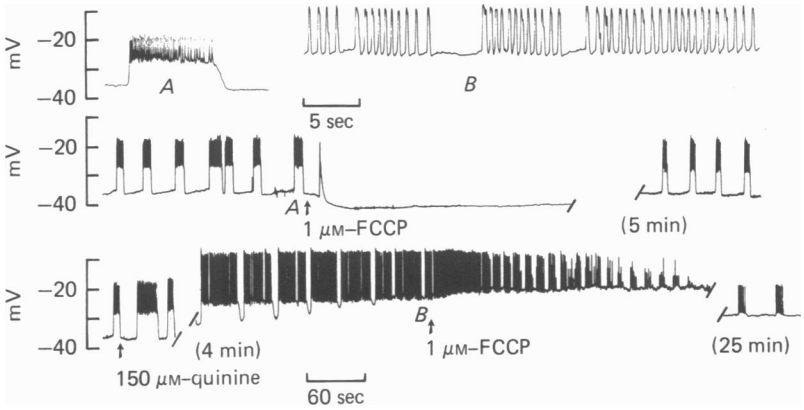

Fig. 6. Reversal of FCCP-induced inhibition of electrical activity by quinine. The upper portion of the figure shows details of spikes during a normal burst stimulated by 11·1 mM-glucose (*A*) and during perifusion with 11·1 mM-glucose plus 100 μM-quinine (*B*) taken from the continuous recording as indicated below the lower traces. The middle trace shows the burst pattern characteristic of this cell in 11·1 mM-glucose and the effects of addition of 1 μM-FCCP for 1·5 min (arrow below trace indicates time at which solution was switched to one containing FCCP). The trace was interrupted for 5 min; the end of the middle trace shows the recording of the burst pattern after removal of FCCP. The lower trace shows the effects of addition of 150 μM-quinine to the glucose-containing perifusion medium. The depolarization was gradual; the trace has been interrupted for 4 min. 9 min after addition of quinine, 1 μM-FCCP was added as indicated by the arrow (just after *B*); quinine and FCCP were both removed after 10 min. After 6 min, no more spikes were detected and the membrane potential depolarized by about 5 mV. The trace was again interrupted for 25 min; at end of the lower trace, the burst pattern is shown to have partially recovered. Experiment N139.

presence of 11·1 mM-glucose, then 22·2 mM-glucose in the same cell. The voltage records from this experiment are presented in Fig. 7. It can be seen that NaN_3 was equally effective in hyperpolarizing the membrane in the presence of both glucose concentrations, taking just over 10 sec in both cases. It would seem unlikely, then, that glucose acts directly on the β-cell membrane to reduce the $[Ca^{2+}]_i$-activated P_K.

Another observation to be made from the data presented in Fig. 7 is that the recovery of activity after removal of azide took considerably longer in the presence of 22·2 mM-glucose, although the inhibitor was present for the same time in both cases, 70 sec. In 22·2 mM-glucose the rate of calcium entry into the β-cell during the spike activity is higher than in 11·1 mM. In the former case the mitochondrial loading of calcium would be facilitated by the presence in the cytoplasm of higher

levels of calcium and glucose. Thus, in 22·2 mM-glucose, on addition of the inhibitor the intracellular Ca released from the mitochondria would be correspondingly greater. The duration of the silent phase perhaps represents then the time required to reduce the cytoplasmic calcium concentration to its level before the addition of NaN_3.

In the absence of glucose, the membrane potential was found to depend strongly upon the extracellular potassium concentration, $[K^+]_o$ (Atwater *et al.* 1978), the experimental points best fitting a curve calculated to fit the constant field equation when the ratio P_K/P_{Na} was taken to be about 70. In two experiments, the mem-

