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The control of ^{86}Rb efflux from rat isolated pancreatic islets by the sulphonylureas tolbutamide and glibenclamide

E.K. Matthews & P.A. Shotton[1]

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

1 The efflux of ^{86}Rb from rat isolated pancreatic islets preloaded with the isotope and perifused *in vitro*, has been used to monitor the effects of sulphonylureas on the potassium permeability, P_K, of pancreatic β-cells.

2 Tolbutamide (5 μM to 5 mM) had a dual effect, causing initially a decrease in ^{86}Rb efflux (the 'on' response) which was rapidly superseded on drug removal by a large phasic increase in ^{86}Rb efflux (the 'off' response). Each kinetic response had a different dose-dependency: the 'on' response was half-maximal at tolbutamide concentrations of 0.02 mM, maximal at 0.2 mM and decreased by concentrations > 0.2 mM whereas the 'off' response was half-maximal at 0.07 mM, maximal at 0.7 mM, with further increases in concentration (up to 5 mM) causing no further change in magnitude.

3 Analysis of the time- and concentration-dependency of tolbutamide action, by presenting increasing concentrations (0 to 1.4 mM) of tolbutamide as a ramp or step function, established a critical dependence of the kinetics of ^{86}Rb efflux during and after exposure to tolbutamide upon the initial rate of increase of the tolbutamide concentration rather than its final steady state.

4 In the presence of quinine (10 μM), D600 (50 μM), or tetraethylammonium (20 mM), the secondary increase in ^{86}Rb following tolbutamide (0.7 mM) removal was totally inhibited. Co^{2+} (2.56 mM) not only blocked the secondary 'off' response but also potentiated the initial 'on' response of tolbutamide.

5 Glibenclamide produced a rapid decrease in ^{86}Rb efflux but at a much lower concentration (10 μM) than tolbutamide and with no 'off' response apparent over a wide range of concentration (1 to 100 μM); moreover the decrease in ^{86}Rb efflux was sustained and only slowly reversible.

6 It is concluded that tolbutamide has two opposing actions on islet β-cell ^{86}Rb efflux, and therefore P_K: (i) a tendency to increase a calcium-sensitive P_K by stimulating calcium entry into the cell and (ii) a decrease in P_K that may be due to a direct effect on the calcium-sensitive P_K itself. The more sustained pharmacological action of glibenclamide is explained by the longer-lasting decrease in P_K that it produces.

Introduction

The control of pancreatic β-cell membrane permeability is of central importance in the stimulation of insulin release by a variety of substances. Depolarization and electrical spiking activity are associated with and may precede the secretion of insulin following stimulatory concentrations of D-glucose (Dean & Matthews, 1968; 1970a), the sulphonylureas tolbutamide and glibenclamide (Dean & Matthews, 1970a; Matthews & Sakamoto, 1975), certain amino-acids (Henquin & Meissner, 1981) and a variety of neuronal and hormonal stimuli. The depolarization produced by glucose is believed to be mediated by a decrease in the potassium permeability, P_K, of the pancreatic β-cell membrane (Sehlin & Täljedal, 1975; Boschero *et al.*, 1977; Henquin, 1978); calcium entry through voltage-dependent channels then ensues (Dean & Matthews, 1970b; Matthews & Sakamoto, 1975) and culminates in the exocytosis of insulin-containing secretory granules.

Although the sulphonylurea compounds have long

[1]Present address : Ciba-Geigy Pharmaceuticals PLC, Wimblehurst Road, Horsham, W. Sussex.

been in clinical use as hypoglycaemic agents their mode of action at the cellular and molecular level has yet to be determined. Studies using ^{32}S-labelled suphonylureas (Hellman *et al.*, 1971) and dextran-linked tolbutamide (Bowen & Lazarus, 1974) indicate that the stimulation of insulin release by these agents may be mediated through a receptor site located at the cell membrane. Both tolbutamide and glibenclamide elicit an alteration of β-cell calcium handling (Malaisse *et al.*, 1972; Kloppel & Schaffer, 1976) and the divalent ion cobalt reduces the depolarization produced by tolbutamide and prevents the appearance of electrical spiking activity (Meissner *et al.*, 1980). Tolbutamide has also been shown to modify the rate of loss of ^{86}Rb from islets prelabelled with the isotope, suggesting that, since pancreatic islets are composed of $>70\%$ β-cells, the drug affects principally β-cell potassium permeability (Henquin, 1980; Matthews & Shotton, 1982). In the present work, therefore, islet cell ^{86}Rb efflux has been studied in detail as an index of changes in β-cell membrane P_K in order to resolve the mechanisms by which the sulphonylureas stimulate the release of insulin.

Methods

Pancreatic islets were isolated from male Sprague-Dawley rats (180–300 g) by a modification of the collagenase digestion procedure of Moskalewski (1965). The rats used were maintained on a diet of CRM pellets in a temperature-controlled room (22.5°) with an 11.5 h light/12.5 h dark cycle. They were killed by cervical dislocation and exsanguination. Each pancreas was excised rapidly, chopped finely, rinsed in Krebs solution (composition given below), and digested for between 6 and 12 min in a solution of 40 mg collagenase in 5 ml Krebs solution. The resulting suspension was washed 5 times, and the isolated islets harvested under a binocular microscope using a siliconized pasteur pipette. Groups of 50 islets were placed in each of 6 siliconized glass tubes and incubated for one hour in 1 ml Krebs solution containing between 25 and 100 μl $^{86}RbCl$ solution to give an activity of between 350 KBq and 1.8 MBq per tube.

After incubation the islets were washed three times in Krebs solution before transfer to glass perifusion chambers, approx. volume 0.25 ml, incorporating sintered glass filters to support the islets. The flow rate of the system was 1 ml min^{-1}.

