Effects of putative activators of K⁺ channels in mouse pancreatic β -cells

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1 The vasodilator and antihypertensive properties of pinacidil, cromakalim (BRL 34915), nicorandil and minoxidil sulphate may be due, at least in part, to their ability to open K⁺ channels in vascular smooth muscles. In this study, mouse pancreatic islets were used to determine whether these drugs affect insulin release by acting on K⁺ channels of β -cells. Their effects were compared to those of diazoxide.

2 Diazoxide caused a dose-dependent inhibition of insulin release by islets incubated with 15 mM glucose (93% at 100 μ M). Pinacidil inhibited release by 36 and 72% at 100 and 500 μ M, respectively. Cromakalim and nicorandil were less effective (35 and 25% inhibition at 500 μ M). Minoxidil sulphate increased insulin release at 500 μ M.

3 In the presence of 7 mM glucose and in the absence of Ca²⁺ (to avoid activation of Ca²⁺-dependent K⁺ channels), ⁸⁶Rb efflux from islet cells was increased by 100–500 μ M pinacidil and 500 μ M nicorandil, which were, however, less potent than diazoxide. Cromakalim was ineffective, whereas 500 μ M minoxidil sulphate decreased the efflux rate. In the absence of glucose and presence of Ca²⁺, 500 μ M cromakalim and minoxidil sulphate inhibited ⁸⁶Rb efflux.

4 Like diazoxide, pinacidil (500 μ M) abolished glucose-induced electrical activity in β -cells and hyperpolarized the membrane.

5 ATP-sensitive K⁺ currents were studied in single β -cells by the whole cell patch-clamp technique. Pinacidil increased the current less than did diazoxide. In contrast, cromakalim and minoxidil sulphate decreased K⁺-currents whilst nicorandil was without effect.

6 It is concluded that pinacidil, like diazoxide, inhibits insulin release from β -cells by opening ATP-sensitive K⁺ channels, whereas the smaller inhibitory effects of cromakalim and nicorandil may involve actions other than on K⁺ channels in these cells. Minoxidil sulphate potentiates glucose-induced insulin release, probably by inhibiting ATP-sensitive K⁺ channels. However, all these effects of the vasodilators are only seen at high concentrations and are thus unlikely to occur *in vivo*.

Introduction

Regulation of insulin release by many secretagogues involves a coordinated control of K⁺ and Ca²⁺ permeabilities of the β -cell membrane (Henquin, 1987; Petersen & Findlay, 1987). Studies with intracellular microelectrodes and measurements of ionic fluxes first showed that a decrease in K⁺ permeability of the plasma membrane underlies glucose-induced depolarization to the threshold potential, at which voltage-dependent Ca²⁺ channels are activated (Sehlin & Täljedal, 1975; Henquin, 1978; Atwater *et al.*, 1978).

Subsequent patch-clamp studies demonstrated that this decrease is due to closure of ATP-sensitive K⁺ channels (Ashcroft *et al.*, 1984; Cook & Hales, 1984; Rorsman & Trube, 1985). Hypoglycaemic sulphonylureas, the most important pharmacological stimulators of insulin release, appear to induce a similar sequence of events by directly closing the same K⁺ channels (Henquin, 1980; Henquin & Meissner, 1982; Sturgess *et al.*, 1985; Trube *et al.*, 1986). On the other hand, the hyperglycaemic sulphonamide, diazoxide, antagonizes the effects of glucose or tolbutamide by opening these ATP-sensitive K⁺ channels (Henquin & Meissner,

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1982; Trube *et al.*, 1986; Dunne *et al.*, 1987; Zünkler *et al.*, 1988).

A new class of drugs, able to relax smooth muscles apparently by opening K^+ channels, is currently arousing great interest because of their potential use as vasodilator and antihypertensive agents. The best characterized of these drugs are pinacidil, cromakalim (BRL 34915), nicorandil and minoxidil sulphate (Cook, 1988; Hamilton & Weston, 1989).

The objective of the present study was to determine whether these putative activators of K^+ channels affect pancreatic β -cell function. Their effects were compared to those of diazoxide. Our aims were first to gain more insight into the tissue-specificity of the action of the K^+ channel activators and, second, to predict whether alterations of glucose homeostasis could be expected in patients eventually treated with these drugs.