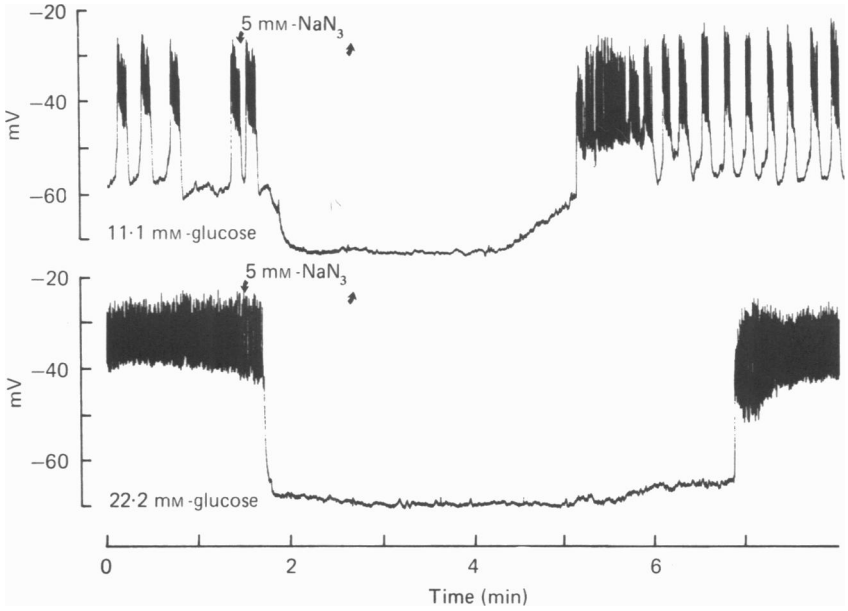

Fig. 7. Effects of azide on electrical activity stimulated by 11·1 and 22·2 mM glucose. Upper trace shows the burst pattern induced by 11·1 mM-glucose. 5 mM-NaN_3 was introduced for 70 sec, as indicated by the arrows. Lower trace shows continuous activity induced by 22·2 mM-glucose (from the same cell). 5 mM-NaN_3 was perifused for 70 sec, as shown by the arrows. Experiment N125.

brane potential dependence upon $[K^+]_o$ was measured in the glucose-free medium before and during exposure to 1 mM-KCN. There was no change in the measured potentials at $[K^+]_o > 8$ mM and only a slight increase of 2 or 3 mV in the potentials between $[K^+]_o < 0{\cdot}1$ mM and $[K^+]_o = 8$ mM was observed.

This observation is confirmed in recent results by A. C. Boschero (personal communication) showing that 1 mM-KCN enhanced Rb^+ efflux from isolated rat islets nearly fourfold in the presence of 16·7 mM-glucose and only twofold in the absence of glucose. These observations indicate that the Ca^{2+} liberated from mitochondria by KCN in the absence of glucose may be less than in the presence of glucose, thus allowing the cell to pump the extra Ca^{2+} out more easily. Alternatively, Simons (1975) found in red cells that the $[Ca^{2+}]_i$-induced increase in K^+ fluxes was saturated at $[Ca^{2+}]_i = 3\ \mu M$; thus the $[Ca^{2+}]_i$-activated P_K in the β-cell may have similar saturation characteristics and may already be near maximal in the absence of glucose.

DISCUSSION

The experiments reported here provide evidence for the existence in the β-cell membrane of a potassium permeability which has properties of the $[Ca^{2+}]_i$-activated P_K described in other tissues (see Introduction for references). There are two main observations to support this: (1) quinine, known to block $[Ca^{2+}]_i$-activated P_K in other systems, depolarizes the β-cell membrane while increasing the input resistance, and (2) mitochondrial inhibitors, known to raise intracellular Ca^{2+} and to stimulate P_K in other systems, hyperpolarize the β-cell membrane while lowering the input resistance. The similarities between exposure to glucose and exposure to quinine and also between removal of glucose and application of mitochondrial inhibitors indicate the existence of a $[Ca^{2+}]_i$-activated P_K in the β-cell membrane. This permeability, high in the absence of glucose and inhibited in the presence of glucose, can account for the observed changes in membrane potential, resistance and permeability to K.

Inhibition of the $[Ca^{2+}]_i$-activated P_K has been attributed to two different mechanisms in other systems: (1) a decrease in the intracellular concentration of Ca^{2+}, and (2) a change in affinity for Ca^{2+} at the gating site. In *Helix aspersa* neurones, Meech (1974) found that membrane resistance depends on $[Ca^{2+}]_i$ in an exponential fashion, being unaffected at $[Ca^{2+}]_i = 10^{-7}$ M, decreasing by 25% at 9×10^{-5} M and by 90% at $1{\cdot}5 \times 10^{-7}$ M. Atwater *et al.* (1978) measured a 30% decrease in input resistance upon removal of glucose. This could indicate an increase in $[Ca^{2+}]_i$ of nearly one order of magnitude in the β-cell upon removal of glucose.

