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COOLING DISSOCIATES GLUCOSE-INDUCED INSULIN RELEASE FROM ELECTRICAL ACTIVITY AND CATION FLUXES IN RODENT PANCREATIC ISLETS

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SUMMARY

1. Insulin release and β-cell membrane potentials in response to glucose at 37 and 27 °C have been measured simultaneously in single, micro-dissected, perifused islets of Langerhans from normal mice.

2. Insulin release and ^{45}Ca outflow in response to glucose at 37 and 27 °C have been measured simultaneously from perifused islets isolated by collagenase digestion from normal rats.

3. The effect of cooling on β-cell membrane potassium permeability was assessed by changes in measured membrane potential and input resistance (in the mouse) and by changes in ^{86}Rb outflow (in the rat).

4. Resting and active β-cell membrane parameters (i.e. membrane potential, spike frequency, input resistance, ^{45}Ca outflow and ^{86}Rb outflow), in both mouse and rat islets, were affected only slightly by cooling to 27 °C, with temperature coefficients of 2 or lower.

5. At 27 °C glucose-stimulated insulin release was inhibited completely in mouse islets and almost completely in rat islets. The temperature coefficients in both preparations were greater than 5.

6. It is concluded that β-cell electrical activity and changes in membrane permeability induced by glucose are not consequences of insulin release.

INTRODUCTION

Since electrical activity was first measured in cells from islets of Langerhans (Dean & Matthews, 1968), there has been speculation as to whether the action potentials, or spikes, represent an early event in stimulus–secretion coupling, i.e. calcium influx (Dean & Matthews, 1970; Pace, Stillings, Hover & Matschinsky, 1975; Matthews & Sakamoto, 1975; Atwater, Ribalet & Rojas, 1978; Ribalet & Beigelman, 1980), or whether they result from the exocytotic fusion of the granule with the cell membrane,

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i.e. incorporation of membrane material with different properties or simple leak current at the instant of fusion (Matthews & Dean, 1970; Meissner & Schmelz, 1974; Meissner, 1976; Meissner & Preissler, 1980). A large number of studies have been performed which illustrate the similarities between electrical activity and insulin release (Dean & Matthews, 1970; Meissner & Atwater, 1976; Meissner, 1976; Frankel, Atwater & Grodsky, 1981) but in all cases insulin release and electrical activity were measured in different laboratories. Differences in methodology and cross-comparison between data taken from mouse islets (electrical) and rat islets (insulin release) make such studies difficult to interpret.

The measurement of radioactive tracer movements in islets has been used to assess the participation of changes in membrane ion permeability in the process of insulin release; although such measurements represent unidirectional ion movements, they nevertheless have shown a close parallel with direct membrane potential measurements. Methods for the simultaneous measurement of radioactive tracer movements and insulin release have been in use for some time (e.g. Malaisse, Brisson & Baird, 1973) but a method for the simultaneous measurement of electrical activity and insulin release has been described only recently (Scott, Atwater & Rojas, 1981). The experiments presented here include simultaneous measurements of electrical activity and insulin release from single micro-dissected mouse islets and these results are compared with simultaneous measurements of ion fluxes and insulin release performed on rat islets prepared by collagenase digestion. Using insulin release as a common denominator, this approach allows comparison between electrical activity in mouse islets with ion fluxes from rat islets.

The present study shows that by lowering the temperature from 37 to 27 °C, insulin release can be dissociated from glucose-induced electrical activity and stimulated calcium outflow, as well as from glucose-induced inhibition of potassium permeability.

METHODS

Albino mice and rats of both sexes were used in these experiments. Animals had access to food and water until they were killed by decapitation just before beginning each experiment.

The modified Krebs solution used during the experiments on mouse islets contained (mM): NaCl, 120; $NaHCO_3$, 25; KCl, 5; $CaCl_2$, 2·5; and $MgCl_2$, 1·1. The solutions were equilibrated with 95 % O_2/5 % CO_2 at 37 °C and were cooled to 27 °C using an electronically controlled Peltier element at the perifusion chamber inlet; this arrangement prevented changes in the pH (HCO_3^- remained constant at 25 mM throughout). The temperature of the chamber was monitored continuously using a microthermistor placed next to the islet. Except where indicated in the Figures, the temperature did not vary more than 0·3 °C.