Methods

Measurements of insulin release, ^{86}Rb efflux and β -cell membrane potential

All experiments were performed with islets of fed female NMRI mice (25–30 g) killed by decapitation. For measurement of the membrane potential of β -cells with an intracellular microelectrode, a piece of pancreas was fixed in a small chamber and continuously perfused (Meissner & Schmelz, 1974). β -Cells were identified by the typical electrical activity that they display in the presence of a stimulatory concentration of glucose. For all other experiments, islets were isolated after collagenase digestion of the pancreas (Lacy & Kostianovsky, 1967). The effect of the various test agents on insulin release was measured with incubated islets. Immediately after isolation, the islets were preincubated for 45 min at 37°C , in a medium containing 15 mM glucose and 2.5 mM Ca^{2+} . Batches of 3 islets were then incubated for 60 min in 1 ml of medium containing 15 mM glucose, 2.5 mM Ca^{2+} and appropriate concentrations of the test agents. A portion of the medium was taken at the end of the incubation for measurement of insulin, with rat insulin used as standard (Henquin & Lambert, 1975). For measurement of the efflux of ^{86}Rb (used as tracer for K), the islets were first loaded with the tracer during 90 min in a medium containing 15 mM glucose and 2.5 mM Ca^{2+} (Garrino & Henquin, 1988). They were then transferred to a dynamic system of perfusion permitting monitoring of ^{86}Rb efflux (Henquin, 1978). The concentrations of glucose and Ca^{2+} used for these experiments are indicated in the legend to each figure.

The solutions used for measurements of insulin release, ^{86}Rb efflux and β -cell membrane potential

had the following ionic composition (mM): NaCl 120, KCl 4.8, CaCl_2 2.5, MgCl_2 1.2, NaHCO_3 24. Ca^{2+} -free solutions were prepared by replacing CaCl_2 with MgCl_2 ; the concentration of total residual Ca^{2+} was less than $20\ \mu\text{M}$. All solutions were gassed with O_2/CO_2 (94:6) to maintain a pH of 7.4 and were supplemented with $1\ \text{mg ml}^{-1}$ bovine serum albumin fraction V (Boehringer, Mannheim, Germany), except for electrophysiological recordings.

Patch clamp measurements in β -cells

Immediately after isolation, mouse islets were dispersed into single cells and clusters of cells, which were then cultured for 1–4 days in 35 mm plastic Petri dishes (Plant, 1988). Patch-clamp recordings were performed with an LM-EPC 7 patch-clamp amplifier (List Electronic, Darmstadt, F.R.G.). Pipettes with resistances of 4–6 M Ω were made of borosilicate glass (Dow Corning, Midland, MI, U.S.A.) and coated with Sylgard. Capacity currents were compensated using the patch-clamp amplifier. Series resistance compensation was not used in most experiments. When quantitative measurements were made, the currents and thus the voltage error were small. Experiments were performed in the plastic Petri dishes, reduced in volume to about 0.6 ml by a Plexiglas insert. The dish was continuously perfused at approximately $2\ \text{ml min}^{-1}$ with solutions at room temperature (20 – 22°C). Only single cells were used for whole-cell recordings. β -Cells were recognised by the presence of single channel currents with an amplitude of approximately 5 pA at 0 mV (single channel conductance around 70 pS) in the cell-attached mode, and by the increase in current in the whole-cell mode during dialysis with low ATP (0.3 mM) pipette solutions. In the cell-attached mode, β -cells, identified as described above, respond to an increase in the bath glucose concentration from 1 to 15 mM with electrical activity.

ATP-sensitive K^+ currents were isolated from other membrane currents in β -cells in the whole-cell mode using a pipette solution with low free Ca^{2+} concentration (50 nM), and an appropriate pulse protocol. The membrane potential was held at $-70\ \text{mV}$, close to the resting potential of the cell. Voltage steps of 100 ms duration to $-80\ \text{mV}$ and $-60\ \text{mV}$, separated by a 100 ms gap, were applied at a frequency of 0.1 Hz. Pulses were generated by computer and the current responses digitized at 2.5 kHz stored on floppy disk (Hof, 1986). In some experiments currents were digitized (Sony PCM 501 ES) and stored on video tape. Currents were displayed on a pen recorder, or the holding current and maximum responses to pulses of $+10\ \text{mV}$ and $-10\ \text{mV}$ were measured from records stored on computer.

