The oral hypoglycemic agent, U-56324, inhibits the activity of ATP-sensitive potassium channels in cell-free membrane patches from cultured mouse pancreatic B-cells

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U-56324, a hypoglycemic agent derived from nicotinic acid, inhibited the activity of ATP-sensitive potassium channels in excised patches from mouse pancreatic B-cells. The effect of U-56324 on channel activity was reversible and concentration-dependent while it had no effect on single channel conductance. The positional isomer, U-59588, which has relatively little hypoglycemic activity, had no effect on channel properties. U-56324, at the same concentrations, had no effect on calcium-activated potassium channels. The basis for the potentially antidiabetic properties of U-56324 may therefore be due to direct and specific inhibition of ATP-sensitive potassium channels.

Pancreatic B-cell; Potassium channel; Patch clamp; ATP

1. INTRODUCTION

Some members of the sulfonylurea class of compounds have proven invaluable as hypoglycemic agents in the treatment of non-insulin-dependent diabetes mellitus [1]. The basis for their clinical efficacy is thought to be due to inhibition of ATP-sensitive potassium channels (K(ATP) channels) in the plasma membranes of B-cells [2,3] and, following the resultant membrane depolarization and calcium influx through voltage-dependent calcium channels, in enhanced insulin secretion [4–7]. A member of another class of compounds chemically related to nicotinic acid, U-56324 (1,2-Dihydro-2-oxo-6-(2,2-dimethylpropyl)-3-pyridinecarboxylic acid), possesses potent hypoglycemic activity and stimulates in vitro insulin secretion [8] (J.R. Colca, personal communication). By analogy with the effect of sulfonylureas on K(ATP) channel activity, we wished to test the hypothesis that the effects of U-56324 on blood glucose concentration and insulin secretion might be explained, at least in part, by effects on K(ATP) channels. We observed that U-56324 exhibited a direct, reversible, concentration-dependent inhibition of K(ATP) channel activity with no effect on the single conductance in excised patches from mouse pancreatic B-cells.

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2. MATERIALS AND METHODS

Islets were isolated from the pancreases of adult female Swiss-Webster mice using collagenase digestion. Single islet cells were mechanically dispersed from the islets in a zero calcium buffer and subsequently plated on polylysine-coated cover slips in RPMI 1640 culture medium. All data were obtained using cells cultured for one week or less.

Following formation of a 10–40 GΩ seal, patches of membrane were excised in either the outside-out or inside-out configuration [9]. Single channel currents were recorded on VCR tape using a PCM data recorder and segments of data were digitized at 1 kHz after filtering at 0.3 kHz by an 8-pole Bessel filter. Amplitude histograms were constructed from which average channel currents were calculated by integrating the current over a time segment of data and dividing by the segment's duration. Single channel conductances were determined from single channel I-V's based on the amplitude histograms. Average channel activity was calculated by dividing average channel current by the single channel current amplitude [10]. The effects of tested compounds were determined by normalizing the average K(ATP) channel activity in the presence of the drug to the mean of control (before drug application) and wash (following drug washout) episode average channel activity.

The bath solution (pipette solution for the inside-out patches) had the following composition (in mM): 120 NaCl, 5 KCl, 3 $CaCl_2$, 2 $MgCl_2$, 10 Hepes, pH = 7.2. The pipette solution (bath solution for inside-out patches) contained (in mM): 140 KCl, 1 EGTA, 1 $MgCl_2$, 0.010 Mg_2ATP, 10 Hepes, pH = 7.2. ATP (10 μM) was present to decrease the rate of rundown of K(ATP) channels commonly observed in excised patches [11–12]. EGTA was included to chelate calcium and thereby suppress activity of K(Ca) channels [13].

U-56324 and U-59588 (1,2-Dihydro-2-oxo-4-(2,2-dimethylpropyl)-3-pyridinecarboxylic acid) were kindly provided by Upjohn (Kalamazoo, MI) and were dissolved in DMSO, and microliter aliquots were added to separate reservoirs containing the bath solution at the appropriate final concentrations. The final DMSO concentration was never more than 0.1% (v/v).