The methods used for making electrical measurements from single micro-dissected mouse islets of Langerhans have been described previously (Atwater *et al.* 1978). The methods used for measuring the time course of insulin release from single micro-dissected mouse islets have been described elsewhere (Scott *et al.* 1981). In the experiments illustrated in Figs. 1, 3 and 4, a single large mouse islet was placed in the chamber. The membrane potential from a β cell in that islet was monitored continuously throughout the experiment and was recorded on one channel of an analog magnetic tape recorder (Racal Thermionic, Store 4). On a separate channel, an event marker was used to mark the period of collections of the perifusate (either 1 min or 20 s). The immunoreactive insulin (IRI) was measured in duplicate samples of the perfusate and the mean value was plotted below the corresponding electrical record on the print-out. The insulin release is represented as dots, plotted at the centre of the collection period, joined by straight lines.

The solutions used for the experiments on rat islets contained (mM): NaCl, 115; $NaHCO_3$, 24; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1, and were continuously equilibrated with 95% O_2/5% CO_2. The methods used for the measurement of ^{86}Rb and ^{45}Ca outflow and insulin release from isolated rat pancreatic islets have been described previously (Herchuelz & Malaisse, 1978; Lebrun, Malaisse & Herchuelz, 1981). After loading with ^{86}Rb or ^{45}Ca in the presence of 16·7 mM-glucose at 37 °C, the islets were placed in a perifusion chamber in a water bath at either 37 or 27 °C. The solutions used during rat islet perifusions were equilibrated at either 37 or 27 °C and the pH adjusted accordingly.

The term 'temperature coefficient' as used here is the ratio of the measured parameter at 37 °C/the measured parameter at 27 °C.

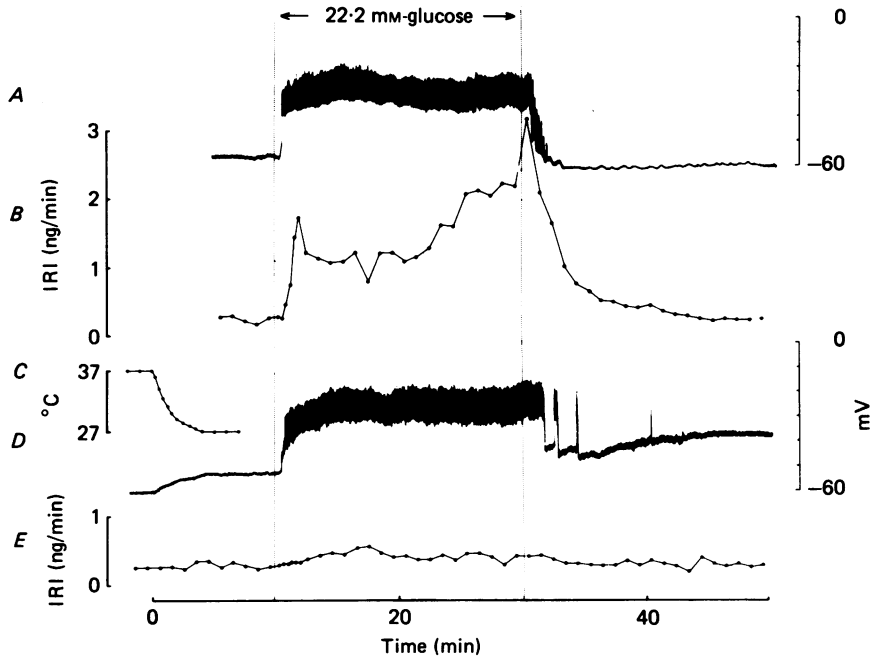

Fig. 1. Membrane potentials, electrical activity and insulin release measured in a single islet exposed to glucose at 37 and 27 °C. *A*, membrane potentials at 37 °C in response to a change from 5·6 to 22·2 mM-glucose during the period indicated at the top of the Figure (vertical calibrations are given at the right). *B*, immuno-reactive insulin (IRI) release from the same islet (vertical calibrations are given at the left). *C*, temperature in the bath near the islet during the change from 37 to 27 °C (vertical calibrations are given at the left). *C* correlates with the traces shown in *D* and *E*. *D*, membrane potentials in response to a change in temperature from 37 to 27 °C and a change from 5·6 to 22·2 mM-glucose at 27 °C (vertical calibrations are given at the right). *E*, IRI release at 27 °C (vertical calibrations at the left).