In human red cells, Lew & Ferreira (1976) found that, depending on the conditions used during the preparation of the cells, the range of $[Ca^{2+}]_i$ which effectively enhanced P_K varied over about an order of magnitude. They concluded that the 'heterogeneous behaviour of ATP-depleted red cell populations in relation to the Ca-induced K flux can be interpreted as resulting from a variable Ca affinity of the K gate' and suggested that changes between high-affinity and low-affinity configurations might be mediated by cellular control mechanisms. In this way, the effects of glucose on the β-cell membrane could be due to a metabolically controlled shift in the affinity for Ca^{2+} at the K gating site. Thus a high concentration of glucose would induce the low affinity configuration and removal of glucose would induce the high affinity configuration. With the data available, it is impossible to establish which of the two mechanisms may be responsible for the inactivation of P_K in the β-cell upon exposure to glucose, and in fact, both may be operative.

DNP has been shown to have two distinct effects on membranes. (i) It increases the permeability to protons in artificial lipid membranes (Liberman & Topaly, 1968), and in mitochondria (Hanstein & Hatefi, 1974) and (ii), it increases the permeability to K ions. The latter effect has been interpreted in two ways. First, because of its mitochondrial effects, the ATP level of the cytoplasm is reduced and the calcium is augmented. This increase in Ca switches on a K permeability (Gardos, 1958). Secondly, DNP stimulates a K permeability increase directly (Haas, Kern & Heinwachter, 1970).

Mitochondrial inhibitors, besides increasing $[Ca^{2+}]_i$ have been observed to lower rapidly the ATP content of islet cells (Ashcroft, Weerasinghe & Randle, 1973). In

order to distinguish between the effects of mitochondrial inhibitors on Ca liberation from mitochindria and ATP depletion in the cell, quinine was used to block the effects of Ca upon P_K. As depicted by the experiment illustrated in Fig. 6, quinine reversed the hyperpolarization and rapid blockage of electrical activity upon addition of FCCP to the perifusion medium. Thus, it seems probable that at least the first action of mitochondrial inhibitors is due to activation of P_K by the induced increase in intracellular Ca^{2+} rather than any reduction in ATP. In the presence of quinine, the first action of FCCP was to induce slight depolarization, enhancing spike frequency. The subsequent cessation of electrical activity, after 5 min in the continued presence of FCCP and quinine, could be explained by inactivation of P_{Ca}, as described previously (Atwater *et al.* 1978), but could also be due to ATP depletion in the cell.

From the observations made in the presence of quinine and earlier with quinidine, the $[Ca^{2+}]_i$-activated P_K appears to play an important role not only in the initial depolarization induced by glucose, in the recognition of a range of stimulatory glucose levels and in the determination of the glucose threshold level for activation of electrical activity, but also in the burst pattern of electrical activity and in the generation of the biphasic response (Atwater & Beigelman, 1976). Thus, if the membrane potential recorded in the presence of quinine represents the potential of the β-cell when $[Ca^{2+}]_i$-activated P_K is completely blocked, then the depolarization during the active phase of the burst may indicate that P_K is inhibited in order to induce a depolarization sufficient to stimulate electrical activity.

If this is the case, then the drop in input resistance during the active phase of glucose-induced bursts may be due to an increased membrane area due to coupling of the cells (this possibility is discussed in Atwater *et al.* 1978).

The repolarization during the silent phase of the burst may be due to an increased P_K, in turn due to an increased $[Ca^{2+}]_i$ resultant from the spike activity. Thus, the burst pattern may be due to an oscillation in $[Ca^{2+}]_i$ near the membrane. The gradual response to glucose could be explained in terms of an increasing buffering power for Ca^{2+} in the cell or to a gradual change in the affinity for Ca^{2+} at the K gate in the cell membrane.

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REFERENCES

ARMANDO-HARDY, M., ELLORY, J. C., FERREIRA, M. G., FLEMINGER, S. & LEW, V. L. (1975). Inhibition of the calcium-induced increase in the potassium permeability of human red blood cells by quinine. *J. Physiol.* **250**, 32–33*P*.

