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Inhibitory effect of glybenclamide on mitochondrial chloride channels from rat heart

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ABSTRACT

Glybenclamide is used as a pharmacological tool in studies of mitochondrial functions supposing its main role to block ATP-dependent potassium (K_{ATP}) channel. The aim of this study was to test whether glybenclamide might interact with the mitochondrial chloride channels. Mitochondrial membranes, isolated from rat heart muscle, were incorporated into lipid bilayer membrane and single chloride channel currents were measured in 250/50 mM KCl *cis*/*trans* solutions. The observed chloride channels ($N = 11$) with mean conductance 120 ± 14 pS were sensitive to glybenclamide, which decreased the open probability ($IC_{50} = 129$ μM) and affected the channel gating kinetics ($IC_{50} = 12$ μM) by perturbing its open state. It did not influence the channel conductance or reversal potential. These results indicate that glybenclamide interacts with chloride channels what should be taken into consideration, when glybenclamide is used as a specific inhibitor of K_{ATP} channels.

1. Introduction

The sulfonylurea derivative glybenclamide (GLYB) is used as oral hypoglycemic agent to treat non-insulin dependent diabetes mellitus [1,2]. The antidiabetic sulfonylureas in general bind to high affinity sulfonylurea receptors, which are the structural elements of ATP-sensitive potassium (K_{ATP}) channels, and so inhibit the channels [3,4]. The property of GLYB to inhibit K_{ATP} channel is widely used as a pharmacological tool in numerous studies including mitochondria [3].

Effect of GLYB on mitochondrial function is complex. It activates cyclosporine A-sensitive mitochondrial permeability transition, induces swelling of mitochondria, increases calcium efflux, inhibits K+ and Na+ uniports, decreases the mitochondrial membrane potential, inhibits respiration and interferes with mitochondrial bioenergetics or reduced intracellular ATP level [4–8]. The complexity of the GLYB effects indicates that more than one molecular mechanism is involved.

Since it was observed that GLYB inhibited cystic fibrosis transmembrane regulator (CFTR), swelling-activated, and Ca^{2+}-activated Cl^- channels in cardiac myocytes and plasma membrane of cultured cells [9–11] it was of interest to know whether GLYB interacts with mitochondrial chloride channels what might contribute to understand its complex mitochondrial effect.

We used the lipid bilayer technique to examine the effects of GLYB on the activity of chloride channels derived from rat heart mitochondria at single channel level. We found that GLYB inhibits these channels by open-channel block mechanism.

2. Material and methods

2.1. Chemicals

Lipids were from Avanti Polar Lipids (Alabaster, AL, USA). Protease inhibitors were from Roche Diagnostics GmbH (Mannheim, Germany). All other chemicals including GLYB were purchased from Sigma–Aldrich (Germany). GLYB was prepared as 100 mM stock solution in DMSO. The final concentration of DMSO in the bath solutions was <0.5%, which, by itself, did not affect chloride currents.

2.2. Isolation of mitochondrial membrane vesicles

Mitochondria from the hearts of male Wistar rats were isolated as described in details in our previous study [12]. All procedures were approved by the State VET and Nutritive Administration of Slovak Republic. In brief, the hearts were excised after thoracotomy. The ventricles were separated and homogenized. The tissue suspension was processed in several steps of differential centrifugation until the final membrane fraction was obtained. The membrane fraction was exposed to sonication at 35 kHz. The purity of this fraction was analyzed as described in [12]. The final membrane fraction consisted mostly of outer and inner mitochondrial

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membranes. The membrane fraction was aliquoted and stored at −80 °C until use. All procedures were performed at 4 °C, and isolation buffers contained a mixture of protease inhibitors (1 μM leupeptin, 1 μM pepstatin, 1 mM benzamidine, 1 μM aprotinin, and 0.2 mM Pefabloc SC).

2.3. Bilayer lipid membrane (BLM) measurement

The native vesicle was fused into BLM formed across an aperture (diameter approx. 0.1 mm) separating the *cis* and *trans* chambers as described in our previous study [12]. The composition of the solutions (in mM) was: *trans*: 50 KCl, 2 $MgCl_2$, 0.4 $CaCl_2$, 1 EGTA, 10/5 HEPES/Tris, 7.4 pH (intracellular side), and *cis*: 250 KCl, 2 $MgCl_2$, 0.1 $CaCl_2$, 10/5 HEPES/Tris, 7.4 pH (matrix side). The single channel current was measured by the bilayer clamp amplifier (BC-525C, Warner Instrument, Hamden, CT, USA), filtered at 1 kHz cut off frequency and the data were analyzed by Clamfit10 software (Axon Instrument, USA). All voltages reported refer to the *trans* side; the *cis* side was grounded. Under our conditions, the positive current amplitude that increased at the application of positive voltages means a flux of chloride anions from the *cis* to the *trans* side. All procedures were carried out at room temperature (22 °C).

The channel conductance (G), the reversal potential (E_{rev}), calculated from the current–voltage relationship, and a relative ionic Cl^-/K^+ selectivity was evaluated as in our previous study [12]. The single channel open probability (P_{open}) was determined from 3 min recordings at control and each concentration of GLYB. The data are shown as either mean ± standard deviation (conductance, reversal potential, current amplitude) or median and interquartile range: lower quartile – Q1 and upper quartile – Q3 (P_{open}, mean open time τ_{open}, mean closed time τ_{closed}).

