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OPPOSITE EFFECTS OF TOLBUTAMIDE AND DIAZOXIDE ON $^{86}Rb^+$ FLUXES AND MEMBRANE POTENTIAL IN PANCREATIC B CELLS

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Abstract—The effects of tolbutamide and diazoxide on $^{86}Rb^+$ fluxes, $^{45}Ca^{2+}$ uptake, insulin release and B cell membrane potential have been studied in rat or mouse islets. In the presence of 3 mM glucose, tolbutamide rapidly and reversibly decreased Rb^+ efflux from perifused islets and depolarised B cells. The effect on Rb^+ efflux was paradoxically more marked with 20 than 100 μg/ml tolbutamide, at least in the presence of extracellular calcium. Addition of tolbutamide to a medium containing 6 mM glucose and calcium increased Rb^+ efflux transiently with 20 μg/ml and permanently with 100 μg/ml. The drug also inhibited Rb^+ influx in islet cells, but had little effect on Rb^+ net uptake. Diazoxide rapidly, steadily and reversibly increased Rb^+ efflux in a dose-dependent manner (20–100 μg/ml). When 20 μg/ml tolbutamide and diazoxide were combined in the presence of 3 mM glucose, only a slight decrease in Rb^+ efflux was observed. The depolarisation of B cells normally produced by tolbutamide was markedly reduced and the electrical activity completely suppressed by diazoxide. In the presence of 10 mM glucose, diazoxide increased Rb^+ efflux from the islets and hyperpolarised B cells. Tolbutamide, tetraethylammonium and quinine reversed the increase in Rb^+ efflux, the inhibition of Ca^{2+} uptake and the suppression of insulin release produced by diazoxide. Tolbutamide rapidly reversed the hyperpolarisation and restored electrical activity. It is suggested that the stimulation and inhibition of insulin release by tolbutamide and diazoxide are due to their respective ability to decrease and to increase the K permeability of the B cell membrane. This change in K permeability leads either to depolarisation and stimulation of Ca^{2+} influx or to hyperpolarisation and inhibition of Ca^{2+} influx.

Tolbutamide is the prototype of hypoglycaemic sulphonylureas widely used to treat non-insulin-dependent diabetic patients. Although it is generally accepted that their primary site of action is located at the B cell membrane [1], the mechanisms by which these drugs stimulate insulin release are not completely understood (for review, see [1, 2]). Recent studies have emphasized the importance of the changes in ionic movements produced in pancreatic B cells by the sulphonylureas [3–9].

Another sulphonamide, diazoxide, possesses hyperglycaemic properties, which make it a useful agent in the management of chronic hypoglycaemic states. An inhibition of insulin release [10], by a direct effect on pancreatic B cells demonstrated *in vitro* [11–13], accounts, at least in part, for its hyperglycaemic action. However, the underlying cellular mechanisms remain obscure [14].

Evidence has accumulated recently indicating that changes in the potassium permeability of the pancreatic B cell membrane play a crucial role in the regulation of insulin release (for review, see [15]). There appears to exist a causal relationship between the ability of various physiological or pharmacological agents to decrease K permeability and to induce insulin release or to increase K permeability and to inhibit insulin release. The present study is an attempt to define the effects of tolbutamide and diazoxide on the potassium permeability of pancreatic B cells, by measuring their modifications of $^{86}Rb^+$ fluxes in isolated rat islets and by recording their influence on the membrane potential of single B cells. Preliminary results have been reported in abstract form [16].

MATERIALS AND METHODS

Insulin release and $^{86}Rb^+$ fluxes were measured in parallel experiments performed with islets isolated by collagenase digestion of the pancreas of fed male Wistar rats. The medium utilized was a Krebs–Ringer bicarbonate buffer, supplemented with 5 mg/ml bovine serum albumin [6]. When $CaCl_2$ was omitted from the medium, it was replaced by $MgCl_2$; when tetraethylammonium chloride was used, the concentration of NaCl was decreased to maintain isoosmolarity. The perifusion system utilized to monitor insulin release or the efflux of $^{86}Rb^+$ from preloaded islets [17, 18] and the technique used to measure the uptake of $^{86}Rb^+$ or $^{45}Ca^{2+}$ by islet cells [6], have been described previously. A few experiments were also performed with islets of female NMRI mice, in order to validate the comparison of experiments made in rat islets with electrophysiological measurements made in mouse B cells.

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Electrophysiological recordings were made in partially microdissected islets of female NMRI mice. Details of the method have been published previously [19]. The medium used was not supplemented with albumin and did not contain PO_4 or SO_4 salts. We did not find that omission of these anions significantly modified the electrical activity. Single cells were impaled with microelectrodes and B cells were identified by their typical electrical activity in response to an intermediate concentration (10 mM) of glucose [19, 20]. Membrane potential was monitored on an oscilloscope and an ink recorder and continuously recorded on tape. The figures shown were obtained by playback of the tape to an ink recorder with high frequency response (Brush Accuchart, Gould).

Tolbutamide or compound [17710] (Hoechst A.G., Frankfurt, West Germany) and diazoxide (Schering Corp., Bloomfield, N.J.) were freshly dissolved each day in NaOH (0.1 M) and aliquots of these stock solutions were added to the appropriate media. A23187 was dissolved in dimethylsulfoxide. Compound [17710] was kindly provided by Prof. M. M. Loubatières (University of Montpellier, France).

Results are presented as means ± S.E.M. or S.D. for efflux experiments. The statistical significance of differences between experimental groups was assessed by Student's *t* test for unpaired data.