Figure 1 Effects of various concentrations of the test agents on insulin release from mouse islets incubated in the presence of 15 mM glucose and 2.5 mM Ca²⁺: diazoxide (●), pinacidil (○), cromakalim (▲), nicorandil (△), minoxidil sulphate (■). Results are expressed as a percentage of control release measured within the same experiment. Insulin release by control islets averaged 7.77 ± 0.39 ng per islet 60 min^{-1} ($n = 74$). Values are means for 11–32 batches of islets; s.e.mean shown by vertical lines.

Two types of solution were used for patch-clamp experiments. The bath solution contained (mM): NaCl 140, KCl 5.6, CaCl₂ 2.6, MgCl₂ 1.2, HEPES 10, and was titrated to pH 7.4 with NaOH. Pipettes were filled with a solution containing (mM): KCl 135, MgCl₂ 4, CaCl₂ 2, EGTA 10, Na₂ATP 0.3, HEPES 10. This solution was titrated to pH 7.15 with KOH.

Drugs

Diazoxide was provided by Schering Corp. (Bloomfield, NJ, U.S.A.). The other substances were obtained from the following sources: pinacidil [(±)-N-cyano-N-4-pyridyl-N-1,2,2-trimethylpropylguanidine, monohydrate] from Leo Pharmaceutical Products (Ballerup, Denmark); minoxidil (2,4-diamino-6-piperidinylpyridine 3-oxide) sulphate from The Upjohn Co. (Kalamazoo, MI, U.S.A.); cromakalim (BRL 34915) [(±)-6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol] from Beecham Pharmaceuticals (Harlow, UK) and

Figure 2 Effects of diazoxide and pinacidil on ⁸⁶Rb efflux from mouse islets perfused with a calcium-free medium containing 7 mM glucose. The experiments were performed in the absence (●) or in the presence (○) of 100 μM tolbutamide. Controls without addition of test substance are shown by the broken lines. Values are means for 4–5 experiments with s.e.mean shown by vertical lines.

nicorandil [N-(nitroxy-2-ethyl)pyridinecarboxamide-3] from Rhône-Poulenc (Vitry, France). ⁸⁶RbCl was purchased from the Radiochemical Centre (Amersham, Bucks, UK). All other reagents were from Merck A.G. (Darmstadt, Germany).

Stock solutions of diazoxide and pinacidil (40 mM) were prepared in NaOH, whereas stock solutions of cromakalim, nicorandil and minoxidil sulphate (500 mM) were prepared in dimethylsulphoxide. These stock solutions were used for up to five days with similar results. Aliquots of the stock solutions were added to the appropriate media just before use. Although without effect, dimethylsulphoxide alone was added to appropriate control solutions ($1 \mu\text{l ml}^{-1}$).

Presentation of results

Electrophysiological experiments are illustrated by recordings which are representative of the indicated number of experiments, performed with different mice. Other results are presented as means $\pm$ s.e.mean for the indicated number of experiments or batches of islets. The statistical significance of differences between means was assessed by Student's *t* test or by analysis of variance followed by Dunnett's multiple comparisons test. Differences were considered significant at $P < 0.05$.

Results

Effects on insulin release and ^{86}Rb efflux

Figure 1 shows the effects of the different test agents on insulin release by islets incubated in the presence of 15 mM glucose. Diazoxide caused a dose-dependent inhibition with almost complete suppression (93%) of secretion at 100 μM . Pinacidil also effectively inhibited release at 100 μM (36%) and 500 μM (72%). Cromakalim and nicorandil were clearly less effective causing only 35 and 25% inhibition, respectively, at 500 μM . On the other hand, minoxidil sulphate significantly potentiated insulin release at 500 μM (by 48%).

Addition of 100 μM diazoxide or 500 μM pinacidil to a Ca^{2+} -free medium containing a substimulatory concentration of glucose (7 mM) caused a marked and sustained increase in ^{86}Rb efflux from perfused islets (Figure 2). This effect was rapidly reversible on removal of either diazoxide or pinacidil and antagonized in the presence of 100 μM tolbutamide.

The maximum change in ^{86}Rb efflux induced by the different test agents is shown by Figure 3. The effect of pinacidil was significant at 100 and 500 μM , but was much smaller than that of diazoxide. Nicorandil only marginally accelerated the efflux rate at

Figure 3 Maximum effect of the test agents on ^{86}Rb efflux from mouse islets perfused with a calcium-free medium containing 7 mM glucose. The results were obtained from experiments similar to those shown in Figure 2. Test agents were used at 100 μM (open columns) or 500 μM (hatched columns). The change in ^{86}Rb efflux is given as the difference between the actual efflux rate immediately before addition of the test agent (36–40 min) and after addition of the test agent (46–50 min). Values are means for 4–6 experiments with s.e.mean shown by vertical bars.