RESULTS

Effects of temperature on glucose-induced electrical activity and insulin release from mouse islets

Single mouse islets of Langerhans were perifused with modified Krebs solution. Membrane potentials were recorded from one of the β cells of the islet while the perifusate was collected and later assayed for insulin content.

Fig. 1 shows the effect of changing the glucose concentration from 5·6 to 22·2 mM

and back to 5·6 mM at 37 and 27 °C. The upper half of Fig. 1 shows the membrane potential (*A*) and insulin release (*B*) before, during and after a 20 min pulse of 22·2 mM-glucose when the temperature of the perifusing solution was 37 °C; the lower half of the Figure shows the membrane potential (*D*) and insulin release (*E*) measured from the same islet when a 20 min pulse of 22·2 mM-glucose was applied after the temperature was lowered to 27 °C, as indicated by the temperature scale (*C*).

At 37 °C, the change from 5·6 to 22·2 mM-glucose resulted in a depolarization of the membrane from −55 mV to −30 mV within 1 min. Constant spike activity was seen throughout the 22·2 mM-glucose pulse. Following the change back to 5·6 mM-glucose, continuous spike activity was maintained for 1 min and then bursts of electrical activity were seen for a further minute as the membrane repolarized to −60 mV. At 37 °C, the rate of insulin release had increased above basal levels within 3 min of the change to 22·2 mM-glucose. After 15 min in high glucose the rate of insulin release was 9 times greater than the basal rate. The change back to 5·6 mM-glucose resulted in a transient increase in insulin output, after which the release gradually fell to basal level with a time constant of 5 min.

A decrease in temperature from 37 to 27 °C in 5·6 mM-glucose lowered the membrane potential from −60 to −50 mV with no change in the basal level of insulin release. When the glucose concentration was raised to 22·2 mM at 27 °C there was little or no increase in insulin output from the islet. This observation contrasts with the apparent lack of effect of the lower temperature on β-cell electrical activity. When the glucose concentration was raised to 22·2 mM at 27°C the membrane depolarized further, and within 1 min of the glucose elevation constant spike activity was observed. Upon switching back to 5·6 mM-glucose, the continuous spike activity persisted for 2 min before the membrane repolarized. Occasional bursts of electrical activity were seen up to 11 min after the return to 5·6 mM-glucose.

Effects of cooling on glucose-stimulated ^{45}Ca outflow and insulin release from rat islets

Electrical activity has been demonstrated to involve an increase in Ca conductance of the β-cell membrane (Matthews & Sakamoto, 1975; Atwater *et al.* 1978; Ribalet & Beigelman, 1980). The glucose-stimulated efflux of ^{45}Ca from islets has been shown to reflect Ca entry (Herchuelz, Thonnart, Sener & Malaisse, 1980). The effect of cooling on ^{45}Ca outflow from perifused rat pancreatic islets was investigated in experiments paralleling those shown in Fig. 1.

Fig. 2 compares the ^{45}Ca outflow (upper part) and insulin release (lower part) during exposure to glucose at 37 and 27 °C. At 37 °C, a rise in the glucose concentration from zero to 16·7 mM provoked first a decrease and then a rapid increase in the rate of ^{45}Ca outflow associated with a typical biphasic increase in insulin release. When the same experiment was carried out at 27 °C the release of insulin induced by glucose was delayed and greatly suppressed. This contrasts with the much more modest effect of the low temperature upon the changes in the rate of ^{45}Ca efflux induced by glucose. At 27 °C, a rise in the glucose concentration from zero to 16·7 mM clearly evoked the dual modification of the rate of ^{45}Ca outflow seen at 37 °C, consisting of an initial fall followed by a secondary rise. At the low temperature, the initial fall (calculated for each experiment with respect to the rate of ^{45}Ca outflow observed from minutes 40–44) and secondary rise (calculated for each experiment with respect to the

minimum rate of ^{45}Ca outflow) displayed almost the same magnitude at 27 °C as that observed at 37 °C, although occurring with a considerable delay. The outflow rate reached a minimum about 7 min after exposure to glucose at 27 °C, compared with about 2 min at 37 °C. Peak outflow occurred after about 14 min of glucose exposure at 27 °C, compared with about 7 min at 37 °C. The temperature coefficient for basal