RESULTS

Effects of tolbutamide on $^{86}Rb^+$ efflux at various glucose concentrations. In the presence of 3 mM glucose, the rate of $^{86}Rb^+$ efflux from perifused islets declined slowly and regularly (Fig. 1, broken line). Addition of a low concentration of tolbutamide (20 μg/ml) to the medium resulted in a rapid and important decrease in the efflux rate. When the concentration of tolbutamide was subsequently raised to 100 μg/ml, $^{86}Rb^+$ efflux rate increased, but remained below control values. This paradoxical effect was not due to the sequence of stimulation. When 100 μg/ml tolbutamide was applied first, a fall in $^{86}Rb^+$ efflux was observed, but a much more pronounced decrease followed the switch to the lower concentration of sulphonylurea (Fig. 1, middle panel). The absolute rates of efflux measured in the presence of either 20 or 100 μg/ml tolbutamide were not affected by the sequence of stimulation. The effect of tolbutamide was completely reversible, as evidenced by the acceleration of $^{86}Rb^+$ efflux which followed removal of the sulphonylurea.

In the absence of extracellular Ca^{2+} (Fig. 1, right panel), 100 μg/ml tolbutamide decreased $^{86}Rb^+$ efflux much more ($P < 0.001$) than in the presence of the cation. Reintroduction of Ca^{2+} in the medium resulted in a large increase in the efflux rate.

The same experiments were repeated in the presence of 6 mM glucose, a concentration just above the threshold for stimulation of insulin release in this system [18]. In these conditions, the rate of $^{86}Rb^+$ efflux was low and stable (Fig. 1, lower panels), but tolbutamide increased it. The effect was only transient with 20 μg/ml and permanent with 100 μg/ml. When the high concentration of tolbutamide was applied first, a large increase in efflux occurred, followed by a return to control values upon lowering the concentration of the drug to 20 μg/ml. In the absence of Ca^{2+} (Fig. 1, right panel), 100 μg/ml tolbutamide no longer increased, but decreased $^{86}Rb^+$ efflux. Reintroduction of Ca^{2+} in the presence of tolbutamide markedly accelerated the efflux rate.

The increase in $^{86}Rb^+$ efflux produced by the two concentrations of tolbutamide was much smaller in the presence of a higher concentration of glucose (15 mM), (not shown).

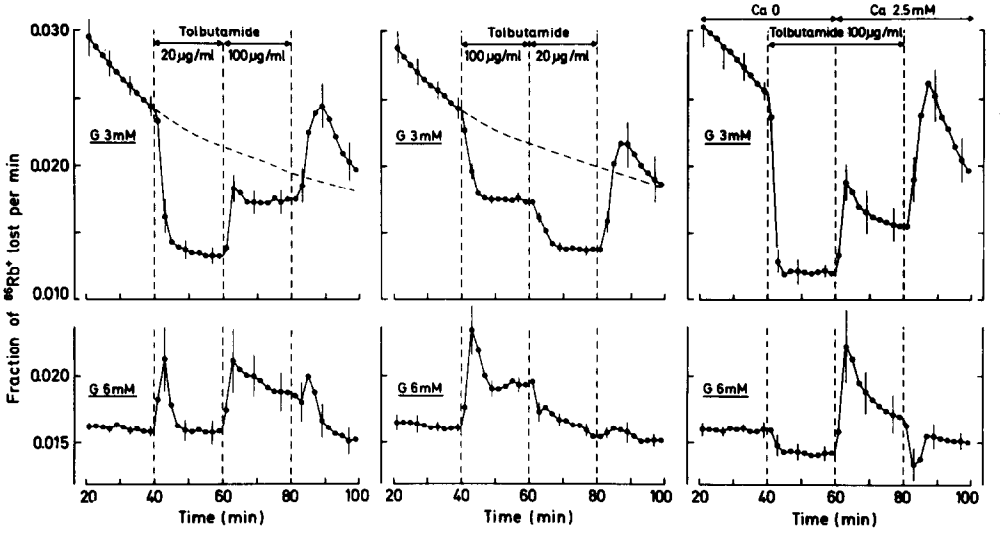

Fig. 1. Effects of tolbutamide on $^{86}Rb^+$ efflux from rat islets perifused in the presence of 3 or 6 mM glucose (G). Tolbutamide was added for the periods and at the concentrations indicated in the upper part of the figure. In the right panel, calcium was omitted from the medium until 60 min. The broken line in the upper panels corresponds to control islets without tolbutamide. Values are means ± S.D. of 4 experiments in each group.

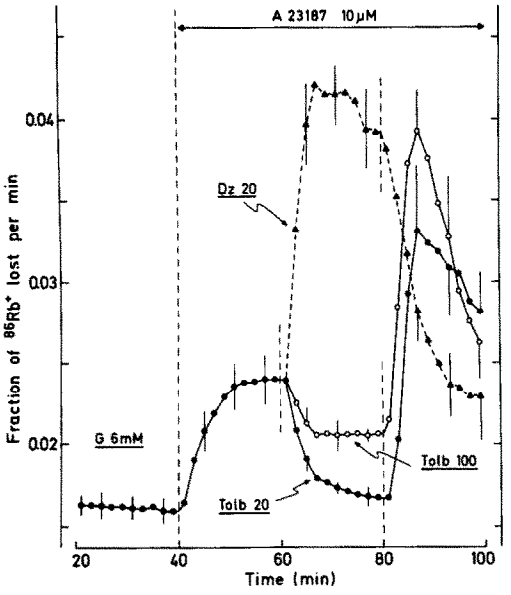

Fig. 2. Effects of tolbutamide and diazoxide on the increase in $^{86}Rb^+$ efflux produced by the divalent cation ionophore A23187 in rat islets perifused in the presence of 6 mM glucose. Diazoxide (Dz 20 μg/ml) or tolbutamide (Tolb 20 or 100 μg/ml) was added only between 60 and 80 min. The ionophore (10 μM) was present from 40 to 100 min. Values are means ± S.D. of 3 experiments in each group.

