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THE ANTIDIABETIC SULFONYLUREA GLIBENCLAMIDE IS A POTENT BLOCKER OF THE ATP-MODULATED K^+ CHANNEL IN INSULIN SECRETING CELLS

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SUMMARY. The ATP-sensitive K^+ channel of RINm5F insulinoma cells is activated after an intracellular ATP depletion. This activation can be followed by $^{86}Rb^+$ efflux. Once activated by ATP depletion, the K^+ channel can be blocked by the hypoglycemic drug, glibenclamide. The blockade is of a high-affinity type ($K_{0.5}$ = 0.06 nM). Recording of the activity of ATP-sensitive K^+ channels with the patch-clamp technique confirmed that they could be completely blocked with 20 nM glibenclamide.

INTRODUCTION. Insulin secretion from pancreatic β-cells is stimulated by glucose which evokes a cyclical pattern of electrical activity (1). The slow wave of depolarization that follows glucose application seems to be due to closure of a K^+ channel which is regulated by intracellular ATP (2-7). Sulfonylureas are used in the treatment of diabetes mellitus (8). Like glucose, sulfonylureas induce electrical activity in pancreatic islets (1, 9-11) and it may be that the two pathways of glucose and sulfonylurea-induced insulin release (2, 5, 12) may converge at the level of the ATP-regulated K^+ conductance. This paper shows that one of the sulfonylureas, glibenclamide, is a very potent blocker of the ATP-regulated K^+ channel in an insulin secreting cell line.

MATERIALS AND METHODS. Cell culture. RINm5F cells, an insulin producing cell line, derived from a rat islet cell tumor, was grown as described previously (13, 14). Cells were plated at a density of 200 000 cells/well (Falcon 24-well tissue culture plates).

$^{86}Rb^+$ efflux experiments. Efflux studies were performed in 24-well culture plates at 37°C and after overnight equilibration of cells in RPMI 1640 medium

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supplemented with 10% fetal calf serum, 0.1 μCi/ml of $^{86}RbCl$ and 0.2 μCi/ml of L-[^{3}H]leucine (internal marker of cell recovery). After removing the medium, cells were preincubated, for various times as indicated, in a medium containing 120 mM NaCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM KCl with 20 mM Hepes/NaOH buffer, at pH 7.5, supplemented by 0.1 μCi/ml $^{86}RbCl$, 0.24 μg/ml oligomycin, 1 mM 2-deoxy-D-glucose and ligands as indicated in the Figures. $^{86}Rb^+$ efflux studies were initiated by removing the preincubation medium and incubating the cells with 200 μl/well of the same medium without $^{86}Rb^+$, oligomycin and 2-deoxy-D-glucose. Efflux were stopped as indicated by removing this latter medium and washing the cells three times with 1 ml of 0.1 M $MgCl_2$ at 37°C. Cells were extracted with 2 x 1 ml of 0.1 N NaOH and counted. Internal concentrations of ATP were measured by the luciferase-luciferin technique using an intracellular volume of 1 μl/10^6 cells (15) corresponding to 4 μl/mg of cell protein.

Electrophysiological measurements. Unitary membrane currents were recorded from membrane patches excised in the inside-out configuration (16). The membrane potential was clamped at -60 mV by a voltage-clamp amplifier (Biologic, France). Currents were digitized at 0.5 ms intervals by a digital oscilloscope (Nicolet, USA) and stored on hard-disk by computer (Hewlett and Packard, USA) for further analysis. Pipettes were coated with Sylgard resin to reduce current noise. The composition of both bath and pipette solution was (in mM) : KCl, 150; $MgCl_2$, 2; EGTA, 4; HEPES-KOH, 10; pH 7.2.

RESULTS AND DISCUSSION. Since the ATP-modulated K^+ channel is known to be permeable to Rb^+ (17), a technique has been devised in which $^{86}Rb^+$ flux experiments have been used to measure the activity of this channel type. Control RINm5F cells have an internal ATP concentration of 3.8 ± 0.3 mM. ATP depletion was obtained by treating the cells with oligomycin in the presence of 2-deoxy-D-glucose. A decrease of the internal ATP concentration created a component of $^{86}Rb^+$ efflux that was inhibited by 0.1 μM glibenclamide (Fig. 1, A and B). An intracellular ATP depletion for 20 min increased the rate constant of

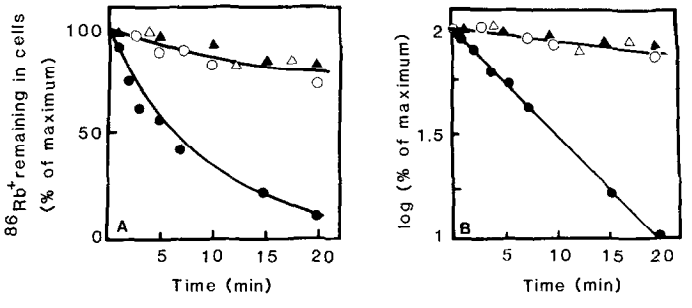

Figure 1. Kinetics of $^{86}Rb^+$ efflux from RINm5F cells. **A**, Kinetics of $^{86}Rb^+$ efflux without depletion of ATP, in the absence (△) or in the presence of 1 μM glibenclamide (▲), and after depletion of ATP for 20 min, in the absence (●) or in the presence of 0.1 μM glibenclamide (○). **B**, Semi-log plots of $^{86}Rb^+$ efflux shown in A.