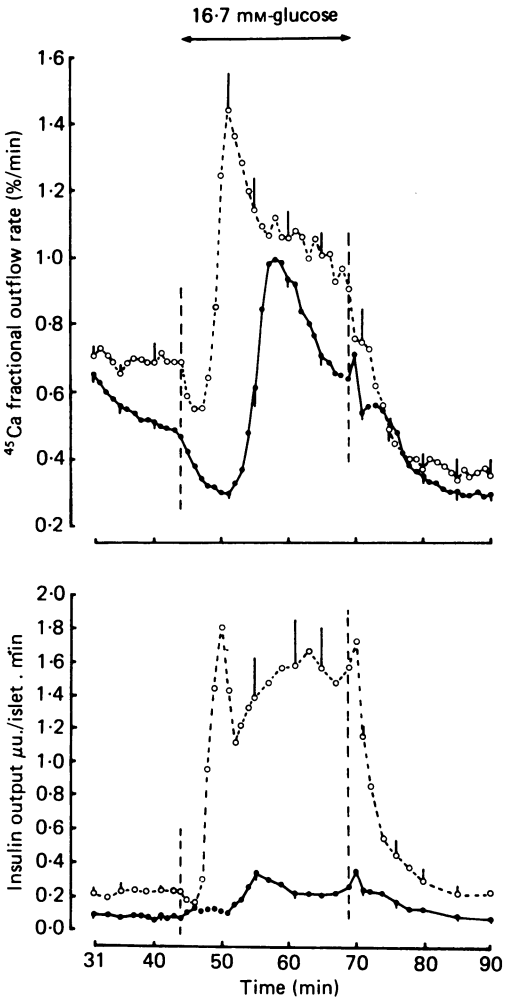

Fig. 2. Effects of glucose on ^{45}Ca outflow and insulin release at 37 and 27 °C. Effects of 16·7 mM-glucose on the rate of ^{45}Ca outflow (upper part) and insulin release (lower part) from rat pancreatic islets at 37 °C (○---○) and at 27 °C (●——●). Values are given as the mean ± S.E. of mean ($n = 4$ at 37 and $n = 8$ at 27 °C). For comparison of IRI from rat islets with IRI from mouse islets in Figs. 1, 3 and 4 note that 25 μu. is equivalent to 1 ng insulin.

^{45}Ca outflow (at minute 44) was 1·6. The peak ^{45}Ca outflow showed a temperature coefficient of 1·45, while total stimulated ^{45}Ca outflow showed a temperature coefficient of 1·98. The time to peak outflow, with respect to minimum outflow, had a temperature coefficient of 0·73.

The total release of insulin recorded during the period of glucose stimulation (minutes 45–68) at 27 °C averaged only 11 % of that observed at 37 °C ($P < 0.0001$). The temperature coefficient for glucose-induced insulin release from rat islets was 5·1 for peak release and 9·1 for total release.

In summary, these experiments (Figs. 1 and 2) show a dissociation of insulin release from Ca entry (as reflected by glucose-induced electrical activity in mouse islets and glucose-induced calcium outflow in rat islets).