Effects of tolbutamide on Ca-*activated* $^{86}Rb^+$ *efflux.* As previously reported [21], in the presence of calcium, the divalent cation ionophore A23187 increased $^{86}Rb^+$ efflux from the islets (Fig. 2). Tolbutamide inhibited this effect with a greater efficacy at low (20 μg/ml) than at high concentration (100 μg/ml). Removal of the sulphonylurea was followed by a large increase in $^{86}Rb^+$ efflux. This effect of tolbutamide was not secondary to an interference with the stimulation of Ca^{2+} uptake by the ionophore. On the contrary, tolbutamide potentiated, in a dose-dependent manner, the uptake of Ca^{2+} triggered by A23187 (Table 1).

Effects of tolbutamide on $^{86}Rb^+$ *uptake by incubated islets.* The uptake of the tracer $^{86}Rb^+$ was measured after 5 min of incubation, to evaluate the entry of K^+ in islet cells, and after 60 min, to estimate its net uptake under conditions approaching equilibrium. After 5 min, $^{86}Rb^+$ uptake was inhibited by about 33 and 55% in the presence of 20 and 100 μg/ml tolbutamide, respectively (Table 2). This inhibition by tolbutamide was of rapid onset, as preincubation of the islets with the drug for 30 min before measurement of $^{86}Rb^+$ uptake did not increase it. Net uptake of $^{86}Rb^+$ was also decreased by tolbutamide (Table 2). However, this effect of the sulphonylurea was much less marked than after short (5 min) incubation, particularly at the lower concentration of the drug (<10%). The stimulation of $^{86}Rb^+$

Table 1. Effects of diazoxide and tolbutamide on Ca^{2+} uptake by rat islets

Test conditions	Ca^{2+} uptake (pmol/islet)
a) Incubation for 30 min	
Glucose 6 mM	10.1 ± 0.7 ††
Glucose 6 mM + A23187 10 μM	17.9 ± 1.2
Glucose 6 mM + A23187 10 μM + Tolbutamide 20 μg/ml	23.0 ± 1.6 ††
Glucose 6 mM + A23187 10 μM + Tolbutamide 100 μg/ml	26.3 ± 1.9 ††
Glucose 6 mM + A23187 10 μM + Diazoxide 20 μg/ml	13.7 ± 1.3 †
b) Incubation for 60 min	
Glucose 10 mM	29.8 ± 1.6 §
Glucose 10 mM + Diazoxide 20 μg/ml	10.3 ± 0.5
Glucose 10 mM + Diazoxide 20 μg/ml + Tetraethylammonium 20 mM	16.7 ± 0.9 §
Glucose 10 mM + Diazoxide 20 μg/ml + Quinine 50 μM	25.8 ± 1.3 §
Glucose 10 mM + Diazoxide 20 μg/ml + Tolbutamide 20 μg/ml	29.9 ± 1.3 §

After preliminary incubation for 30 min in the presence of 3 mM glucose alone, batches of seven islets were incubated for 30 or 60 min in 100 μl of medium layered on silicone oil. The medium had the indicated composition and contained 0.25 mM [6,6'-^{3}H]sucrose (0.1 Ci/mmole) and 2.5 mM $^{45}CaCl_2$ (10 mCi/mmole). At the end of the incubation period, the islets were separated from the radioactive solution by centrifugation through the oil. Values are means ± S.E.M. of 8 batches of islets (2 separate experiments) in (a) and of 15 batches (3 separate experiments) in (b).

Significance levels: † $P < 0.05$ and †† $P < 0.001$ vs 6 mM glucose + 10 μM A23187. § $P < 0.001$ vs 10 mM glucose + 20 μg/ml diazoxide.

Table 2. Effects of tolbutamide and diazoxide on $^{86}Rb^+$ uptake by rat islets

Test conditions	$^{86}Rb^+$ uptake (pmol K^+/islet)				
	Controls	Tolbutamide (20 μg/ml)	Tolbutamide (100 μg/ml)	Diazoxide (20 μg/ml)	Diazoxide (100 μg/ml)
a) Incubation for 5 min					
Glucose 3 mM	204 ± 6 (26)	135 ± 4 (14) $^{+++}$	99 ± 4 (14) $^{+++}$	286 ± 15(12) $^{+++}$	277 ± 16(12) $^{+++}$
b) Incubation for 5 min with 30 min of preincubation in same conditions					
Glucose 3 mM	206 ± 4 (26)	138 ± 7 (14) $^{+++}$	91 ± 4 (14) $^{+++}$	267 ± 8 (14) $^{+++}$	260 ± 9 (14) $^{+++}$
c) Incubation for 60 min					
Glucose 3 mM	614 ± 15(33)	565 ± 12(16) $^{+}$	376 ± 14(16) $^{+++}$	568 ± 20(17)	529 ± 20(17) $^{++}$
Glucose 10 mM	876 ± 18(25)	792 ± 25(14) $^{+}$	724 ± 16(14) $^{+++}$	930 ± 35(14)	971 ± 25(14) $^{++}$

In experimental series (a) and (c), islets were first preincubated for 30 min in the presence of 3 mM glucose alone; in series (b), the preincubation medium also contained tolbutamide or diazoxide at the same concentration as in the subsequent incubation. Each test substance was studied in 3 or 4 separate experiments. Batches of six islets were then incubated for 5 or 60 min in 100 μl of medium layered on silicone oil. The medium had the indicated composition and contained 0.25 mM [6,6'-^{3}H]sucrose (0.1 Ci/mmole) and 0.05 mM $^{86}Rb^+$ used as tracer for K^+ (3 mCi/mmole of K^+). The measured $^{86}Rb^+$ uptake was expressed as pmole of K^+.