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

$$\text{rate coefficient } (\text{min}^{-1}) = \frac{\Delta x}{\Delta t\, x_t}$$

where Δx is the amount of radioactivity collected in each collection interval, Δt is the length of the interval, and x_t is the total amount of rubidium released up until the start of the interval.

To create a gradient of tolbutamide concentration (see Results) a standard concentration-gradient forming technique was used. A reservoir of Krebs solution containing tolbutamide at the desired final concentration was connected to a second reservoir containing an equal quantity of Krebs solution alone. Fluid was drawn from the second reservoir into the perifusion system, and as the level fell, fluid from the first reservoir flowed in to keep the level of fluid in both reservoirs equal. This created a linearly increasing gradient of tolbutamide concentration from 0 mM to the desired concentration. By substituting the dye methylene blue for tolbutamide and pumping the fluid through a flow-cell in a spectrophotometer, the excellent reproducibility of the gradient-forming apparatus was confirmed.

Islets were perifused in a Krebs-Henseleit medium of the following composition (mmol l^{-1}): NaCl 118, $CaCl_2$ 2.56, KCl 4.7, $MgCl_2$ 1.13, NaH_2PO_4 1.15, $NaHCO_3$ 25, supplemented with 2.8 mM D-glucose and, after islet isolation, by 0.5 g 100 ml^{-1} bovine serum albumin. Solutions were freshly made each day, and gassed with 95% O_2/5% CO_2 before and during each experiment, giving a final pH of 7.4. All solutions used contained D-glucose 2.8 mM and experiments were conducted at 37°C maintained by a thermostatically controlled water bath.

All chemicals were of 'Analar' or comparable quality. ANDA was obtained from the Aldrich Chemical Co., collagenase from Boehringer Mannheim, ^{86}Rb from Amersham International, albumin (bovine serum, fraction V) from Sigma and D600 (methoxyverapamil) from Knoll A.G.

Results

Tolbutamide at a concentration of 0.7 mM caused a pronounced and biphasic alteration of islet cell ^{86}Rb efflux (see Figure 1a). Both the decrease in efflux following tolbutamide addition (the 'on' response) and the increase following its removal (the 'off' response) were concentration-dependent (Figure 1b), but with different dose-dependencies. The 'on' response was half-maximal at a tolbutamide concentration of 0.02 mM, and declined at concentrations grea-

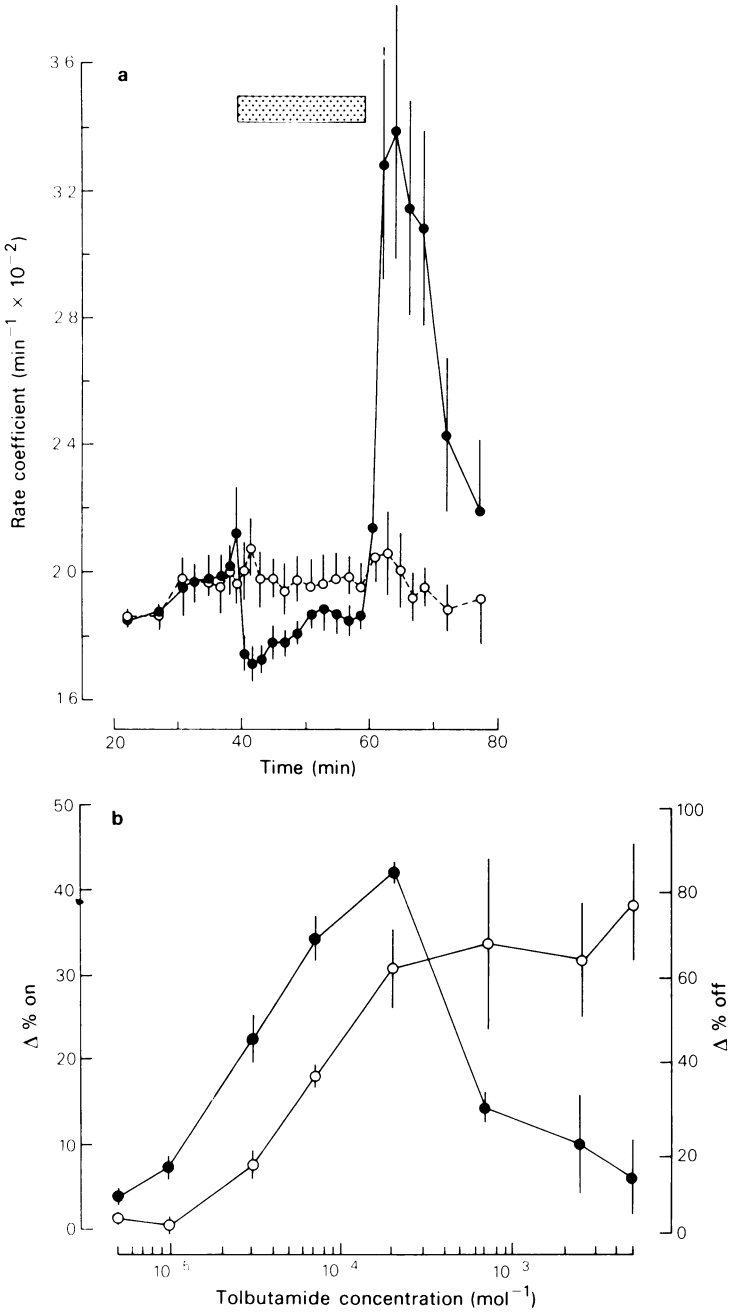

Figure 1 (a) Effects of tolbutamide on the rate coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are means ± s.e.means (vertical lines). Control islets (open circles, $n = 5$) were perifused throughout with Krebs solution. Test islets (filled circles, $n = 7$) were exposed to Krebs solution containing tolbutamide (0.7 mM) during the period indicated by the shaded bar. Significant differences ($P < 0.05$) between control and test values existed from the 40th to the 49th min and from the 63rd to the 69th min. All solutions contained D-glucose 2.8 mM throughout. (b) Effects of different concentrations of tolbutamide on the rate coefficient of ^{86}Rb efflux from isolated pancreatic islets. 'On' response (filled circles) taken as efflux rate at 43rd min of experiment. 'Off' response (open circles) that at 65th min. Values are means ± s.e.means (vertical lines) of between 4 and 7 estimations at each tolbutamide concentration, calculated as the percentage change in efflux from the baseline level.