Figure 4 Effects of the test agents on ^{86}Rb efflux from mouse islets perfused with a glucose-free medium (G0) containing 2.5 mM CaCl_2 . Diazoxide 100 μM ($\blacktriangle$), pinacidil 500 μM ($\triangle$), cromakalim 500 μM ($\circ$), minoxidil sulphate 500 μM ($\bullet$). Controls without addition of test substance are shown by the broken line. Values are means for 4–5 experiments with s.e.mean shown by vertical lines.

500 μM . Cromakalim was ineffective. In contrast, minoxidil slightly decreased ^{86}Rb efflux at 500 μM .

The effect of the test agents on ^{86}Rb efflux from islet cells was also measured during perfusion of the islets with a medium containing calcium but no glucose. Under these conditions 500 μM minoxidil or cromakalim slightly decreased the efflux rate, whereas 100 μM diazoxide and 500 μM pinacidil increased it (Figure 4). Nicorandil at 500 μM was without effect (not shown). None of the drugs modified basal insulin release.

Effects on β -cell membrane potential

In the presence of a stimulatory concentration of glucose, β -cells display typical electrical activity (Figure 5). Diazoxide (100 μM) rapidly abolished this activity and hyperpolarized the membrane. Pinacidil (500 μM) produced a similar, but less rapid and marked effect. In one out of the four cells examined, the inhibition of electrical activity by 500 μM pinacidil was only partial, whereas it was always complete with 100 μM diazoxide. Moreover, the hyperpolarization by 100 μM diazoxide (17.0 ± 2.0 mV) brought the membrane potential to the same

Figure 5 Effects of diazoxide and pinacidil on the membrane potential of single mouse β -cells perfused with a medium containing 15 mM glucose and 2.5 mM CaCl_2 . The drugs were added at the indicated concentrations and for the periods shown by the arrows: (c) is the direct continuation of (b); (a) was obtained in another cell. These records are representative of results obtained in 4 similar experiments.

level as in the absence of glucose, whereas the hyperpolarization by pinacidil was smaller (7.8 ± 1.5 mV) and less sustained (Figure 5).

Effects on ATP-dependent K⁺ currents

Following rupture of the patch membrane and dialysis with an intracellular medium containing an ATP-concentration lower than that in the intact cell, ATP-sensitive K⁺ currents were activated as ATP diffused out of the cell (Figure 6a; Trube *et al.*, 1986). After reaching a maximum, which was maintained for a different time in each individual cell, the currents then declined as run-down of the conductance occurred. The efficacy of isolation of the ATP-sensitive K⁺ current in these experiments is shown by the near complete block of the currents by 100 μM tolbutamide (Figure 6b).

Addition of diazoxide to the external solution reversibly increased the amplitude of the ATP-sensitive K⁺ current (Figure 6), an effect dependent on the time at which the drug was applied. Diazoxide (100 μM) produced only a small increase in the current during the phase in which the current was fully activated, but its effectiveness increased as run-down occurred. Owing to this variability, quantitative estimates of the effects of diazoxide and other

Figure 6 (a) Time-course of activation and run-down of ATP-sensitive K⁺ currents, and the effect of 100 μM diazoxide, in a single β -cell. The upper trace shows a recording of currents from a β -cell, starting some seconds after dialysis of the cell with a pipette solution containing 0.3 mM ATP. Currents were recorded in response to pulses of ± 10 mV from a holding potential of -70 mV. The lower record is a continuation of the upper record and shows the effect of diazoxide during the linear run-down phase. Zero current level is shown by the arrow. (b) Effects of pinacidil, diazoxide and tolbutamide on ATP-sensitive K⁺ currents. The current values shown are the currents at the holding potential of -70 mV: middle trace; at -60 mV: upper trace; and at -80 mV: lower trace. Drugs were added at the indicated concentrations and for the periods shown by the bars.

drugs were first made when run-down had reached an approximately linear phase (Figure 6). Under such conditions, the effects of diazoxide in different cells were similar and could be seen with concentrations as low as 10 μM .

Of the other substances tested, only pinacidil was able to increase the ATP-sensitive K⁺ current in a way similar to diazoxide. At 100 μM , the effect of pinacidil was only marginal and barely visible (data not shown). At 500 μM , however, pinacidil increased the current by $35 \pm 3\%$ (Figure 6b). Diazoxide applied immediately after complete washout of pinacidil increased the current by $257 \pm 20\%$. In contrast, cromakalim and minoxidil sulphate, at 500 μM ,

decreased the current through ATP-sensitive K^+ channels by 42% and 29% respectively (means, $n = 3$), while nicorandil was without effect (data not shown).