Values are means ± S.E.M. of a number of batches of islets given in parentheses.

Significance levels: † $P < 0.05$, †† $P < 0.005$ and ††† $P < 0.001$ vs controls.

net uptake by glucose was not prevented by tolbutamide, although a slight decrease was still present.

Effects of compound [17710]. Compound [17710] is a non-insulinotropic derivative of tolbutamide. Its effects on $^{86}Rb^+$ efflux from perifused islets were tested at the concentration of 20 or 100 μg/ml, in three experiments performed in the presence of 3 and 6 mM glucose. The mean efflux rate of $^{86}Rb^+$ was not significantly modified by the substance (95–101% of controls). At the concentration of 100 μg/ml, compound [17710] did not affect $^{86}Rb^+$ uptake by islets incubated for 5 min (197 ± 5 vs 197 ± 7 pmoles/islet) or for 60 min (640 ± 24 vs 626 ± 34 pmoles/islet; $n = 8$). At the same concentration, it had no significant effect on the membrane potential of B cells, perifused with a medium containing 3 mM glucose (not shown).

Effects of diazoxide on $^{86}Rb^+$ efflux. Addition of diazoxide (20 μg/ml) to a medium containing 3 mM glucose caused a prompt and marked increase in the efflux rate of $^{86}Rb^+$, which subsequently declined but remained clearly higher than in controls (Fig. 3). A rapid fall in $^{86}Rb^+$ efflux followed withdrawal of diazoxide from the perifusion medium. When diazoxide and tolbutamide were added simultaneously, at the concentration of 20 μg/ml, a decrease in efflux occurred, which was similar to that observed with tolbutamide alone during the first 6 min. Thereafter, the efflux rate increased progressively and stabilized at values slightly lower than in controls. Upon removal of diazoxide, the unabated effect of tolbutamide became apparent and the rate of $^{86}Rb^+$ efflux decreased (Fig. 3).

In the presence of a high concentration of glucose (15 mM), diazoxide increased $^{86}Rb^+$ efflux rate, in

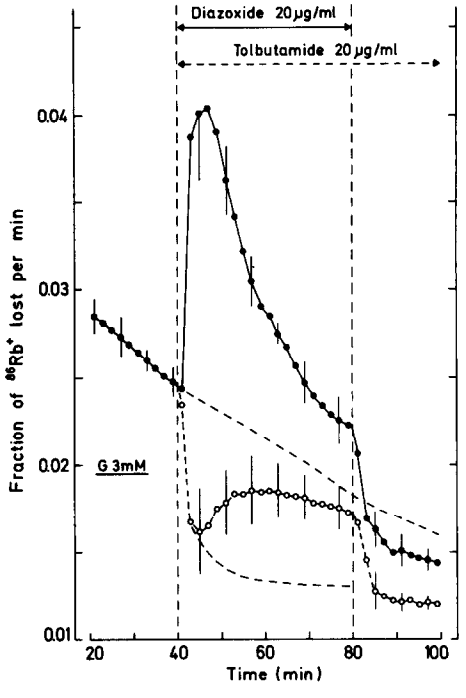

Fig. 3. Effects of diazoxide alone or with tolbutamide on $^{86}Rb^+$ efflux from rat islets perifused in the presence of 3 mM glucose. Diazoxide (20 μg/ml) was present between 40 and 80 min. It was added alone (●) or together with 20 μg/ml tolbutamide (○), which remained present until 100 min. The upper broken line corresponds to controls without any drug; the lower broken line shows the effect of 20 μg/ml tolbutamide alone. Values are means ± S.D. of 4 experiments in each group.

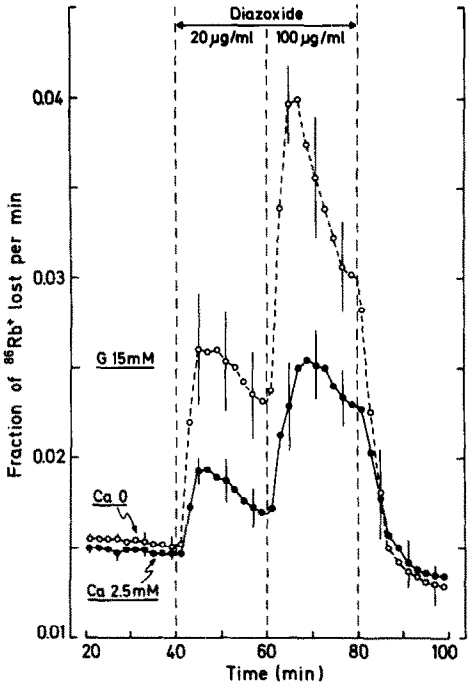

Fig. 4. Effects of diazoxide on $^{86}Rb^+$ efflux from rat islets perifused in the presence of 15 mM glucose. One series of experiments was performed with a medium containing 2.5 mM $CaCl_2$ (●), the other series in a medium devoid of $CaCl_2$ (○). Values are means ± S.D. of 4 experiments in each group.