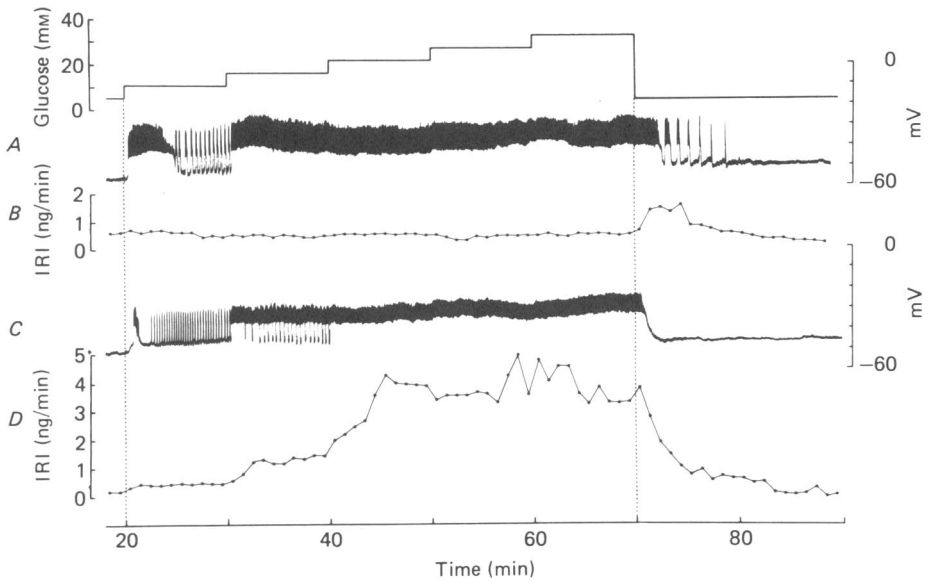

Fig. 3. Effects of increasing glucose concentration on electrical activity, membrane potentials and insulin release at 27 and 37 °C. *A*, membrane potentials at 27 °C in response to glucose at the concentrations indicated at the top of the Figure. *B*, IRI release at 27 °C from the same islet. *C*, membrane potentials from the same β cell as in *A* at 37 °C. *D*, IRI release at 37 °C. In this experiment, the islet was dissected and the β cell impaled in 11·1 mM-glucose at 27 °C. Glucose was removed for 10 min before the start of the sample collection (minute 0). Samples were collected every minute for the duration of the experiment. At minute 100, the temperature was increased to 37 °C over a 2 min period. At minute 110 the glucose was removed again. The records *C* and *D* begin at minute 128.

Effects of increasing glucose concentration on electrical activity, membrane potential and insulin release at 27 and 37 °C

Increasing the glucose concentration in a stepwise fashion in experiments on single islets has been reported to result in a parallel increase in insulin release and electrical activity (Scott *et al.* 1981). The upper half of Fig. 3 shows the membrane potential records (*A*) and the insulin release (*B*) during perifusion with solutions containing 5·6, 11·1, 16·7, 22·2, 27·6 and 33·3 mM-glucose for 10 min at each concentration (as indicated at the top of the Figure) at a constant temperature of 27 °C; the lower half of the Figure shows the records of membrane potential (*C*) and insulin release (*D*) from the same islet during perifusion with the same glucose concentrations at a constant temperature of 37 °C.

The temperature was raised from 27 to 37 °C 38 min before the beginning of the lower traces in Fig. 3. Upon warming, action potentials occurred for a few seconds followed by a hyperpolarization of the membrane of 5 mV. No change in insulin release was detected during the transient electrical activity produced on warming (results not shown).

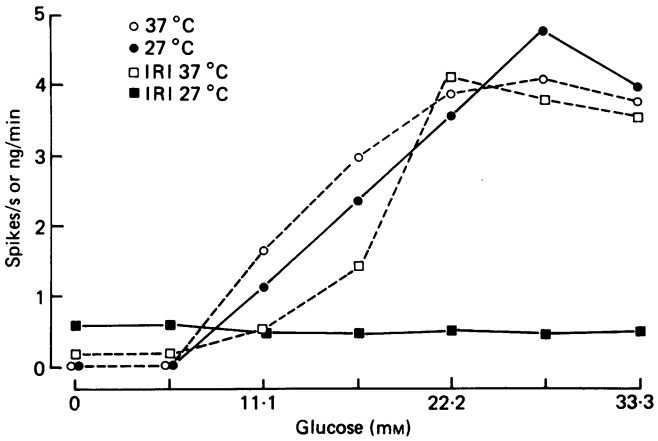

Fig. 4. Spike frequency and insulin output dependence upon glucose concentrations at 37 and at 27 °C. Same experiment as in Fig. 3. The spike frequency was calculated by counting the total number of spikes in 10 s intervals during the last 2 min of exposure to each concentration of glucose, averaging the values and dividing by 10, at 37 °C (○---○) and at 27 °C (●——●). The insulin data represent the average value of the last five measurements at each glucose concentration at 37 °C (□---□) and 27 °C (■——■).

The membrane potential records at 27 and 37 °C, as shown in Fig. 3*A* and *C* respectively, were very similar; 5·6 mM-glucose was subthreshold and increasing the glucose level to 11·1 mM induced a biphasic pattern (Meissner & Atwater, 1976) for electrical activity at both temperatures. There were slight differences, however, in the response to 11·1 mM-glucose. At 27 °C, the first burst of the biphasic response occurred earlier (22 s after the change to 11·1 mM-glucose at 27 °C compared with 45 s at 37 °C) and was more prolonged (270 s at 27 °C compared with 30 s at 37 °C). The steady-state burst pattern in 11·1 mM-glucose was slower at 27 than at 37 °C; during the last 200 s of exposure the burst periodicity was 2·7 bursts/min at 27 °C compared with 8·1 bursts/min at 37 °C. The temperature coefficient for the burst periodicity was 3·0.

