Comparison of the inhibitory effect of ATP-dependent K\(^+\) channel blockers glibenclamide and chlorpropamide, on diazoxide-induced relaxation of rat ileum

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ABSTRACT

Objective: This study was conducted to compare the potential inhibitory effect of ATP-dependent K\(^+\) channel blockers, glibenclamide and chlorpropamide on diazoxide-induced relaxation of isolated rat ileum.

Material and Methods: The study was performed on rat ileum connected to an isotonic transducer and the contractions were recorded on one-channel MD\(_2\) recorder. In this study the relaxant effect of diazoxide on KCl-induced precontracted rat ileum, and its inhibition by different concentrations of glibenclamide and chlorpropamide were studied.

Results: Diazoxide (10\(^{-5}\)-10\(^{-3}\)M) produced dose-dependent relaxation. EC\(_{50}\) for diazoxide-induced relaxation was 8 x 10\(^{-5}\)M. Neither glibenclamide nor chlorpropamide had any effect on the resting tension of the rat ileum preparation. Both glibenclamide and chlorpropamide reversed diazoxide-induced relaxation. The pA\(_2\) values for glibenclamide and chlorpropamide reversal of diazoxide-induced relaxation were 6.3 and 5 respectively.

Conclusion: The results confirm that the inhibitory effect of glibenclamide on diazoxide-induced relaxation in rat ileum is higher than that of chlorpropamide.

KEY WORDS: Diazoxide, oral hypoglycemics, sulfonylureas, rat.

Introduction

Recently, increasing attention has been paid to cell membrane K\(^+\) channels as a site of action for a number of drugs. ATP-dependent K\(^+\) channels have been described in diverse cell types, including cardiomyocytes, pancreatic beta cells, neurons, smooth muscle cells and skeletal muscle cells, where they play important physiological and pathophysiological roles.\(^1\) Blockers of K\(^+\) channels are able to promote insulin secretion from pancreatic beta cells and suppress dysrhythmias of the heart.\(^2\) K\(^+\) channel openers cause relaxation of a variety of smooth muscles. Some antihypertensive agents such as minoxidil and diazoxide have been used clinically for years. They produce their action by opening the K\(^+\) channels.\(^3\)

This study was undertaken to compare the action of two competitive antagonists of ATP-dependent K\(^+\) channels, namely glibenclamide and chlorpropamide against the relaxant effect of diazoxide, a K\(^+\) channel opener, on rat ileum.

Material and Methods

Experimental animals and procedures

Segments of terminal ileum (1-1.5 cm long) were removed from male Wistar rats weighing 250-300 g. The tissue was suspended in 50 ml organ bath containing Tyrode’s solution (mM): NaCl 137, KCl 2.7, CaCl\(_2\) 1.5, MgCl\(_2\) 1, NaHCO\(_3\) 12, NaH\(_2\)PO\(_4\) 0.4, and glucose 5.5 (Merck, Germany), which was bubbled with carbogen (5% CO\(_2\) and 95% O\(_2\)) at 37°C. The tissue was connected longitudinally by surgical thread to an isotonic transducer and the contractions were recorded on one-channel MD\(_2\) recorder. The tissues were allowed to equilibrate for 30 min and the bathing fluid was exchanged by overflow every 10 min.

At the end of the equilibration period, the tissue was contracted by 20 mM KCl and the responses were allowed to reach a plateau. This was obtained within 10-15 min after the addition of KCl. Diazoxide (Sigma) was then added in a cumulative
manner (10⁻²–10⁻³M). Sufficient time was allowed before the next dose and the tissue was washed every 5 min till baseline was restored. Subsequently, the tissue was contracted once again with 20 mM KCl, and at plateau, glibenclamide (10⁻⁹–10⁻³M) or chlorpropamide (3 x 10⁻⁶–10⁻³M) or an equivalent volume of vehicle (dimethyl sulfoxide - Merck, Germany) was added and allowed to equilibrate for 10 min before the addition of diazoxide.

Treatment of data and statistics

The relaxant effects of diazoxide are expressed as percent of inhibition of tension produced by 20 mM KCl. For determination of EC⁵₀, logarithm of (response/ [max response- response]) was plotted against the logarithm of the concentration of diazoxide (agonist). The pA₂ values for glibenclamide and chlorpropamide were determined by the Schild plot, in which the logarithm of (dose ratio-1) is plotted as a function of the negative logarithm of the antagonist concentration (pA₂). The intercept on the abscissa is –log Kᵣ or pA₂. The term of dose ratio is applied to the expression [CY]/[C], which is agonist concentration in the presence and absence of antagonist. Results are means ± SEM. One-way analysis of variance (ANOVA) followed by post hoc Dunnett’s multiple comparisons test was used for comparison of the effect of different concentrations of glibenclamide and chlorpropamide. Differences between means were considered to be significant at P<0.05.

Results

As shown in Figure 1, the cumulative application of diazoxide (10⁻²–10⁻³M) inhibited contractions induced by 20 mM KCl in a concentration-dependent manner. The EC⁵₀ for diazoxide-induced relaxation in this tissue was determined to be 8 x 10⁻³ M. Neither diazoxide nor glibenclamide had any effect on the resting tension of the rat ileum.

Addition of glibenclamide (10⁻²–10⁻³M) or chlorpropamide (3 x 10⁻⁶–10⁻³M) to KCl–contracted ileum produced no change in tension. However, both shifted the dose-response curve of diazoxide rightward in a concentration-dependent manner (Figures 1 and 2). The pA₂ values for glibenclamide and chlorpropamide reversal of diazoxide relaxation were 6.3 and 5 respectively.

Discussion

The K⁺ channel opener diazoxide inhibits KCl–induced contractions at 20 mM in the rat ileum. The effect of diazoxide was antagonized by glibenclamide (pA₂ = 6.3) and chlorpropamide (pA₂ = 5).

Our results are in agreement with a previous report indicating dose-dependent inhibition of rat ileal smooth muscle by K⁺ channel activator lemakalim and the presence of glibenclamide-sensitive K⁺ channel in this tissue.² On the other hand, Davies and co-workers reported that diazoxide had no effect on contractions elicited by KCl or electrical stimulation in rat intestinal smooth muscle, in spite of the relaxant effect of nicorandil, another K⁺ channel modulating agent.² This may be because of significant differences in the pharmacological and electrophysiological profiles of ATP–dependent K⁺ channels in various smooth muscles.³ For example, the sensitivity of ATP–dependent K⁺ channels to compounds such as diazoxide and lemakalim varies and differs between preparations.⁴,⁵

Displacement experiment in the presence of various sulfonylureas also showed greater potency of glibenclamide in comparison with chlorpropamide and tolbutamide in guinea pig isolated small intestine.⁶ Studies of K⁺ channel modulating agents on mouse intestinal smooth muscle also showed greater effectiveness of glibenclamide in comparison with
tolbutamide (another first generation sulfonylurea) against pinacidil-induced relaxation.\textsuperscript{11}

The $pA_2$ value obtained for glibenclamide (6.3) from the concentration-response curves of diazoxide was different from the $pA_2$ obtained for diazoxide and glibenclamide on vascular smooth muscle\textsuperscript{12} (7.2). This may be due to the involvement of different subtype of K$^+$ channels in vascular and gastrointestinal smooth muscles.

This study showed the relaxant effect of diazoxide against KCl (20 mM)-induced contraction in rat ileum and the greater potency of glibenclamide in relation to chlorpropamide in inhibiting diazoxide-induced relaxation of rat ileum.

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References


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