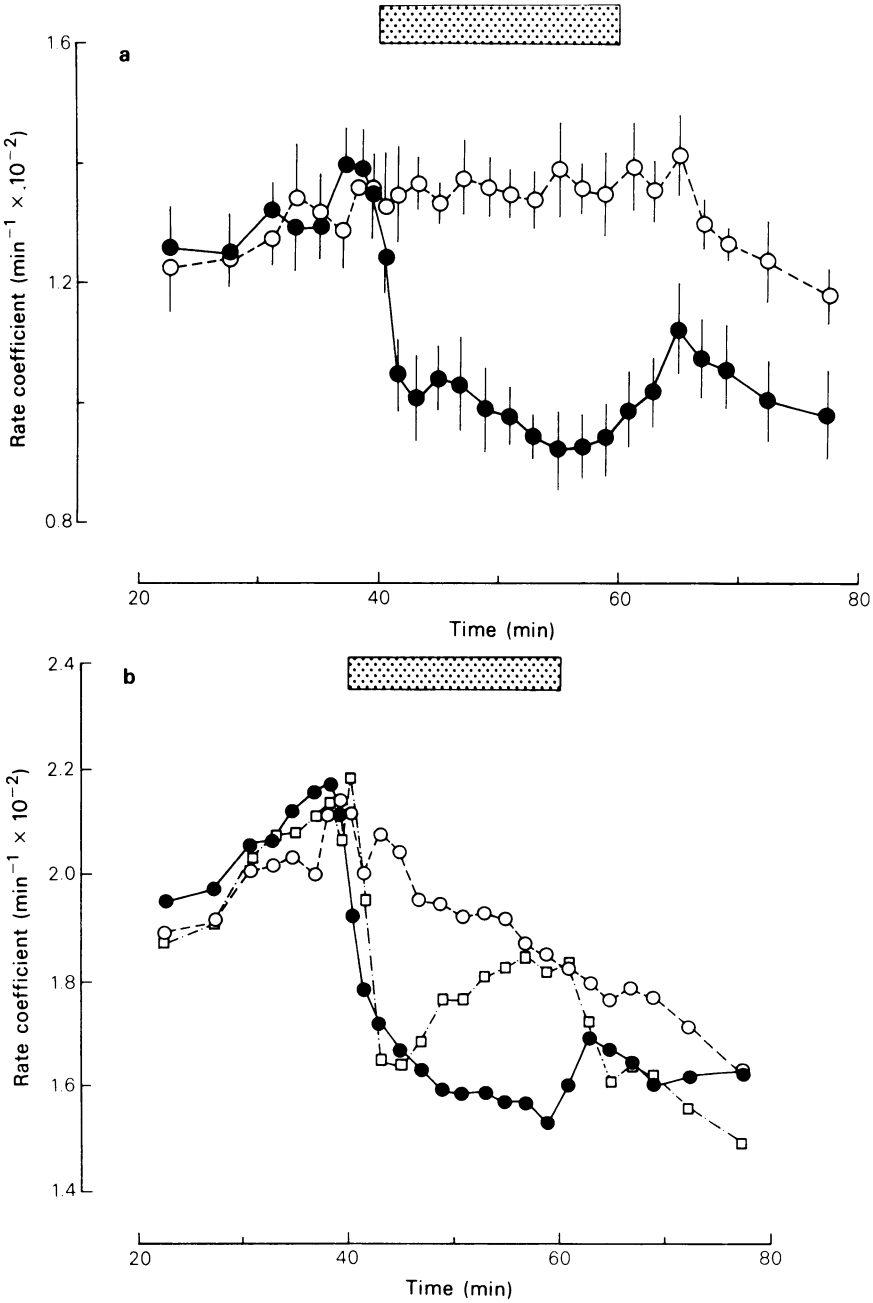

Figure 2 (a) Effects of glibenclamide on the rate coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are means ± s.e.means (vertical lines). Control islets (open circles, $n = 4$) were perifused throughout with Krebs solution containing D-glucose 2.8 mM. Test islets (filled circles, $n = 4$) were exposed to Krebs solution containing glibenclamide (10 μM) during the period indicated by the shaded bar. Significant differences ($P < 0.05$) between control and test values existed from the 41st min to the end of the experiment. (b) Effects of glibenclamide on the rate coefficient of ^{86}Rb efflux from isolated pancreatic islets. Mean values are shown. Standard error bars are omitted for clarity. Control islets (open circles, $n = 4$) were perifused throughout with Krebs solution containing D-glucose 2.8 mM. Glibenclamide, 1 μM (open squares, $n = 5$) or 100 μM (filled circles, $n = 7$) was present during the period indicated by the shaded bar. Significant differences ($P < 0.05$) between control and test values existed from the 41st to the 47th min (glibenclamide 1 μM) and from the 41st to the 61st min (glibenclamide 100 μM).

ter than 0.2 mM, whilst in contrast the 'off' response was half-maximal at a concentration of 0.07 mM and remained near maximal at concentrations ranging from 0.2 to 5 mM.