While the burst frequency was decreased by cooling, burst duration was increased. The average duration of the active phase of the bursts at 27 °C was 9·1 ± 0·4 s (mean ± S.E. of mean; $n = 8$) and at 37 °C was constant at 4 s ($n = 13$); the temperature coefficient was 0·44. The temperature coefficient of the over-all activity, i.e. the combination of these two parameters, is 1·32. The records shown in Fig. 3*A* and *C* are representative of similar observations made in response to 11·1 mM-glucose at the two temperatures in twenty β cells from different islets.

Another difference between the two membrane potential records shown in Fig. 3 is that at 27 °C, 16·7 mM-glucose induced continuous spike activity, while at 37 °C

the burst pattern persisted. Also, the electrical activity continued for 9 min after the switch from 33·3 to 5·6 mM-glucose at 27 °C, while at 37 °C the spike activity ceased within 1 min.

At 37 °C, the release of insulin as a function of glucose was very similar in this islet to that reported earlier (Scott *et al.* 1981). At 27 °C, however, the same concentrations of glucose failed to stimulate release beyond the basal level. In contrast, the transient

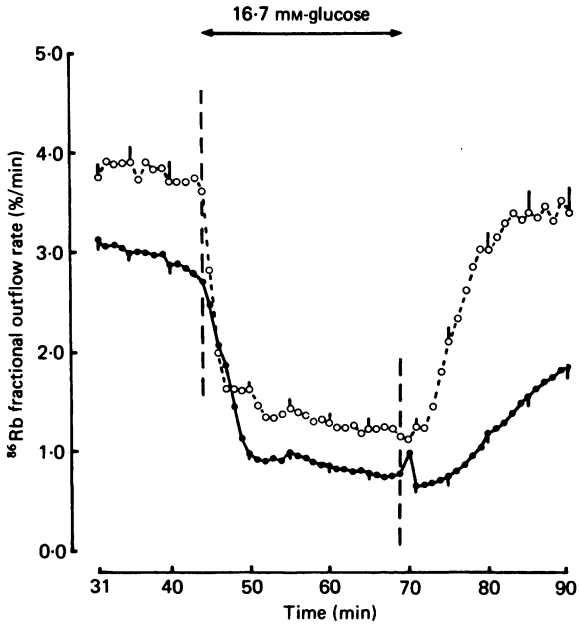

Fig. 5. Effects of glucose on ^{86}Rb outflow at 37 and at 27 °C in rat islets. Effect of a rise in the glucose concentration from 0 to 16·7 mM on the rate of ^{86}Rb outflow from rat pancreatic islets at 37 °C (○---○) and at 27 °C (●——●). Mean values (±S.E. of mean; $n = 4$) for ^{86}Rb outflow are expressed as a fractional outflow rate (i.e. the counts per minute (c.p.m.) of each sample divided by the sum of the c.p.m. left in the islets at the end of the experiment and all the c.p.m. washed out from the islets between the time of collection of the sample and the end of the experiment).

release often measured upon return to basal glucose, sometimes called the 'off response' was not inhibited at the lower temperature (see also in rat islets, Fig. 2).

Fig. 4 illustrates the dependence of spike frequency and insulin release on glucose concentration at 27 and 37 °C (data taken from the experiment in Fig. 3). The electrical activity was not very different at the two temperatures although there was a tendency for the spike frequency at 27 °C to be lower than at 37 °C in the range 11·1–22·2 mM-glucose. At 37 °C the spike frequency was maximal at a concentration of 22·2 mM-glucose, whereas at 27 °C it was maximal at 27·6 mM-glucose. At 27·6 mM-glucose, the average spike frequency was almost 5 spikes/s at 27 °C compared with an average of 4 spikes/s at 37 °C. At 16·7 mM-glucose, the temperature coefficient for spike frequency was 1·26 compared with 0·8 at 27·6 mM-glucose.

Thus, it seems clear that cooling to 27 °C inhibits insulin release without greatly affecting the electrical activity even at supra-maximal stimulatory glucose concen-

trations. The experiments illustrated in Figs. 1, 3 and 4 are representative of similar observations made measuring insulin release in eight experiments and electrical activity in thirty-two experiments.