ARMSTRONG, C. M. & BINSTOCK, L. (1965). Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. gen. Physiol.* **48**, 859–872.

ASHCROFT, S. J. H., WEERASINGHE, L. C. C. & RANDLE, P. J. (1973). Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. *Biochem. J.* **132**, 223–231.

ATWATER, I. & BEIGELMAN, P. M. (1976). Dynamic characteristics of electrical activity in pancreatic β-cells. Effects of calcium and magnesium. *J. Physiol., Paris* **72**, 769–786.

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.* **278**, 117–139.

ATWATER, I., RIBALET, B. & ROJAS, E. (1979). Mouse pancreatic β-cells: tetraethylammonium blockage of the potassium permeability increase induced by depolarization. *J. Physiol.* **288**, 561–574.

BEIGELMAN, P. M., RIBALET, B. & ATWATER, I. (1977). Electrical activity of mouse pancreatic β-cells. II. Effects of glucose and arginine. *J. Physiol., Paris* **73**, 201–217.

BLAUSTEIN, M. P. & HODGKIN, A. L. (1969). The effect of cyanide on the efflux of calcium from squid axons. *J. Physiol.* **200**, 497–527.

BOSCHERO, A. C., KAWAZU, S., DUNCAN, G. & MALAISSE, W. J. (1977). Effect of glucose on potassium ion handling by pancreatic islets. *FEBS Lett.* **83**, 151–154.

BYGRAVE, F. L. (1978). Mitochondria and the control of intracellular calcium. *Biol. Rev.* **53**, 43–79.

CROGHAN, P. C. & LEA, E. J. A. (1972). On the mechanism of uncoupling of oxidative phosphorylation. *J. Physiol.* **224**, 32–33*P*.

DEAN, P. M. & MATTHEWS, E. K. (1970). Glucose-induced electrical activity in pancreatic islet cells. *J. Physiol.* **210**, 225–264.

GARDOS, G. (1958). The function of calcium in the potassium permeability of human erythrocytes. *Biochim. biophys. Acta* **30**, 653–654.

GODFRAIND, J. M., KRNJEVIČ, K. & PUMAIN, R. (1970). Unexpected features of the action of dinitrophenol on cortical neurones. *Nature, Lond.* **228**, 562–564.

GORMAN, A. L. F. & MCREYNOLDS, J. S. (1974). Control of membrane K^+ permeability in a hyperpolarizing photoreceptor: similar effects of light and metabolic inhibitors. *Science, N.Y.* **185**, 620–621.

GRABOWSKI, W., LOBSIGER, E. A. & LUTTGÄU, J. CH. (1972). The effect of repetitive stimulation at low frequencies upon the electrical and mechanical activity of single muscle fibres. *Pflügers Arch.* **334**, 222–239.

HANSTEIN, W. G. & HATEFI, Y. (1974). Trinitrophenol a membrane impermeable uncoupler of oxidative phosphorylation. *Proc. natn. Acad. Sci. U.S.A.* **71**, 288–292.

HAAS, H. G., KERN, R. & HEINWACHTER, M. M. (1970). Electrical activity and metabolism in cardiac tissue: an experimental and theoretical study. *J. membrane Biol.* **3**, 180–209.

HENQUIN, J. C. (1977). Tetraethylammonium potentiation of insulin release and inhibition of rubidium efflux in pancreatic islets. *Biochem. biophys. Res. Commun.* **77**, 551–556.

HENQUIN, J. C. (1978). D-glucose inhibits potassium efflux from pancreatic islet cells. *Nature, Lond.* **271**, 271–273.

HENQUIN, J. C., HOREMANS, B., NENQUIN, M., VERNIERS, J. & LAMBERT, A. E. (1975). Quinine-induced modifications of insulin release and glucose metabolism by isolated pancreatic islets. *FEBS Lett.* **67**, 371–374.

KRNJEVIČ, K. & LISIEWICZ, A. (1972). Injections of calcium ions into spinal motoneurones. *J. Physiol.* **225**, 363–390.

KUBEL, M. (1974). Carrier-mediated transport across black lipid membranes: Resistance of spherical films in the presence of derivatives of phenol and benzoic acid. *Ber. Bunsen-Gesell.* **78**, 548–553.