Gibenclamide was considerably more potent than tolbutamide since at concentrations of 1, 10 and 100 μM it caused a fall in the rate of ^{86}Rb efflux of approximately 25% (Figure 2a, b). At a concentration of 1 μM efflux returned to control values following the initial decrease but at the higher concentrations the depressed level of ^{86}Rb efflux was maintained, even following the removal of the drug, in contrast to the marked increase in efflux seen at the termination of tolbutamide stimulation.

The dual nature of the response to tolbutamide may indicate the superimposition of two or more time-dependent processes. The effects of different rates of stimulus presentation were therefore studied and Figure 3 depicts the changes following a stepwise increase in tolbutamide concentration from 0 to 0.07 mM for a 10 min period followed by a step to 0.7 mM for a further 10 min before returning to 0 mM. Initially the addition of tolbutamide caused a fall in the rate of ^{86}Rb efflux of more than 25%. When the concentration was further increased to 0.7 mM no corresponding change in efflux was evident, but upon removal of tolbutamide the rate of ^{86}Rb efflux increased to a level only 13% higher than control values. In contrast, when tolbutamide was present for the whole 20 min period at a concentration of 0.07 mM or 0.7 mM the increase seen on removal was more than 30% or 60% respectively.

When the tolbutamide gradient was increased in ramp form, from 0 to 0.7 mM over a 20 min period, efflux fell initially by 34% before rising to 74% of the prestimulatory level (Figure 4a). After the removal of tolbutamide the rate of efflux rose by nearly 30% before returning towards control levels. The magnitude of the 'off' response was similar to that following the removal of tolbutamide after stimulation with a constant level of between 0.03 and 0.07 mM, although the area under the tolbutamide concentration/time curve during the ramp stimulation was equivalent to that of a constant tolbutamide concentration of 0.35 mM over the same period.

A similar effect was seen with a ramp stimulation

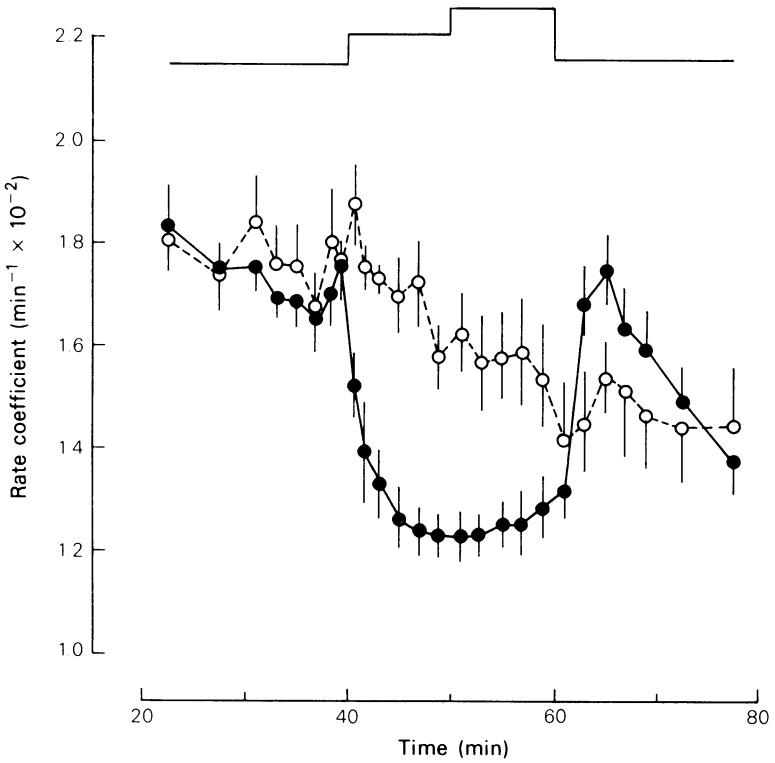

Figure 3 Effects of tolbutamide introduced in steps on the rate coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are means ± s.e.means (vertical lines). Control islets (open circles, $n = 4$) perifused throughout with Krebs solution containing D-glucose 2.8 mM. Test islets (filled circles, $n = 8$) perifused with Krebs solution containing tolbutamide 0.07 mM from 40th to 50th min, and tolbutamide 0.7 mM from 50th to 60th min. Significant differences ($P < 0.05$) between control and test values existed from the 42nd to the 59th min.