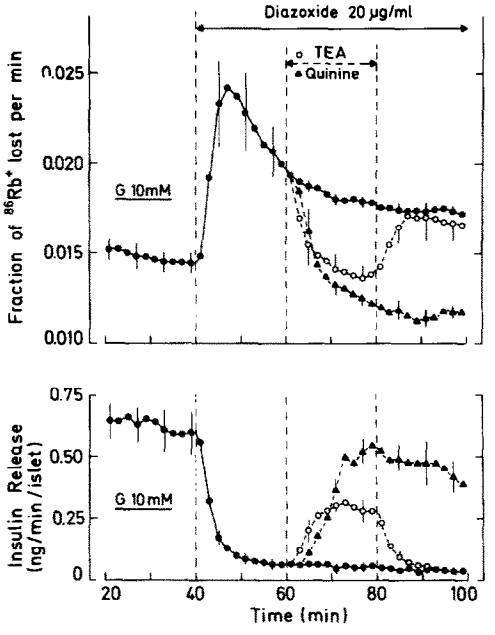

Fig. 6. Effects of diazoxide on $^{86}Rb^+$ efflux and insulin release from rat islets perifused in the presence of 10 mM glucose and their reversal by tetraethylammonium (TEA) or quinine. TEA (20 mM) or quinine (50 μM) was added only between 60 and 80 min. Diazoxide (20 μg/ml) remained present from 40 to 100 min. Values are means ± S.D. of 3 experiments in each group.

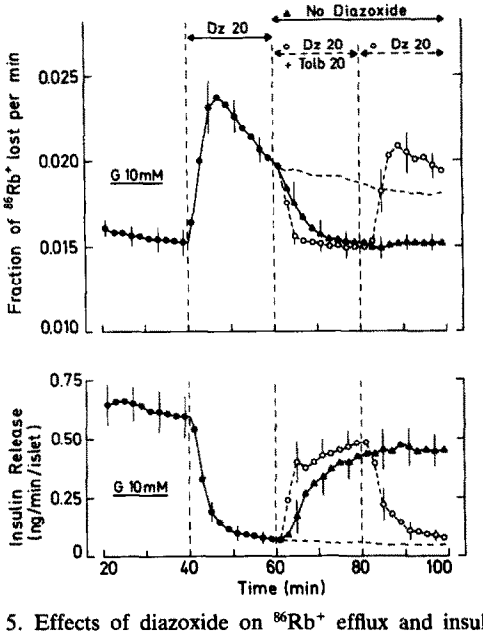

Fig. 5. Effects of diazoxide on $^{86}Rb^+$ efflux and insulin release from rat islets perifused in the presence of 10 mM glucose and their reversal by tolbutamide. In one series of experiments (▲), diazoxide (Dz, 20 μg/ml) was present only from 40 to 60 min. In the other series (○), diazoxide was present until 100 min and tolbutamide (Tolb, 20 μg/ml) was added between 60 and 80 min. The broken line corresponds to control islets with diazoxide alone between 40 and 100 min. Values are means ± S.D. of 4 experiments in each group.

a dose-dependent manner (Fig. 4). This effect did not require the presence of extracellular calcium. On the contrary, the rise in efflux produced by diazoxide was unexpectedly amplified by omission of calcium from the perifusion medium, although the absence of the cation had no effect on the control efflux rate.

Effects of diazoxide on Ca-*activated* $^{86}Rb^+$ *efflux.* When $^{86}Rb^+$ efflux was accelerated by the divalent cation ionophore A23187, diazoxide was still able to increase it markedly (Fig. 2). This effect did not fade out during the 20 min of diazoxide presence, but was quickly reversible upon withdrawal of the drug. It occurred although diazoxide slightly inhibited Ca^{2+}-uptake triggered by A23187 (Table 1).

Correlations between the effects of diazoxide on $^{86}Rb^+$ *efflux and insulin release.* The links between the increase in $^{86}Rb^+$ efflux and the inhibition of insulin release produced by diazoxide have been evaluated in the experiments illustrated by Figs. 5 and 6. In islets perifused with 10 mM glucose, diazoxide (20 μg/ml) caused a simultaneous increase in $^{86}Rb^+$ efflux and decrease in insulin release. The efflux rate reached a peak value 6–10 min after addition of the drug and then stabilized; insulin release was totally suppressed in a monophasic manner (Fig. 5). These effects of diazoxide were reversible as shown by the progressive decrease in $^{86}Rb^+$ efflux rate and the increase in insulin release, which followed removal of the drug. Addition of tolbutamide to a medium containing diazoxide abolished the

increase in $^{86}Rb^+$ efflux and the inhibition of insulin release produced by this latter. Both effects of tolbutamide were reversible and faster than the changes produced by simple withdrawal of diazoxide itself (Fig. 5). Essentially similar results were obtained with mouse islets (not shown). Tetraethylammonium and quinine have been shown to decrease the potassium permeability of islet cells [17, 21]. They reversed the acceleration of $^{86}Rb^+$ efflux produced by diazoxide and, concomitantly, increased insulin release (Fig. 6). The effect of tetraethylammonium was of faster onset and of smaller amplitude than that of quinine on both efflux and release. It was also completely reversible upon removal of the substance, whereas the effect of quinine was not reversible.

Table 1 shows that diazoxide markedly decreased Ca^{2+} uptake stimulated by 10 mM glucose. This inhibition was partially antagonized by tetraethylammonium, almost completely prevented by quinine, and totally suppressed by tolbutamide.

Effects of diazoxide on $^{86}Rb^+$ uptake by incubated islets. $^{86}Rb^+$ uptake measured after 5 min, was increased by roughly 30% in the presence of 20 or 100 μg/ml diazoxide; the effect tended to be lower, although not significantly, when the measurement was not made immediately after addition of diazoxide, but after 30 min of preincubation with the drug. $^{86}Rb^+$ net uptake was slightly decreased by diazoxide in the presence of 3 mM glucose and slightly increased in the presence of 10 mM glucose. These differences were significant only with 100 μg/ml diazoxide.