It has been established that glucose inhibits potassium permeability in the β cell (Atwater *et al.* 1978) and that this inhibition is clearly demonstrated by measurements of the effects of glucose on ^{42}K (or ^{86}Rb) outflow from isolated islets (Henquin, 1978; Boschero & Malaisse, 1979).

Effects of glucose on ^{86}Rb outflow from rat islets at 37 and 27 °C

The fractional outflow rate from ^{86}Rb is shown in Fig. 5 before, during and after exposure to 16·7 mM-glucose. It may be seen that in the absence of glucose (minutes 31–44) as well as in the presence of glucose (minutes 45–68) the ^{86}Rb outflow rate is less at 27 than at 37 °C. In the absence of glucose (at minute 44) the ^{86}Rb outflow rate was 3·7 %/min at 37 °C and was 2·8 %/min at 27 °C. The temperature coefficient for basal ^{86}Rb outflow rate is 1·3. This observation is an indication that cooling inhibits potassium permeability.

Also apparent from Fig. 5 is the fact that glucose rapidly and reversibly inhibits ^{86}Rb outflow to nearly the same extent at 27 and at 37 °C. The ^{86}Rb outflow rate at the end of the period of glucose stimulation (minute 68) was 1·2 %/min at 37 °C and 0·8 %/min at 27 °C. The temperature coefficient for glucose-inhibited ^{86}Rb outflow is 1·5. The major difference between the response at the two temperatures is that at 27 °C the onset of the glucose-induced inhibition of ^{86}Rb outflow is slower. At 27 °C the inhibitory effect of the sugar on the rate of ^{86}Rb outflow was also less rapidly reversible than at 37 °C.

As it has been shown that glucose metabolism is required for the glucose-induced inhibition of the potassium permeability (Boschero & Malaisse, 1979; Henquin, 1980; Carpinelli & Malaisse, 1980), the results presented in Fig. 5 indicate that the metabolic link between glucose utilization and potassium permeability is not significantly affected by a reduction of temperature to 27 °C. In fact, the temperature coefficient of 1·5 for the ^{86}Rb outflow is lower than the temperature coefficient of 2·05 for lactic acid production in rat islets (Malaisse-Lagae, Sener, Lebrun, Herchuelz, Leclercq-Meyer & Malaisse, 1982).

Effects of cooling on β-cell membrane potassium permeability

Reducing the temperature both in the presence and absence of glucose induces a depolarization ranging from 2 to 16 mV. In the absence of glucose, the average decrease in membrane potential upon changing the temperature from 37 to 27 °C was $11{\cdot}0 \pm 1{\cdot}1$ mV mean $\pm$ S.E. of mean; the average resting potential was -58 ± 2 mV at 37 °C and -47 ± 2 mV at 27 °C ($n = 12$). The temperature coefficient for this depolarization is 1·2. The depolarization induced by cooling is still measured in the presence of 2 mM-Co^{2+} ($n = 5$), indicating that depolarization is not due to Ca entry. The depolarization caused by cooling was accompanied by a 10 ± 2 % increase in input resistance ($n = 10$) (for details in methodology see Atwater *et al.* 1978). If cell-to-cell coupling is not significantly changed by cooling, the measurements of membrane potential and input resistance indicate that cooling decreases the membrane potassium permeability. (Cell-to-cell coupling has been found to be unaffected by cooling to

22 °C, as estimated electrically in micro-dissected mouse islets (E. Rojas & G. Eddlestone, personal communication) and by injections of Lucifer Yellow dye in islets isolated by collagenase digestion from mice (P. Meda, personal communication).)

DISCUSSION

Recently we developed a technique for the simultaneous measurements of insulin release and electrical activity from single islets (Scott *et al.* 1981). In those studies there was an indication of dissociation between the two processes in that the time constants were different, release being slower in response than the estimated diffusion time would predict.

The results presented here show that the glucose-induced electrical activity is not inhibited at 27 °C: the onset of spike activity is not delayed, membrane potential at the plateau is not altered, and duration of the active phase of the bursts in 11·1 mM-glucose is increased, as is the duration of individual spikes. It is well known that cooling slows down the membrane gating mechanisms which control ionic fluxes and thus leads to longer-lasting ionic currents (Keynes & Rojas, 1974; Collins & Rojas, 1982). These observations predict that Ca entry into the β cell upon exposure to glucose will not be inhibited by cooling from 37 to 27 °C and may even be somewhat enhanced. Since insulin release is completely blocked by cooling to 27 °C, complete dissociation of electrical activity from the release process has been demonstrated.