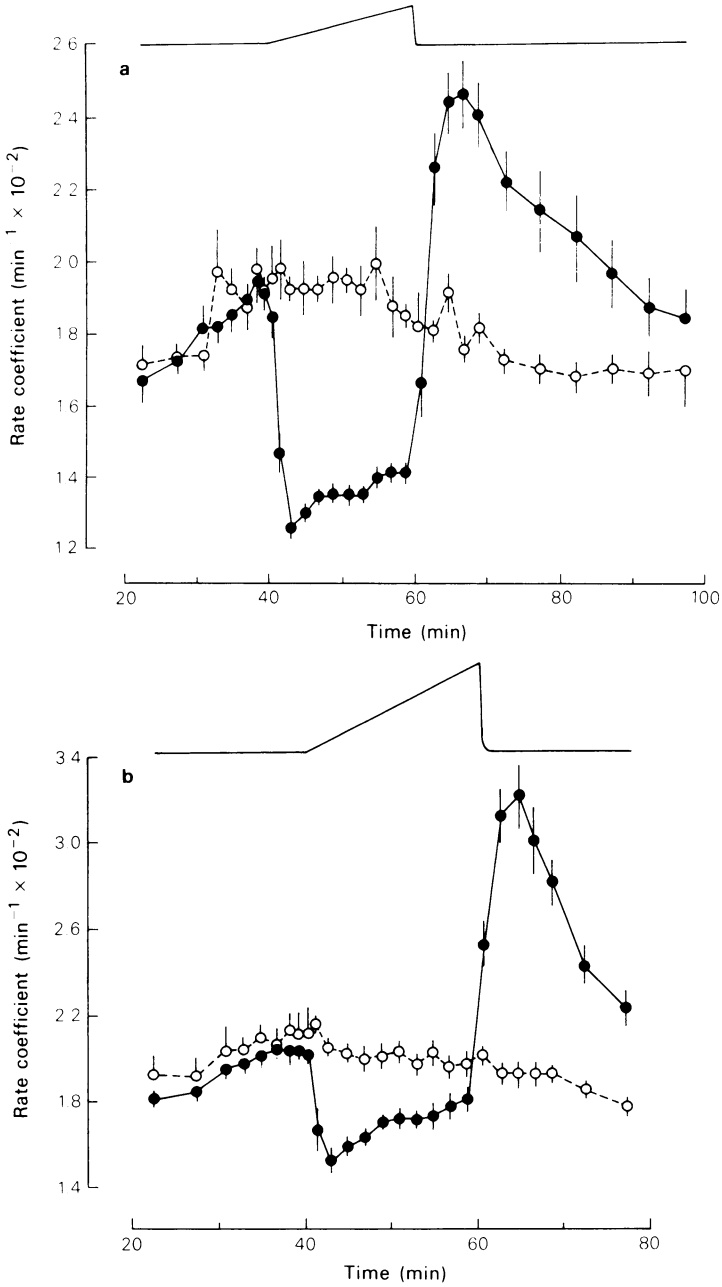

Figure 4 (a) Effects of a progressive increase in tolbutamide concentration on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are means ± s.e.means (vertical lines). Control islets (open circles, $n = 3$) perifused throughout with Krebs solution containing D-glucose 2.8 mM. Test islets (filled circles, $n = 8$) exposed to a gradient of tolbutamide concentration rising from 0 to 0.7 mM between 40 and 60 min as depicted above the figure. Significant differences ($P < 0.001$) between control and test values existed from the 42nd to the 59th min and ($P < 0.05$) from the 63rd to the 77th min. (b) Effect of a progressive increase in tolbutamide concentration on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are means ± s.e.means (vertical lines). Control islets (open circles, $n = 4$) perifused throughout with Krebs solution containing D-glucose 2.8 mM. Test islets (filled circles, $n = 6$) exposed to a gradient of tolbutamide concentration rising from 0 to 1.4 mM between 40 and 60 min as depicted above the figure. Significant differences ($P < 0.005$) between control and test values existed from the 42nd to the 57th min and from the 63rd min to the end of the experiment.

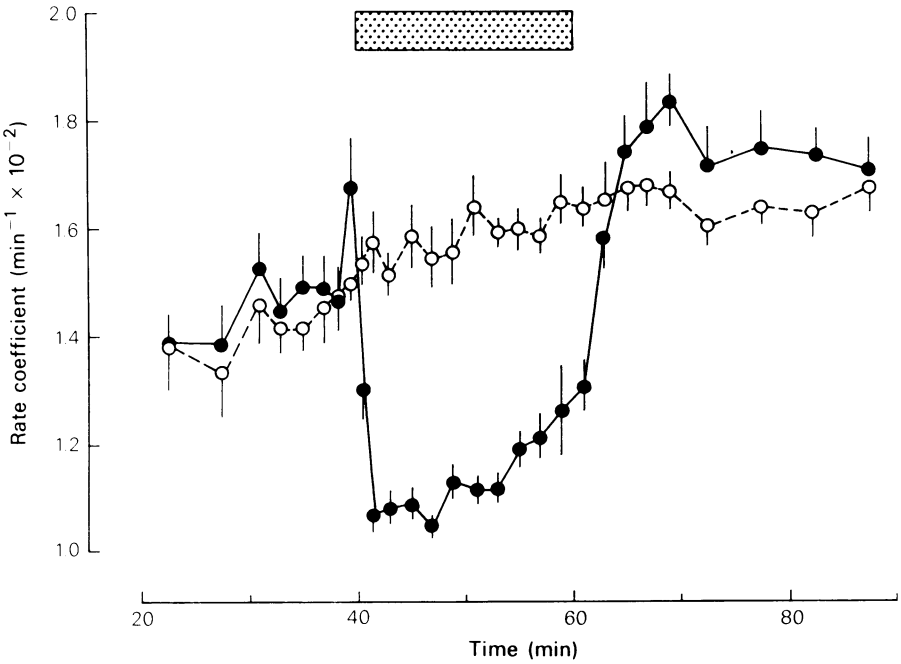

Figure 5 Effects of tolbutamide in the presence of cobalt 2.56 mM on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are mean ± s.e.means (vertical lines). Both control (open circles, $n = 4$) and test islets (filled circles, $n = 6$) were perifused throughout with Krebs solution in which $CaCl_2$ was replaced by $CoCl_2$ 2.56 mM. NaH_2PO_4 was omitted from both solutions to prevent precipitation of cobalt salts. Tolbutamide (0.7 mM) was added to the test solution during the period indicated by the shaded bar. Significant differences ($P < 0.001$) between control and test values existed from the 42nd to the 61st min.

from 0 to 1.4 mM (Figure 4b) but the 'on' response was now at a level only 25% lower than the prestimulus level and the 'off' response was much larger, being 58% greater than the prestimulatory level and not significantly different from the 'off' response seen when a constant level of tolbutamide of between 0.2 and 5 mM is removed after 20 min.

The role of calcium

In view of the known effects of tolbutamide on islet cell Ca^{2+} handling, further experiments examined the possibility that the alteration of ^{86}Rb efflux produced by tolbutamide was secondary to tolbutamide-induced calcium entry.