Discussion

The specific aim of this study was to determine whether drugs known to activate K^+ channels in smooth and cardiac muscle cells produce a similar effect in pancreatic β -cells and thereby alter insulin release. We therefore selected the most appropriate experimental conditions to detect even small effects of the drugs. Insulin release was measured in the presence of a stimulatory concentration of glucose which allows either stimulatory or inhibitory effects of a test substance to be detected. However, measurements of ^{86}Rb efflux under these conditions (15 mM glucose and 2.5 mM $CaCl_2$) are not adequate to demonstrate that a small inhibition of release, such as that produced by a low concentration of diazoxide (15 μM), may also be due to an increase in membrane K^+ permeability (unpublished data). This difficulty is due to the fact that activation of one type of K^+ channel (ATP-sensitive) and the concomitant change in membrane potential reduce the activation of other K^+ channels (voltage, and voltage and Ca^{2+} -sensitive). Thus ^{86}Rb efflux was measured in the presence of 7 mM glucose and in the absence of extracellular Ca^{2+} . Under these conditions, most ATP-sensitive K^+ channels are closed (Misler *et al.*, 1986; Ashcroft *et al.*, 1988) and the activation of Ca^{2+} -dependent K^+ channels is avoided. ^{86}Rb efflux was also measured in the absence of glucose, when a sufficient number (not all) of ATP-sensitive K^+ channels are open to hyperpolarize the β -cell membrane (Ashcroft *et al.*, 1988). ATP-sensitive K^+ currents were measured in the whole cell configuration as described by Trube *et al.* (1986), but the effect of the drugs was tested when the current was more stable (later phase of run-down).

Effects of diazoxide

The effects of diazoxide described here confirm those previously observed with the drug on ATP-sensitive K^+ channels (Trube *et al.*, 1986; Dunne *et al.*, 1987; Znkler *et al.*, 1988), membrane potential, ^{86}Rb efflux and insulin release (Henquin & Meissner, 1982; Henquin *et al.*, 1982). There remains no doubt that diazoxide inhibits insulin release by the following sequence of events: the opening of ATP-sensitive K^+ channels leads to hyperpolarization of the β -cell membrane; this reduces activation of voltage-dependent Ca^{2+} channels and, hence, Ca^{2+} influx.

It has long been known that diazoxide exerts a hypotensive action through direct relaxation of vascular smooth muscles (Koch-Weser, 1976), an effect that may be due to the opening of K^+ channels in these tissues (Cook, 1988; Winquist *et al.*, 1989).

Effects of pinacidil

A recent brief report has shown that 500 μM pinacidil increases ^{86}Rb efflux and inhibits glucose-induced insulin release from rat islets (Lebrun *et al.*, 1988). Our results show that pinacidil mimics all effects of diazoxide. It activated ATP-sensitive K^+ currents, accelerated ^{86}Rb efflux (an effect counteracted by tolbutamide), hyperpolarized the β -cell membrane, suppressed the Ca^{2+} -dependent electrical activity and inhibited insulin release. However, these effects were only produced by a high concentration of pinacidil (100–500 μM) and remained smaller than those produced by 100 μM diazoxide. We, therefore, suggest that pinacidil inhibits insulin release by a similar cellular mechanism as, but with a lower potency than diazoxide. Whether both drugs directly affect ATP-sensitive K^+ channels in the same way remains to be established.

It is well known that pinacidil has a direct arterial vasodilator effect (Kaergaard Nielsen & Arrigoni-Martelli, 1981), which was recently ascribed to its ability to open still unidentified K^+ channels in smooth muscle cells (Bray *et al.*, 1987; Cook *et al.*, 1988).