Chloride channels were readily distinguished from K^+ channels using *cis*/*trans* solutions 250/50 mM KCl. The theoretical E_{rev} for K^+, presuming 100% permeability to K^+ and zero permeability for other ions, is +40.7 mV. In our experimental conditions we controlled purity of Cl^- current by applying voltage of ±30 mV.

3. Results

3.1. Single channel properties of mitochondrial chloride channels

After fusion of a vesicle containing ion channel into BLM we observed the activity of chloride channels with the mean single channel chloride current amplitude at 0 mV equal 2.8 ± 0.4 pA ($N = 11$). All chloride channels were highly active under control conditions, with median P_{open} equal 0.5970 (0.4948–0.7826; Q1–Q3). The mean conductance of the chloride channels was $G = 120 \pm 14$ pS ($N = 6$) and the mean reversal potential was $E_{rev} = -22.4 \pm 2.2$ mV ($N = 6$), giving the selectivity Cl^-/K^+ ratio of 4.2. The chloride channels were under control conditions characterized by median of mean open time $\tau_{open} = 14.14$ ms (7.98–16.50; Q1–Q3) and of mean closed time $\tau_{closed} = 4.04$ ms (2.25–4.83; Q1–Q3). The voltage dependence of P_{open} was bell-shaped and consistent with [13].

3.2. Effect of glybenclamide on mitochondrial chloride channels behaviour

To quantify the effect of GLYB on the activity of the chloride channels, an average chloride current, P_{open}, single channel amplitude histogram, conductance, reversal potential and mean open and close times were measured and evaluated. GLYB was added to the *cis* compartment (matrix side in our experimental conditions). In all experiments ($N = 11$) GLYB had concentration

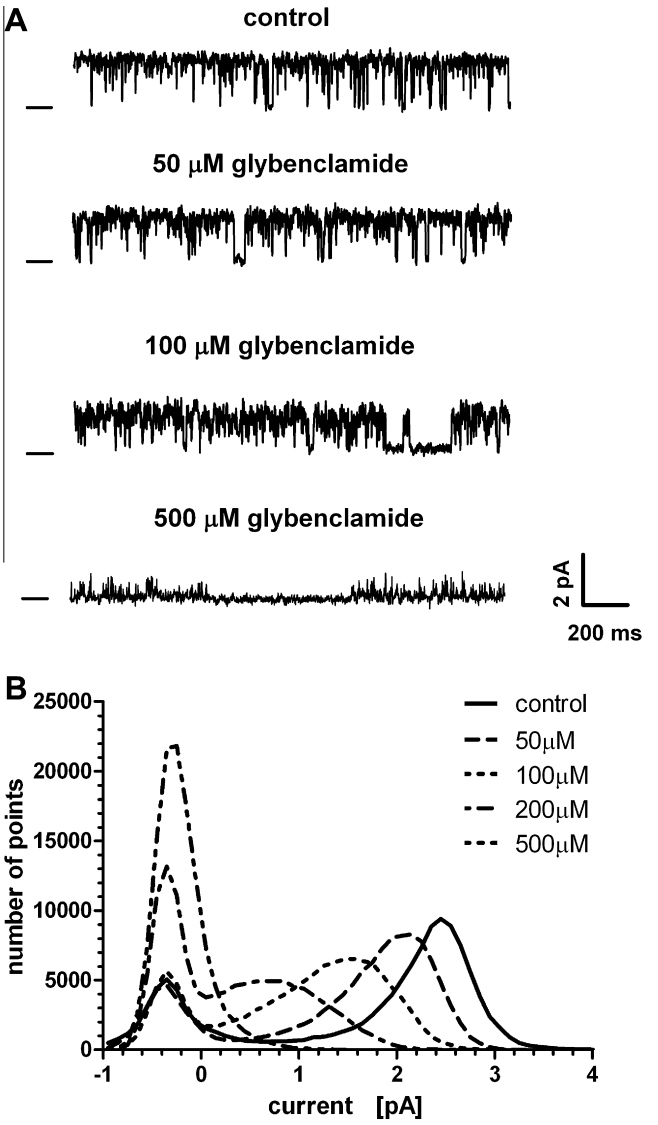

Fig. 1. (A) Effect of GLYB on single channel chloride current. These representative current traces show the decrease of current amplitude with increasing concentration of GLYB applied to the *cis* side at 0 mV. At 500 μM concentration, the openings were to short to be detected with the sampling rate of 4 kHz. The channels open upwards; black line on the left indicated the closed level. (B) Amplitude histogram showing the decrease of current amplitude at different GLYB concentrations. Both decrease of current amplitude and increase of open level noise (increase of peak width) as function of GLYB concentration is seen in the graph.

depended inhibitory effect on the chloride channels (Figs. 1 and 2). An apparent decrease of the single channel current amplitude was also observed (Fig. 1B). However, it is likely that the decreased current amplitude was the consequence of faster gating than 1 kHz cut off filter frequency, because we did not observe any clear open level after application of ⩾100 μM GLYB.

GLYB decreased P_{open} in a concentration dependent manner Fig. 2. The logarithm of GLYB concentration for half-maximal inhibition of activity is equal to 2.11 ± 0.04 (mean, SE; log (μM)), which gives the value of $IC_{50} = 129$ μM (95% confidence interval from 86 to 191 μM, $R^2 = 0.9961$).

To check whether GLYB affects the channel gating, we monitored the mean open time and mean closed time values. These are obtained by fitting the dwell time histograms with single exponential function. Fig. 3A shows the changes of mean open time τ_{open} as function of increasing GLYB concentration. The value of τ_{open} significantly decreased (Kruskal–Wallis test, $P = 0.0011$). Even