Effects of tolbutamide and diazoxide on membrane potential of B cells. In the presence of 10 mM glucose, the membrane potential of B cells exhibited repetitive slow waves with a fast spike activity originating from the plateau level (Fig. 7). On addition of diazoxide (20 μg/ml) to the perifusion medium, the membrane hyperpolarized markedly (Fig. 7A), to −68 mV ± 3 (S.E.M.) in five different mice, and the spike activity ceased. Removal of the drug was followed by a progressive depolarization and finally by reappearance of a normal electrical activity. When tolbutamide was added to a medium containing diazoxide and glucose, a rapid depolarization to the plateau level occurred, with appearance of a continuous spike activity (Fig. 7B). Progressively the frequency of these spikes decreased and the electrical activity changed into regular slow waves. Figure 7C illustrates the potentiation by tolbutamide (20 μg/ml) of the electrical activity induced by 10 mM glucose alone. After addition of the sulphonylurea, the membrane remained depolarized at the plateau level and a continuous spike activity was observed. When diazoxide (20 μg/ml) was subsequently added, the frequency of the spikes decreased and regular slow waves of the membrane potential developed. Discontinuation of diazoxide led to reappearance of the continuous activity (Fig. 7C).

In the presence of a low glucose concentration (3 mM), the membrane potential of B cells was high (−67 mV ± 1 S.E.M., $n = 4$) and no electrical activity was observed. Tolbutamide (20 μg/ml) produced a rapid depolarization and evoked a continuous spike activity (Fig. 8A). Addition of diazoxide

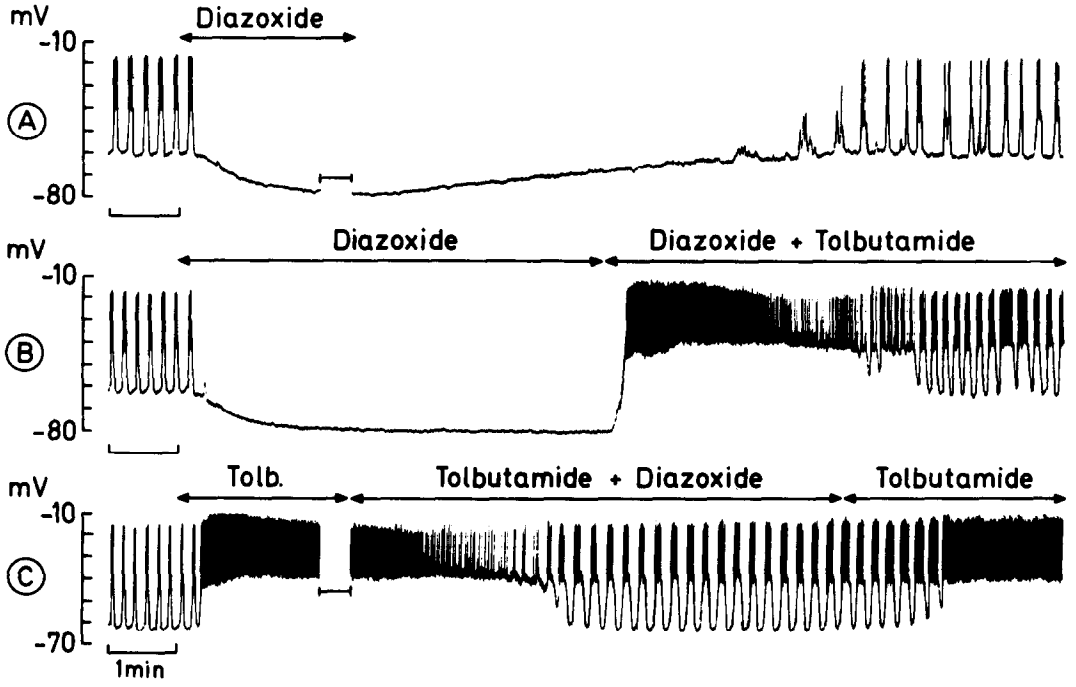

Fig. 7. Effects of diazoxide (20 μg/ml) and tolbutamide (20 μg/ml) on membrane potential of a single mouse B cell. The concentration of glucose was 10 mM. (A) Diazoxide was added for 6 min, the last 4 of which are not shown. (B) Diazoxide was present for 6 min, before addition of tolbutamide. (C) Tolbutamide was present for 9 min before addition of diazoxide, but only the first 2 min of that period are shown. All records were obtained in the same cell. Records B and A and records C and B are separated by an interval of 14 and 8 min in G10 mM alone, respectively. These records are representative of results obtained in 5 different animals.