The divalent ion cobalt, which inhibits the glucose-stimulated entry of calcium (Henquin & Lambert, 1975), reduced the basal efflux of ^{86}Rb from isolated islets by some 25% (see Figure 5). Addition of tolbutamide 0.7 mM caused a significantly greater reduction in ^{86}Rb efflux in the presence of cobalt than in its absence (29 ± 6.6% vs 15 ± 2.3%; $P < 0.001$) and this much larger effect was maintained throughout the period of stimulation. The 'off' response normally seen on removal of tolbutamide was almost totally abolished, the rate of ^{86}Rb efflux increasing only 17% above the prestimulatory level.

The potent Ca^{2+} antagonist D600 at a concentration of 0.05 mM had an even greater effect on basal ^{86}Rb efflux which was reduced to some 46% below the level depicted in Figure 1a and the addition of tolbutamide 0.7 mM now caused no detectable change in ^{86}Rb efflux: control and test values were not significantly different ($P > 0.05$).

The inhibition of the 'off' response by cobalt or D600 suggests that activation of a calcium-dependent potassium permeability underlies the 'off' response. That this is indeed the case is substantiated by the inhibitory effect of quinine, which has previously been shown to inhibit the islet-cell calcium-dependent potassium permeability (Atwater *et al.*, 1979). In the presence of quinine the addition of tolbutamide, instead of reducing islet-cell ^{86}Rb efflux, caused a sustained increase of 30% (Figure 6). However the 'off' response was totally abolished. Unlike quinine, the neurotoxic polypeptide apamin was completely without effect on either basal or tolbutamide-stimulated ^{86}Rb efflux. Apamin inhibits the increase in P_K produced by a raised $[Ca]_i$ in some tissues (Banks *et al.*, 1979), but even at a concentra-

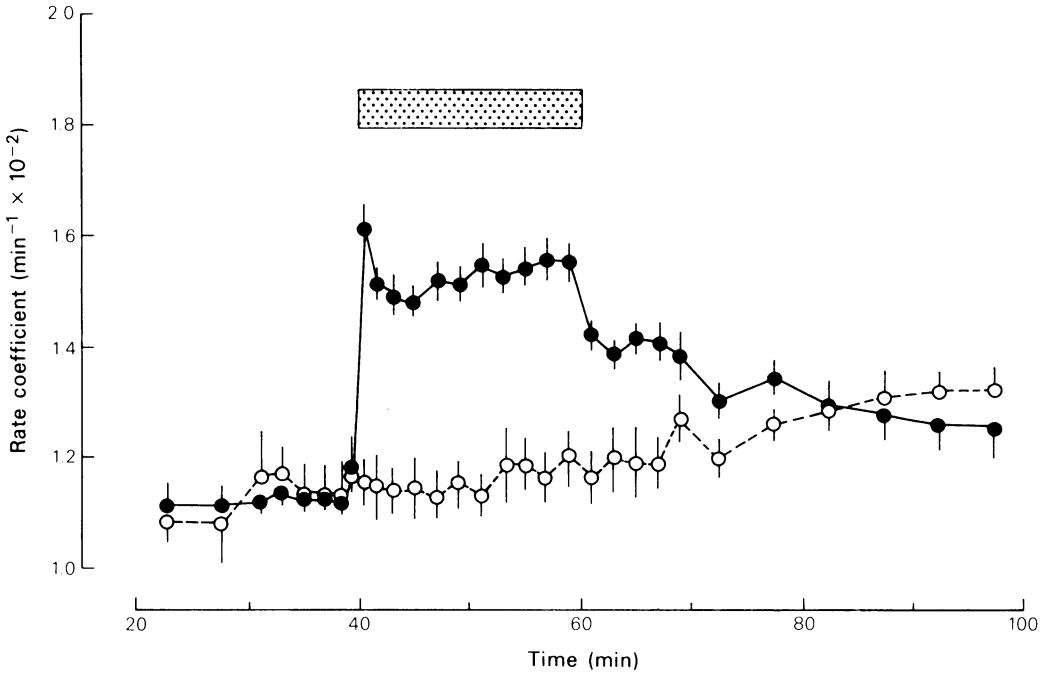

Figure 6 Effects of tolbutamide in the presence of quinine 10^{-5} M on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are means ± s.e.means (vertical lines). Both control (open circles, $n = 5$) and test islets (filled circles, $n = 7$) were perifused throughout with Krebs solution containing quinine 10^{-5} M. Tolbutamide (0.7 M) was added to the test solution during the period indicated by the shaded bar. Significant differences ($P < 0.001$) between control and test values existed from the 41st to the 67th min.

tion of 10^{-7}M, i.e. 10 times higher than that required for complete inhibition of the calcium-activated P_K in isolated hepatocytes, it was ineffective against islet cells.

The effect of tolbutamide may also be mediated in part by a voltage-dependent change in islet-cell P_K. In the presence of the voltage-dependent potassium-channel blocker tetraethylammonium (TEA), tolbutamide caused a change in ^{86}Rb efflux similar to that seen when quinine was present in the perifusion fluid. Addition of tolbutamide caused an increase of ^{86}Rb efflux of more than 30%, which declined slowly to control levels upon removal of the drug (Figure 7). A transient increase in efflux was seen shortly after the removal of tolbutamide, but it was much smaller than the 'off' response that usually occurred.

Discussion

Like D-glucose, the sulphonylureas tolbutamide and glibenclamide both induce a marked decrease in the rate of ^{86}Rb efflux from isolated pancreatic islet cells. However, the removal of tolbutamide is accompanied by a pronounced increase in ^{86}Rb efflux that is sensitive to both the inhibition of Ca^{2+} entry and the presence of agents that are believed to inhibit either voltage- or calcium-dependent activation of islet cell potassium permeabilities. The lack of such an 'off' response upon removal of glibenclamide may be explained by the higher affinity of the drug for a putative membrane-located sulphonylurea receptor; the decrease in ^{86}Rb efflux persists after the removal of the drug, and it has been demonstrated that the electrical changes produced by glibenclamide are maintained for up to 1 hour following its removal (Meissner & Atwater, 1976). The decrease in ^{86}Rb efflux produced by glibenclamide, while taking longer to develop, is also much larger than that following the addition of higher concentrations of tolbutamide.