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3. RESULTS

K(ATP) channels were identified in outside-out patches on the basis of their single channel conductance with assymetrical potassium concentrations across the patch (140 mM K^+ in pipette, 5 mM K^+ in bath, γ = 19.4 ± 0.7 pS, measured between −40 and 0 mV pipette potential), inward-rectification, and the lack of voltage-dependence observed for their probability of opening [14]. Bath application of U-56324 (5 mM) resulted in a rapid (less than 10 seconds) decrease in channel activity that was somewhat more slowly reversible (Fig. 1). U-56324 decreased the average K(ATP) channel activity in a concentration-dependent manner, but had no effect on the single channel current amplitude at any of the concentrations tested (Fig. 2). The single channel conductance was not significantly affected by U-56324 (19.4 ± 0.7 pS in control solution vs 20.4 ± 1.1 pS in U-56324, N = 6). The half-maximal effective concentration (EC_{50}) and Hill coefficient (n) for the effect of U-56324 on average channel activity were estimated by fitting the data in Fig. 2 with the following equation:

$$R = 1 - \frac{[X]^n}{[X]^n + [EC_{50}]^n}$$

where X is drug concentration and R average channel activity at that concentration normalized to the control channel activity. The best-fitting curve yielded an EC_{50} = 233 μM and n = 0.58. The relatively low Hill coefficient could be due to negative cooperativity of drug binding or patch-to-patch variation of EC_{50}. Furthermore, the effect of U-56324 on K(ATP) channel activity was not voltage-dependent when comparing results obtained at 0 mV and −40 mV pipette potentials (Table I).

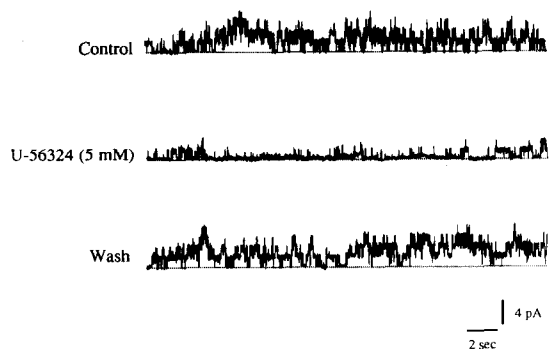

Fig. 1. The effect of U-56324 on K(ATP) channel activity in an outside-out patch. Control K(ATP) channel activity is shown in top trace. Middle record displays channel activity after U-56324 (5 mM) was applied, and the bottom trace was obtained following washout of U-56324. The dashed line in this and subsequent figures with traces denotes current level at which no channels are open. 0 mV pipette potential.

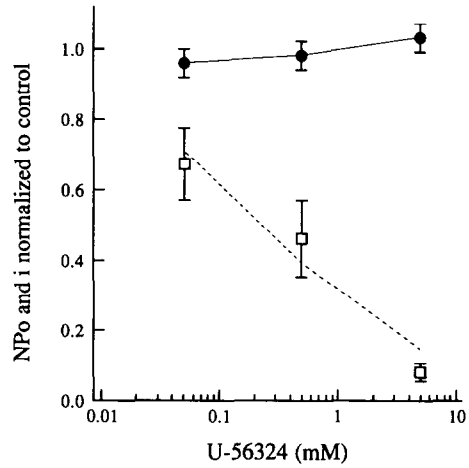

Fig. 2. Concentration–response curves for the effect of U-56324 on average channel activity (*NPo*, open squares) and channel current amplitude (*i*, closed circles) normalized to the mean of data obtained in control saline and following washout of U-56324. All data were taken from outside-out patches at 0 mV pipette potential. The dashed curve is the line-of-best-fit to the average channel activity using the equation given in the text. Each point is the mean of 6 patches, except for 5 mM U-56324 data, which is the mean of 5 patches. Error bars are SEM.

Some patches were obtained that contained calcium-activated potassium channels, as determined by their relatively large current amplitudes and reversal potential [13], and in which K(ATP) channel activity was absent or had rundown. U-56324 (5 mM) had no apparent effect on the properties of these channels (data not shown).

To determine whether the effect of U-56324 on K(ATP) channels is a general property of this class of compounds, the positional isomer U-59588, which has relatively little hypoglycemic activity [8], was also tested. U-59588 (5 mM) had no effect on average K(ATP) channel activity (Fig. 3). Higher concentrations of U-59588 were not tested.

The effects of U-56324 were also tested in inside-out patches containing K(ATP) channels, as shown in Fig. 4. In this experiment, ATP (2 mM) was bath-applied to establish channel identity, and it markedly reduced channel activity, U-56324 (5 mM) was subsequently applied following washout of 2 mM ATP, and this

Table I

Average K(ATP) channel activity, *NPo*, measured in 5 outside-out patches in control solution (mean of activity in equal duration segments of control and drug wash data) and with U-56324 at 0 mV and −40 mV pipette potential. The concentrations of U-56324 are 50 (n = 2), 500(n = 2), and 5000 (n = 1) μM. Values are mean ± SEM

Pipette potential (mV)	Average channel activity (*NPo*)		Reduction in *NPo* (%)
	Control	U-56324	
−40	2.28 ± 0.17	0.99 ± 0.59	57
0	2.68 ± 0.34	0.99 ± 0.61	63