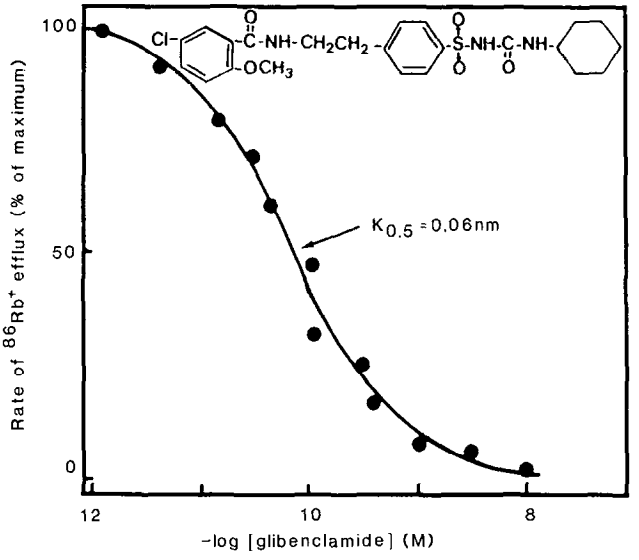

Figure 2. Inhibition by glibenclamide of ATP-sensitive rate of $^{86}Rb^+$ efflux from RINm5F cells. Efflux of $^{86}Rb^+$ was measured as described in Methods, in the presence of increasing concentrations of glibenclamide (●). Time of ATP depletion was 20 min (then $[ATP]_{in}$ = 0.4 mM) and time of $^{86}Rb^+$ efflux was 1 min. Efflux experiments were in duplicate.

efflux, k_{efflux}, from 0.5 x 10^{-5} sec^{-1} to 1.6 x 10^{-3} sec^{-1}. This 32-fold increase of k_{efflux} was abolished by 0.1 μM glibenclamide.

Fig. 2 presents a more detailed analysis of the efficacy of glibenclamide in inhibiting the ATP-sensitive rate of $^{86}Rb^+$ efflux in RINm5F cells. Half-maximum inhibition ($K_{0.5}$) was observed at 0.06 nM glibenclamide.

Complete blockade of the ATP-sensitive K^+ channel already occured near 10 nM glibenclamide. These $^{86}Rb^+$ flux data were completed with electrophysiological experiments using the patch-clamp technique with the same RINm5F cells. Fig. 3 shows that K^+ channels were clearly expressed in the presence of a low concentration of ATP (1 μM) on the cytoplasmic face of an excised patch as previously observed (3, 18). A high internal ATP concentration (2.5 mM), similar to those that are physiologically present in a β-cell, closed these K^+ channels. K^+ channels activated by lowering the cytoplasmic ATP concentration to 1 μM were completely blocked by 20 nM glibenclamide (Fig. 3). Therefore, there is an excellent consistency between flux measurements and electrophysiological data.

These results suggest that glibenclamide will probably be a very useful ligand to study the molecular aspects of structure and function of the ATP-

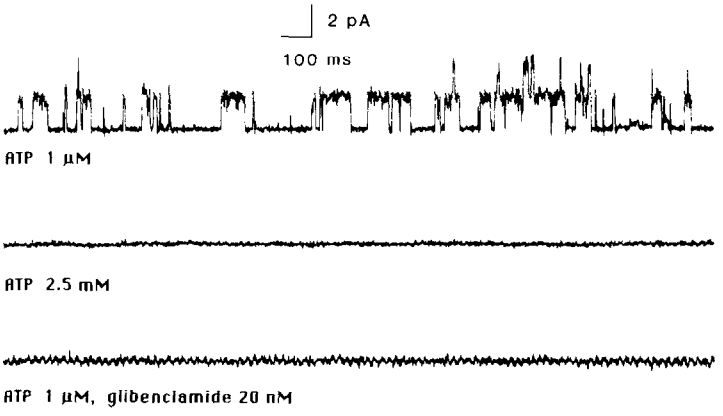

Figure 3. Single channel currents recorded from inside-out patches. An upward deflection in the traces corresponds to an inward current. The membrane potential was -60 mV and the currents were filtered at 500 Hz. ATP-regulated K^+ channels, that opened at low concentrations of ATP (1 µM, upper trace) could be blocked by either 2.5 mM ATP (middle trace) or 20 nM glibenclamide (lower trace).

sensitive K^+ channel as well as the pathologies associated with this type of channel. Its efficacy to block the ATP-sensitive K^+ channel ($K_{0.5}$ = 0.06 nM) is as good or better than efficacies of tetrodotoxin or saxitoxin that has recently played a major role in our current understanding of the Na^+ channel or of (+)PN 200-110 that has been an essential 1,4-dihydropyridine to decipher the molecular properties of one type of Ca^{2+} channels.

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