Tolbutamide thus has at least two actions on islet cell rubidium, and therefore potassium, efflux with different but parallel, dose-dependencies. The major component of the 'off' response appears to be due to the activation of a calcium-dependent P_K, as is demonstrated by the marked inhibition of the 'off' response in the presence of either cobalt or quinine. Cobalt reduces the depolarization produced by tolbutamide and prevents the appearance of spike potentials (Meissner *et al.*, 1980), by blocking the

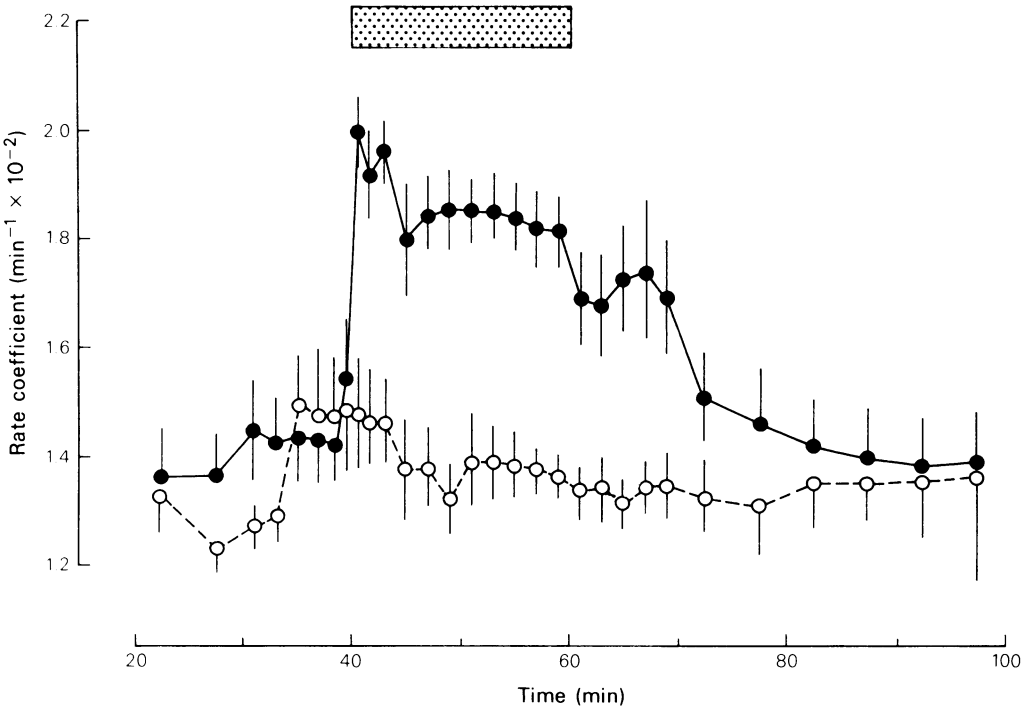

Figure 7 Effects of tolbutamide in the presence of tetraethylammonium (TEA) 20 mM on the coefficient of ^{86}Rb efflux from isolated pancreatic islets. Values are means ± s.e.means (vertical lines). Both control (open circles, $n = 7$) and test islets (filled circles, $n = 8$) were perifused throughout with Krebs solution containing TEA 20 mM. Tolbutamide (0.7 mM) was added to the test solution during the period indicated by the shaded bar. Significant differences ($P < 0.01$) between control and test values existed from the 41st to the 69th min.

entry of calcium through voltage-dependent channels. It has been suggested (Matthews, 1975) that the entry of calcium may stimulate a calcium-sensitive potassium permeability that, by repolarizing the cell, limits further calcium entry. The calcium-activated P_K of red blood cells is blocked by quinine and quinidine (Armando-Hardy *et al.*, 1975). Quinidine blocks the silent phases between bursts of islet cell electrical activity (Atwater & Beigelman, 1976; Ribalet & Beigelman, 1980); quinine potentiates the insulinotropic action of glucose (Henquin *et al.*, 1975) and depolarizes the β-cell membrane whilst increasing the input resistance (Atwater *et al.*, 1979). Henquin (1979) has demonstrated that the presence of quinine or the absence of external calcium abolishes the increase in ^{86}Rb efflux produced by the calcium inonophore A23187. All these findings point to the existence of a functional calcium-activated P_K in islet cells, and the results described in this paper demonstrate that such a Ca-activated P_K plays an integral part in the islet β-cell response to tolbutamide stimulation. This P_K differs in nature from that found in hepatocytes since, in common with that of the red blood cell, the calcium-dependent increase in islet cell ^{86}Rb efflux is unaffected by the presence of apamin.

The potentiation of the tolbutamide 'on' response in the presence of Co^{2+} suggests that the action of the sulphonylurea in decreasing ^{86}Rb efflux is opposed simultaneously by the tendency of calcium entry to increase it, although the reduced depolarization produced by tolbutamide in the presence of Co^{2+} may play a part in the potentiation of the 'on' response since the electrical gradient driving ^{86}Rb efflux would be reduced and any voltage-dependent P_K would be less active. The existence of two opposing actions of tolbutamide provides an explanation for the finding that tolbutamide momentarily increases islet cell ^{86}Rb efflux when this is first lowered by the presence of a stimulatory level of glucose (Henquin, 1980; Malaisse *et al.*, 1980). Under these conditions tolbutamide may directly stimulate inward calcium transport.