The dissociation between ^{45}Ca outflow and insulin release at 27 °C described here confirms similar studies from other laboratories (Hellman & Andersson, 1978; Wollheim & Sharp, 1981). In agreement with the electrical data, the experiments comparing glucose-induced ^{45}Ca outflow from rat islets at 37 and 27 °C also show that the magnitude of the glucose-induced Ca movements is not greatly altered (Fig. 2*A*). The time course of these Ca movements is, however, significantly slower at 27 °C.

The glucose-stimulated ^{45}Ca outflow has been attributed to Ca-induced liberation of ^{45}Ca from intracellular stores following the stimulated Ca entry into the cell (Herchuelz & Malaisse, 1978; Herchuelz *et al.* 1980). Since no delay was observed in the onset of electrical activity (i.e. Ca entry) at 27 °C, the delay in ^{45}Ca outflow could be due to an alteration of the Ca exchange within the cell.

The β-cell membrane potassium permeability in the absence of glucose has been shown to be mainly due to the Ca^{2+}-activated potassium channels (Atwater, Dawson, Ribalet & Rojas, 1979; Henquin, 1979). Several observations presented here lead to the conclusion that cooling the β cell inhibits potassium permeability: cooling decreases the fractional ^{86}Rb outflow rate; cooling depolarizes the β cell and increases the measured input resistance. Nevertheless, no increase in basal insulin release was detected in either rat or mouse islets by cooling to 27 °C. The inhibitory effect of lowering the temperature on potassium permeability may be a direct one on the membrane channels (Barrett, Magleby & Pallotta, 1982) or may be an indirect one due to altered handling of cell Ca (Atwater, Dawson, Scott, Eddlestone & Rojas, 1980).

At 37 °C, glucose-induced insulin release from rat islets (Fig. 2, lower part) compares well with the release from mouse islets (Fig. 1*B*). In both preparations the

release was biphasic (although the first phase is not seen regularly from mouse islets), the second phase reaching steady state after 20 min of exposure to glucose. The steady-state release from single mouse islets was greater than that measured from the average rat islet at both sub- and supra-threshold values (Scott *et al.* 1981). This is probably due to the very large islets used to allow detection of insulin from a single islet (minimum diameter 0·2 mm). Insulin release from the mouse islet (Fig. 3) at 37 °C in response to 16·7 mM-glucose was 1·4 ng/islet min while from rat islets it averaged 1·5 μu./islet min or 0·06 ng/islet min (Fig. 2). Thus, at 37 °C in the mouse islets used in these studies the rate of release was about 25 times higher than that measured in rat islets. The basal release from mouse islets was not affected by cooling to 27 °C (Figs. 1 and 3) while in rat islets it appeared to be reduced (Fig. 2). At 27 °C, glucose did not increase insulin release above basal levels in mouse islets (Figs. 1 and 3) whereas the glucose-stimulated release from rat islets was greatly reduced but not completely abolished (Fig. 2).

Glucose metabolism is known to be a prerequisite for the glucose-induced decrease in potassium permeability, changes in ^{45}Ca outflow and increase in insulin release (Herchuelz & Malaisse, 1978; Boschero & Malaisse, 1979; Carpinelli & Malaisse, 1980; Atwater *et al.* 1980; Henquin, 1980). The temperature coefficient (Q_{10}) for glucose utilization in rat islets is reported to be 2·05 (Malaisse-Lagae *et al.* 1982). The temperature coefficients for the various parameters measured in the present study fall into two groups: 2 or less for membrane ion permeabilities as measured electrically or by tracer fluxes, and 5 or greater for insulin release. It is clear that the limiting process in the release of insulin, normally triggered by glucose, has a high temperature coefficient and that this step is independent of glucose metabolism, potassium permeability and Ca movements.

Since insulin release by exocytosis has actually been induced by cooling to very low temperatures (Dahl & Henquin, 1978), it is unlikely that cooling to 27 °C affects exocytosis *per se*. Thus, it is necessary to propose that a highly temperature-dependent step is involved in the secretion process somewhere between the voltage-dependent Ca entry and the exocytotic step. The large temperature coefficient indicates that a membrane process may be involved.

In conclusion, insulin release stimulated by glucose, can be dissociated from β-cell electrical activity, as well as from the changes in ^{86}Rb and ^{45}Ca outflow normally associated with the stimulatory process.

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