LASSEN, U. V., PAPE, L. & VESTERGAARD-BOGIND, B. (1976). Effect of calcium on the membrane potential of *Amphiuma* red cells. *J. membrane Biol.* **26**, 51–70.

LEA, E. J. A. & CROGHAN, P. C. (1969). The effect of 2,4-dinitrophenol on the properties of thin phospholipid films. *J. membrane Biol.* **1**, 225–237.

LEW, V. L. (1970). Effect of intracellular calcium on the potassium permeability of human red cells. *J. Physiol.* **206**, 35–36*P*.

LEW, V. L. & FERREIRA, H. G. (1976). Variable calcium sensitivity of a potassium-selective channel in intact red cell membranes. *Nature, Lond.* **263**, 336–338.

LEW, V. L. & FERREIRA, H. G. (1977). The effect of calcium on potassium permeability of red cells. In *Membrane Transport in Red Cells*, ed. ELLORY, J. C. & LEW, V. L., pp. 93–100. London: Academic.

LIBERMAN, E. A. & TOPALY, V. P. (1968). Selective transport of ions through bimolecular phospholipid membranes. *Biochim. biophys. Acta* **163**, 125–136.

MALAISSE, W. J., BOSCHERO, A. C., KAWAZU, S. & HUTTON, J. C. (1978). The stimulus secretion coupling of glucose-induced insulin release XXVII. Effect of glucose on K^+ fluxes in isolated islets. *Pflügers. Arch.* **373**, 237–242.

MATTHEWS, E. K. (1975). Calcium and stimulus-secretion coupling in pancreatic islet cells. In *Calcium Transport in Contraction and Secretion*, ed. CARAFOLI, E., pp. 203–210. London: North-Holland Publishing Co.

MATTHEWS, E. K. & SAKAMOTO, Y. (1975). Electrical characteristics of pancreatic islet cells. *J. Physiol.* **246**, 421–437.

MEECH, R. W. (1974). The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J. Physiol.* **237**, 259–277.

MEECH, R. W. (1976). Intracellular calcium and the control of membrane permeability. In *SEB Symposium, Calcium in Biological Systems*, pp. 161–191. Cambridge, University Press.

MEISSNER, H. P. (1976). Electrical characteristics of the β-cells in pancreatic islets. *J. Physiol., Paris* **72**, 757–767.

MEISSNER, H. P. & ATWATER, I. (1976). The kinetics of electrical activity of β-cells in response to a 'square wave' stimulation with glucose or glibenclamide. *Hormone Metab. Res.* **8**, 11–16.

MEISSNER, H. P. & SCHMELZ, H. (1974). Membrane potential of β-cells in pancreatic islets. *Pflügers. Arch.* **351**, 195–206.

MEISSNER, H. P. & SCHMIDT, H. (1976). The electrical activity of pancreatic β-cells of diabetic mice. *FEBS Lett.* **67**, 371–374.

PACE, C. S. & PRICE, S. (1972). Electrical responses of pancreatic islet cells to secretory stimuli. *Biochem. biophys. Res. Commun.* **46**, 1557–1563.

RANSOM, B. R., BARKER, J. L. & NELSON, P. G. (1975). Two mechanisms for post stimulus hyperpolarizations in cultured mammalian neurones. *Nature, Lond.* **256**, 424–425.

ROJAS, E. & HIDALGO, C. (1968). Effect of temperature and metabolic inhibitors on ^{45}Ca outflow from squid giant axons. *Biochim. biophys. Acta* **163**, 550–556.

SEHLIN, J. & TÄLJEDAL, I-B. (1975). Glucose-induced decrease in Rb^+ permeability in pancreatic β-cells. *Nature, Lond.* **253**, 635–636.

SIMONS, T. J. B. (1975). Resealed ghosts used to study the effect of intracellular calcium ions on the potassium permeability of human red cell membranes. *J. Physiol.* **246**, 52–54*P*.

VASSORT, G. (1975). Voltage-clamp analysis of transmembrane ionic currents in guinea-pig myometrium: evidence for an initial potassium activation triggered by calcium influx. *J. Physiol.* **252**, 713–734.