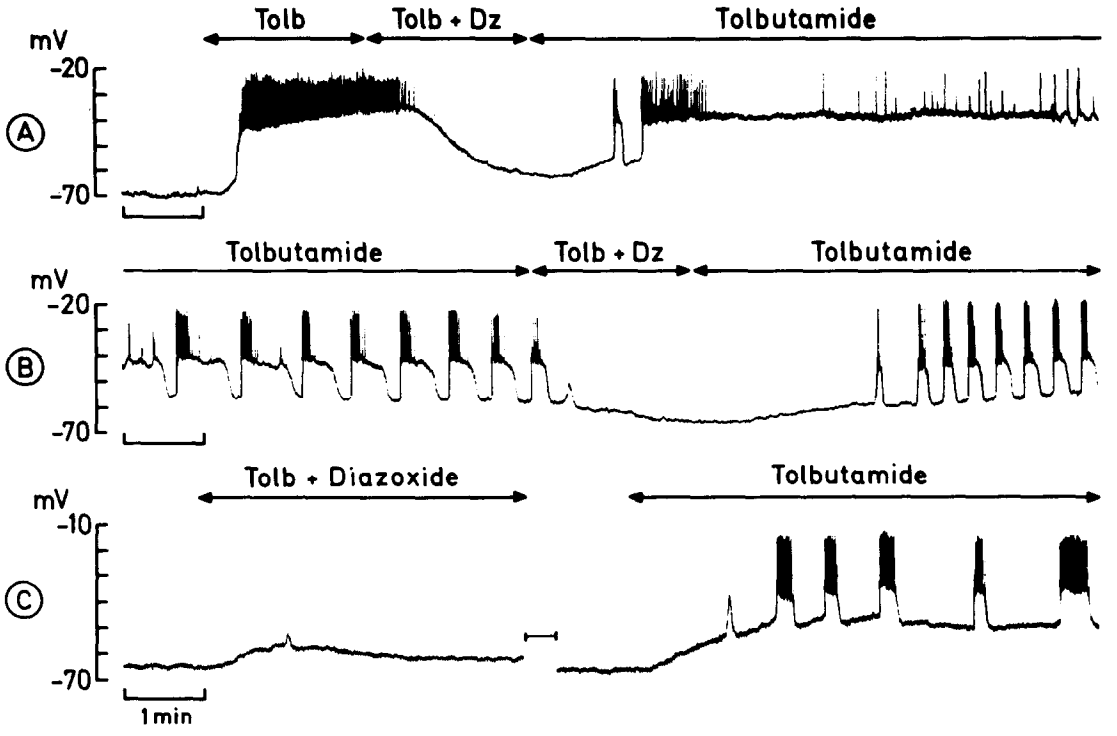

Fig. 8. Effects of diazoxide (20 μg/ml) and tolbutamide (20 μg/ml) on membrane potential of a single mouse B cell. The concentration of glucose was 3 mM. Record B is the direct continuation of record A. Record C was obtained in another cell. The concentration of glucose was decreased from 10 to 3 mM 10 min before addition of tolbutamide alone (A) or with diazoxide (C). In C, an interval of 8 min in glucose 3 mM alone is not shown. These records are representative of results obtained in 4 different animals.

(20 μg/ml) was followed by suppression of this activity and hyperpolarization, whereas progressive depolarization and resumption of activity occurred upon withdrawal of the drug. For several minutes, the membrane remained depolarized at the plateau level and only few spikes were observed. Afterwards the membrane potential exhibited slow waves and spike activity appeared on the plateau (Fig. 8B). They were also suppressed by diazoxide which, again, reversibly hyperpolarized the B cell membrane.

When tolbutamide and diazoxide were added simultaneously, a slight and reversible depolarization of the B cell occurred, but no electrical activity was observed (Fig. 8C). A subsequent stimulation with tolbutamide alone induced a more marked depolarization with slow waves of electrical activity.* In four different mice, the depolarization between the resting membrane potential and the plateau potential averaged 32 mV ± 1 (S.E.M.) with tolbutamide alone; on the other hand the maximum depolarization was only 8.5 mV ± 1 when tolbutamide and diazoxide were combined.

* The characteristics of the depolarization and of the electrical activity induced in B cells by low concentrations of tolbutamide are markedly influenced by several factors like the glucose concentration, the length of glucose deprivation, and the duration of stimulation. (H.P.M., unpublished observations).

DISCUSSION

The present results clearly show that tolbutamide and diazoxide exert opposite effects on $^{86}Rb^+$ fluxes in pancreatic islet cells and on the membrane potential of B cells. They strongly suggest that the potassium permeability of the B cell membrane is a primary target for the action of these sulphonamides.

Effects of tolbutamide. Plasma levels of tolbutamide in diabetic patients are below 60 μg/ml [22], but much higher concentrations have generally been used for *in vitro* studies. The actual difference is even greater, owing to the strong binding of tolbutamide to albumine, the concentration of which is several-fold higher in plasma than in incubation buffers. Recent experiments have shown that lower concentrations of tolbutamide are sufficient to stimulate insulin release by isolated islets [6, 9] or the perfused pancreas [23] and to induce electrical activity in B cells [7]. Furthermore, it has become evident that certain effects of the high concentrations of tolbutamide qualitatively differ from those obtained with lower concentrations [6, 7]. Their relevance to the clinical effects of the drug may thus be questioned.

Earlier experiments with 100–200 μg/ml tolbutamide have shown that the drug decreases the rate of $^{86}Rb^+$ efflux from islet cells [24–26]. The present study demonstrates that this effect is more marked at lower concentrations and was thus underesti-

mated. These results, combined with the evidence that the drug depolarizes B cells, reinforce the suggestion [6, 7, 24] that the decrease in $^{86}Rb^+$ efflux produced by tolbutamide in whole islets (comprising 75% of B cells) reflects a decrease in the potassium permeability of the B cell membrane. It is likely that this decrease in potassium permeability underlies the depolarization, which subsequently activates voltage dependent Ca-channels [6, 7]. The observation that the tolbutamide analogue, compound [17710], devoid of effects on $^{86}Rb^+$ fluxes, does not change membrane potential and calcium fluxes or insulin release [9] lends support to that proposal.