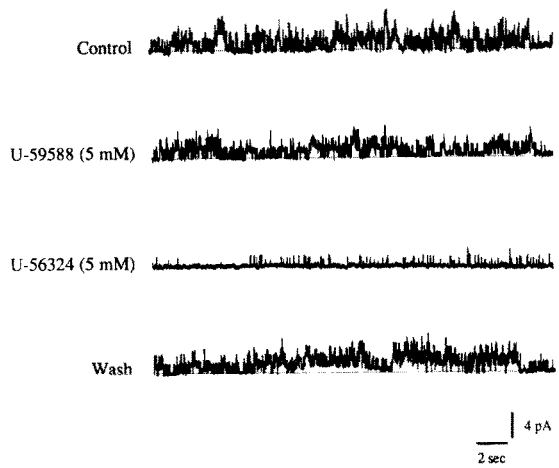

Fig. 3. The positional isomer U-59588 does not decrease channel activity in outside-out patches. First record depicts control K(ATP) channel activity, and the second in the presence of U-59588 (5 mM). U-56324 (5 mM) was then applied to the patch and significantly reduced channel activity (third trace). The fourth trace was obtained after washout of U-56324. 0 mV pipette potential.

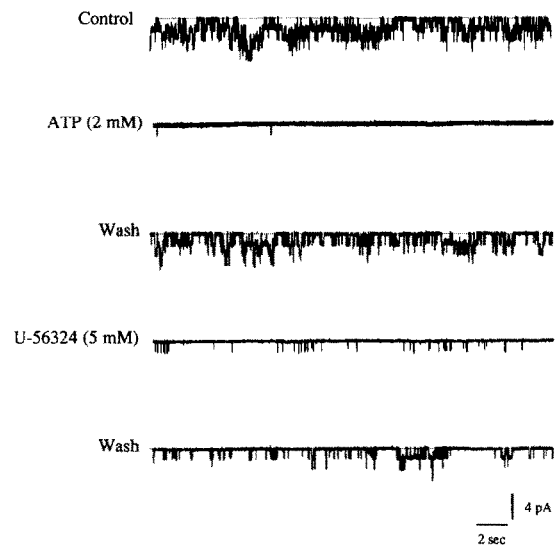

Fig. 4. The effect of U-56324 on K(ATP) channel activity in an inside-out patch. Control K(ATP) channel activity is represented in the first trace, with the second obtained in the presence of ATP (2 mM). The third record was obtained following washout of ATP and the fourth after application of U-56324 (5 mM). The final trace depicts channel activity after washout of U-56324. 0 mV pipette potential.

resulted in a reversible inhibition of channel activity. As with the outside-out patches, the drug reduced the probability of opening and/or number of active K(ATP) channels, but had no effect on the channel current amplitude.

4. DISCUSSION

The data support the hypothesis that, as with sulfonylurea compounds, the efficacy of U-56324 as a hypoglycemic agent and stimulator of insulin secretion are due, at least in part, to a reduction in K(ATP) channel activity in pancreatic B-cells through a direct effect on these channels. U-56324 reduced K(ATP) channel activity over a concentration range similar to that for its concentration-dependent stimulation of in vitro insulin secretion from rat pancreatic islets (J.R. Colca, personal communication).

The effect of U-56324 on K(ATP) channels bears some similarities with the reported effects of sulfonylurea compounds. U-56324, like tolbutamide and glyburide, reduced K(ATP) channel activity without affecting the single channel current amplitude or conductance through a direct action on the channel or closely associated protein [15]. Furthermore, the reduction in K(ATP) channel activity for both was not voltage-dependent [15] (Table I). Both U-56324 and the sulfonylureas can inhibit K(ATP) channel activity from either side of the plasma membrane [16], suggesting that they bind to sites within the plasma membrane. For the known potassium channel types in the B-cell membrane, the sulfonylureas are thought to act specifically on K(ATP) channels [3,15,17]. We observed no action of U-56324 on K(Ca) channels, but have not tested its possible effects on voltage-dependent, 'delayed rectifier' potassium channels [17].

The estimated EC_{50} for the reduction in K(ATP) channel activity by U-56324 (233 μM) is considerably higher than reported EC_{50} values for the sulfonylureas, which range from the low nanomolar to low micromolar concentrations [3,18,19]. The concentration–response data shown here were obtained solely from excised, outside-out patches. Differences in concentration-response parameters for sulfonylurea inhibition of K(ATP) channels between excised and intact-cell configurations have been reported [15,16], which may be due to the presence of ADP when the latter configurations are used [20]. Whether U-56324 binds to the same sites as the sulfonylureas to alter channel activity remains for future studies.

In summary, U-56324, a compound derived from nicotinic acid that possesses hypoglycemic activity and stimulates insulin secretion, also directly inhibits the activity of K(ATP) channels from mouse pancreatic B-cells. Although other actions are possible, we speculate that, as with sulfonylurea compounds, the basis for these potentially antidiabetic effects resides in its specific and direct effect on K(ATP) channels.

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