It is clear that Co^{2+} reduces the basal rate of ^{86}Rb outflow even in the absence of extracellular calcium, an observation also made by Lebrun *et al.* (1981). The observation led these authors to conclude that cobalt has a direct effect on islet cell P_K, but they did

not consider the possibility that cobalt may, in addition to its calcium-antagonist property, also possess a degree of quinine-like activity on the calcium-activated P_K despite the fact that both quinine and cobalt produce very similar effects. This action would also explain the inhibitory effect of cobalt on the fast depolarization phase that precedes each burst of glucose-induced electrical activity (Meissner & Preissler, 1979), if the calcium-activated P_K is operative in the resting state. The experiments described here show that in fact a calcium-activated P_K makes a substantial contribution to the overall resting ^{86}Rb efflux, since quinine reduces the basal rate of efflux even in the absence of any stimulation (see also Carpinelli & Malaisse, 1980; Herchuelz *et al.*, 1981; Henquin, 1982).

The reversal of the tolbutamide 'on' response in the presence of quinine is reminiscent of a similar increase in islet cell ^{86}Rb efflux that occurs upon the addition of a stimulatory level of glucose to a medium already containing quinine (Carpinelli & Malaisse, 1980; Lebrun *et al.*, 1982; Henquin, 1982). This rise in efflux was not affected by the simultaneous presence of TEA or removal of external calcium. Islet cell depolarization also causes an increase in ^{86}Rb efflux (Matthews & Shotton, 1982; 1983) and, since both glucose and tolbutamide induce depolarization of islet cells, it is likely that the increase in ^{86}Rb efflux produced by these stimulants in the presence of quinine is due to a similar phenomenon, the effect being unmasked when ^{86}Rb efflux is already lowered by the presence of quinine. It is interesting that a similar effect occurs on the addition of tolbutamide in the presence of TEA. Although TEA is believed to act primarily by inhibiting the voltage-dependent gating of potassium channels it has recently been shown to inhibit the calcium-activated P_K in molluscan neurones (Cottrell, 1982). Thus the similarity of its effects to those of quinine may well be due to an inhibition of the same calcium-dependent P_K, particularly since the effects of quinine and TEA on islet cell ^{86}Rb efflux are not additive (Carpinelli & Malaisse, 1980).

The major importance of calcium entry in the control of islet cell permeability is emphasized by the total absence of any effect of tolbutamide in the presence of the calcium antagonist D600. At the concentration used in these experiments (50 μM) D600 totally abolishes the spike activity in mouse pancreatic β-cells due to the addition of either D-glucose or tolbutamide (Matthews & Sakamoto, 1975). The total absence of any decrease in ^{86}Rb efflux upon the addition of tolbutamide may be a consequence of the very much reduced resting rate of efflux produced by the presence of D600. Only quinine was able to produce a comparable decrement in basal Rb efflux, and it is probable that this level may represent the lower limit of islet cell potassium permeability.

In the squid axon the voltage-dependent calcium channel inactivates in a time-dependent manner following stimulation (Baker *et al.*, 1973). It is clear that a similar inactivation process occurs in islet cells. Thus prior stimulation by a low concentration of tolbutamide attenuates the 'off' response following the termination of a subsequent stimulatory period with a higher concentration of tolbutamide. The effect is also apparent when a 'ramp' or gradient of tolbutamide concentration is applied. It appears that the 'off' response following removal of tolbutamide is dependent upon the drug concentration during the early phase of stimulation, and that subsequent alteration of the tolbutamide concentration after this 'critical period' has no influence on the magnitude of the 'off' response. The response to the steeper concentration gradient (from 0 to 1.4 mM in 20 min, see Figure 7) indicates that the first few minutes of stimulation are the most critical. The 'off' response to tolbutamide in this experiment was equivalent to that produced by a constant level of tolbutamide of 0.2 mM or greater, a concentration which is reached approximately 3 min after starting the tolbutamide gradient. If, as seems likely, such a pattern of response is due primarily to the partial inactivation of calcium entry during maintained stimulation then

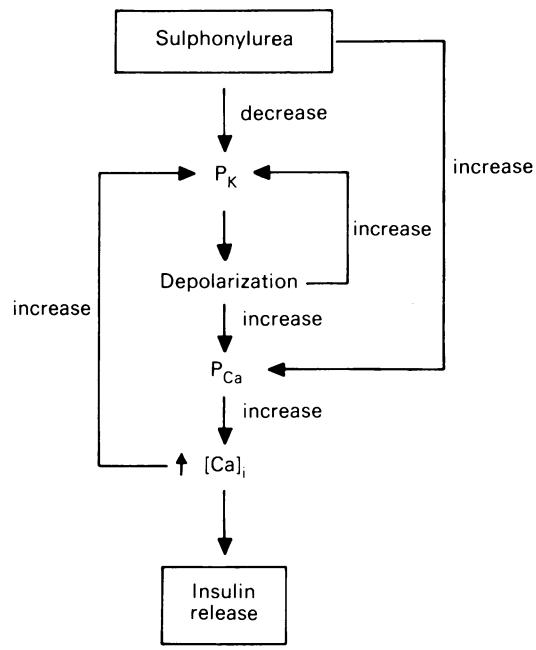

Figure 8 The interacting effects of sulphonylureas on islet β-cell P_K and P_{Ca} leading to insulin release.

this would explain the well-known phasic nature of tolbutamide-stimulated insulin release.

In conclusion, tolbutamide appears to have at least two divergent effects on islet cell ^{86}Rb efflux (see Figure 8). Like glucose, the dominant activity is one of causing a decrease in efflux. However tolbutamide also causes an increase in efflux dependent upon the entry of calcium. Under normal conditions this latter effect is masked during the presence of tolbutamide but is apparent following its removal. For glibenclamide the decrease in K-permeability is evidently not overcome by the entry of Ca^{2+} and build up of $[Ca]_i$, pointing to a more potent and long-lasting inhibitory effect on the calcium-dependent K-channel.

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