The inhibition of $^{86}Rb^+$ influx by tolbutamide was directly dose-dependent, whereas the decrease in $^{86}Rb^+$ efflux was less marked at high concentrations, at least in the presence of extracellular calcium. This unexpected behaviour was observed not only in control conditions, but also when the Ca-sensitive K permeability of islet cells [21] was increased by the ionophore A23187. In contrast to low concentrations of tolbutamide which produce regular slow waves of the membrane potential, these high concentrations of tolbutamide produce a permanent depolarisation of B cells [7]. One must assume, therefore, that they exert an additional effect, which increases $^{86}Rb^+$ efflux slightly and thus tends to mask the genuine effect of the sulphonylurea on K-channels. Two possibilities deserve a short comment. First, tolbutamide could interfere with the normal functioning of the electrogenic [20] Na/K pump of B cells. It has been speculated earlier that such an inhibition contributes to the permanent depolarization of B cells by glibenclamide [4]. Recent unpublished experiments (HPM and JCH) could support this possibility, which would also be consistent with the tendency of tolbutamide to increase Na^+ uptake in islet cells [8] and with its inhibition of $^{86}Rb^+$ influx. However, the real significance of this latter observation cannot be fully appreciated until discrimination of K^+ fluxes in ouabain-sensitive active transport and ouabain-insensitive passive exchange is completed. Secondly, an increased inward depolarising current, carried by Ca^{2+} ions, could also be involved in the permanent depolarisation produced by the high concentrations of tolbutamide. The partial repolarisation observed when cobalt is superimposed on tolbutamide [7] is consistent with this possibility.

Either of the above-mentioned possibilities could also explain the Ca^{2+}-dependent increase in $^{86}Rb^+$ efflux produced by 100 μg/ml tolbutamide in the presence of glucose. It should be noted, however, that other substances like leucine [27] or theophylline [28] also increase $^{86}Rb^+$ efflux slightly when the potassium permeability of islet cells has been reduced by glucose.

From studies in artificial systems, it has been inferred [29, 30] that the primary effect of hypoglycaemic sulphonylureas is a facilitation of Ca^{2+} transport across B cell membranes, thanks to their ionophoretic capacity. The biological relevance of such an effect, observed only with high concentrations of the drugs, was not supported by a recent investigation evaluating the changes in Ca^{2+} fluxes produced by tolbutamide in isolated islets [9]. The present results provide no support, but cannot rule out, such a side effect of high concentrations of tolbutamide.

Effects of diazoxide. Plasma levels of diazoxide in treated patients range between 10 and 50 μg/ml, with approximately 90% of the drug bound to albumin [31, 32]. It is thus reasonable to assume that the effects observed in these *in vitro* experiments with 20 μg/ml diazoxide may be relevant to the therapeutic action of the drug.

It has been reported that glucose oxidation by isolated islets is inhibited by 200 μg/ml diazoxide [33], but not by 120 μg/ml diazoxide [34]. It is thus unlikely that an impairment of glucose metabolism accounts for the effects observed here with an even lower concentration (20 μg/ml). Diazoxide-inhibition of glucose-stimulated Ca^{2+} uptake by islet cells has also been reported [35, 36] and is confirmed here. At first sight, a blockade of Ca-channels by diazoxide could thus suffice to explain the inhibition of glucose-induced insulin release. However, such a property would not explain why diazoxide does not inhibit insulin release induced by potassium [37] or arginine [38, 39].

The observation that, in the presence of 10 mM glucose, diazoxide rapidly increases $^{86}Rb^+$ efflux from islet cells, combined with the evidence that the drug hyperpolarises B cells, strongly suggests that the primary effect of diazoxide is to increase the potassium permeability of the B cell membrane. This hyperpolarisation may, in turn, inactivate voltage-dependent Ca-channels and, consequently, inhibit Ca^{2+} influx, electrical activity and insulin release. Such a sequence is in complete agreement with the reversal of all diazoxide effects by tolbutamide. Thus, the sulphonylurea decreased the accelerated $^{86}Rb^+$ efflux, depolarised B cells, normalised Ca^{2+} uptake, led to reappearance of electrical activity and restored insulin release. That sequence is further supported by the partial or complete normalisation of $^{86}Rb^+$ efflux rate, Ca^{2+} uptake and insulin release by tetraethylammonium and quinine. The effects of these two latter agents on B cells have been ascribed to their ability to decrease the potassium permeability [17, 21]. It is also pertinent to note that quinine is unable to restore insulin release inhibited by a blocker of Ca-channels such as D600; furthermore D600 does not increase $^{86}Rb^+$ efflux and does not polarise the B cell membrane (unpublished observations). Finally this mode of action of diazoxide also explains why the drug does not inhibit insulin release triggered by a high concentration of extracellular K^+, and even by arginine. The depolarisation of B cells by arginine is attended by an increase in $^{86}Rb^+$ efflux from islet cells [27] and, thus, does not result from a primary decrease in the potassium permeability of the B cell membrane.

It is known that diazoxide suppresses insulin release induced by tolbutamide in the absence or in the presence of a non-stimulatory concentration of glucose [37, 40]. The present experiments show that 20 μg/ml diazoxide abolishes the electrical activity induced by 20 μg/ml tolbutamide without completely suppressing the depolarising effect of the sulphonylurea. This is consistent with the persistence of a slight decrease in $^{86}Rb^+$ efflux when both drugs are added together. The interaction tolbutamide–diaz-

oxide is extremely dose-dependent [40]: at a given ratio, the insulinotropic effect of tolbutamide is completely suppressed in the absence of glucose, although the sulphonylurea can restore insulin release suppressed by diazoxide in the presence of 8.3 mM glucose. Similar observations were made here with $^{86}Rb^+$ fluxes and membrane potential. One possible explanation is that both glucose and tolbutamide concur to antagonise the increase in potassium permeability of the B cell membrane by diazoxide.

In conclusion, it is proposed that the stimulation and inhibition of insulin release by clinically relevant concentrations of tolbutamide and diazoxide are due to their respective ability to decrease and to increase the potassium permeability of the pancreatic B cell membrane. This change in potassium permeability leads to depolarisation or hyperpolarisation of B cells with either activation or inactivation of voltage-dependent Ca-channels and finally stimulation or inhibition of Ca^{2+} influx.

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