Effects of cromakalim

It has been reported in abstract form (Wilson *et al.*, 1988) that 10 μM cromakalim did not affect (basal?) insulin release by rat islets or interfere with the stimulatory effect of a very high concentration (50 μM) of glibenclamide. Our results show that high concentrations of cromakalim slightly inhibited glucose-induced insulin release. There is no evidence, however, to suggest that this effect is due to an increase in K^+ permeability of the β -cell membrane, since ^{86}Rb efflux was never increased. On the contrary, cromakalim decreased it at least in the absence of glucose and inhibited ATP-sensitive K^+ currents. These unexpected observations could be due to the fact that cromakalim is only a partial agonist of K^+ channels with antagonistic effects at high concentrations, or that the K^+ channels of β -cells are markedly different from K^+ channels of muscle cells. None of these explanations would satisfactorily explain the slight inhibition of insulin release. It is thus pertinent to point out that cromakalim can

inhibit contractile activity at concentrations lower than those required to increase ⁸⁶Rb efflux (Quast, 1987; Hollingsworth *et al.*, 1987). Whether this reflects an action of the drug at a site distinct from K⁺ channels, is still unresolved.

Cromakalim activates ATP-sensitive K⁺ channels in heart muscle cells (Escande *et al.*, 1988; Sanguinetti *et al.*, 1988), and indirect evidence suggests that similar K⁺ channels could be activated in smooth muscle cells (Wilson *et al.*, 1988; Winquist *et al.*, 1989). In the heart, activation of ATP-sensitive K⁺ channels by cromakalim could only be demonstrated at 37°C. It is unlikely that this phenomenon explains our inability to detect an increase in K⁺ permeability by cromakalim. Thus ⁸⁶Rb efflux was measured at 37°C and, in the absence of glucose, an inhibitory effect of cromakalim occurred. This is consistent with the inhibition of ATP-sensitive K⁺ currents measured at room temperature.

Effects of nicorandil

The slight acceleration of ⁸⁶Rb efflux produced by 500 µM nicorandil suggests that an increase in membrane K⁺ permeability of β-cells could underlie the small inhibition of insulin release produced by the same concentration of the drug. Since no effect on ATP-sensitive K⁺ currents was detected, it is possible that nicorandil affects another type of K⁺ channel. However, we cannot exclude the possibility that the effect was too small to be detected by the method used. We can also not rule out an additional action of nicorandil which would contribute to the inhibition of insulin release. It has been suggested that the vasodilator action of nicorandil is not only due to K⁺ channels opening but also to an unidentified mechanism mediated by the nitro group present in the nicorandil molecule (Weir & Weston, 1986). Inhibition of glucose-induced insulin release by sodium nitroprusside has been reported (Laychock, 1981).

Effects of minoxidil sulphate

The hypotensive effects of minoxidil (Freyburger *et al.*, 1981) are currently attributed to the opening of unidentified K⁺ channels in vascular muscles by the active metabolite minoxidil sulphate (Meisner *et al.*, 1988). We unexpectedly found that a high concentration of minoxidil sulphate decreased ⁸⁶Rb efflux and

ATP-sensitive K⁺ currents. This small effect was insufficient to trigger insulin release in the absence of glucose. However, since minoxidil sulphate decreased ⁸⁶Rb efflux also in the presence of 7 mM glucose (in contrast to cromakalim which decreased it only in the absence of glucose), it is possible, though not proven, that the drug potentiates glucose-induced insulin release by decreasing K⁺ permeability of the β-cell membrane.

Conclusions

Although pinacidil, nicorandil, cromakalim and minoxidil sulphate affect insulin release *in vitro*, this 'side-effect' is unlikely to occur *in vivo* and to perturb glucose homeostasis of patients treated with these agents. These effects on β-cells were only observed at concentrations which are 1–3 orders of magnitude higher than those necessary to affect contractility of vascular muscles. Of the drugs tested here, which are known or thought to activate K⁺ channels in muscle cells, only diazoxide and pinacidil decreased insulin release by opening K⁺ channels in β-cells. Cromakalim and minoxidil sulphate were rather found to inhibit K⁺ channels, whilst nicorandil did not affect them in β-cells. These observations add to the accumulating evidence that the effects of certain drugs on K⁺ channels may be very tissue-specific. With the reservation that it has not yet been demonstrated that diazoxide relaxes muscle solely by opening K⁺ channels, it is interesting to note that its effects on contraction (Winquist *et al.*, 1989) and on insulin secretion occur in the same concentration-range. In contrast, tolbutamide and glibenclamide must be used at much higher concentrations to affect K⁺ permeability in heart muscle cells (Belles *et al.*, 1987; Escande *et al.*, 1988; Sanguinetti *et al.*, 1988) than in pancreatic β-cells (Henquin & Meissner, 1982; Garrino *et al.*, 1985; Zünkler *et al.*, 1988). This may reflect subtle differences in the structure of K⁺ channels (even of the same type) between tissues or in their mechanisms of control.

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