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Effects of tolbutamide, glibenclamide and diazoxide upon action potentials recorded from rat ventricular muscle

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Drugs which influence the electrical activity of insulin-secreting B cells of mammalian islets of Langerhans by closing (tolbutamide and glibenclamide) or opening (diazoxide) ATP-sensitive potassium channels were applied to the ventricular muscle of the rat. Action potentials were recorded from the ventricular epicardium of perfused intact rat hearts. Tolbutamide (0.5–2.0 mM), glibenclamide (0.01–0.1 mM) and diazoxide (0.5 mM) each evoked a dose-dependent increase (7–33%) in the duration of the ventricular action potential measured at 50% of repolarization. These drugs were without effect upon the resting membrane potential or the peak of the action potential. Single-channel recordings of ATP-sensitive K^+ channels were obtained from excised membrane patches of enzymatically isolated rat ventricular myocytes. Tolbutamide and diazoxide inhibited openings of ATP-sensitive K^+ channels. Diazoxide inhibited ATP-sensitive K^+ channel openings in the presence of ATP. Diazoxide did not evoke opening of ATP-sensitive K^+ channels. It is concluded that these drugs could act to increase the duration of the cardiac action potential by inhibiting openings of ATP-sensitive K^+ channels.

Introduction

In recent years the target of certain antidiabetic drugs has been identified as a potassium-selective ion channel which can be closed by intracellular adenosine triphosphate (see Refs. 1 and 2 for recent reviews concerning the ATP-sensitive K^+ channel). In the membranes of insulin-secreting cells this channel is closed by the sulfonylurea drugs tolbutamide and glibenclamide and it is opened by the sulfilamide drug diazoxide [3–8]. Similar ATP-sensitive K^+ channels occur in cardiac muscle cells [9], where tolbutamide and glibenclamide have also been shown to evoke channel closure [10,11]. The effects of diazoxide upon cardiac ATP-sensitive K^+ channels have not been described.

Our original intention was to compare the effects of the sulfonylureas and diazoxide upon action potentials recorded from an intact beating cardiac preparation under quasi-ischaemic conditions created by the application of metabolic poisons. In our initial experiments we were surprised to find that the sulfonylureas not only reversed the metabolic poison evoked reduction of action potential duration, but actually extended it further than had been recorded under control conditions. We therefore repeated these experiments without poisoning the heart. We found that the sulfonylureas induced an elongation of the duration of the normal (i.e., unpoisoned) ventricular action potential, and that diazoxide evoked the same response as tolbutamide and glibenclamide.

Materials and Methods

Action potential recording

Adult male Wistar rats weighing 160–200 g were anesthetized with pentothol (50 mg/200 g). The heart was rapidly removed from the thoracic cavity and immersed in Tyrode's solution. The aorta was cannulated and the heart was transferred to a plexiglass experiment chamber (capacity 8 ml) where it was perfused with Tyrode's solution by gravity (approx. 75 cm water column).

Glass microelectrodes filled with 3 mM KCl and having tip resistances of 15–25 MΩ were used to measure membrane potential. Ventricular epicardial cells were impaled using standard floating microelectrode techniques. The heart was paced at 1.5–3 Hz by bipolar stimulating electrodes placed upon the surface of the

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ventricle. Recorded action potentials were amplified by a WPI M707 amplifier and displayed on the screen of a Tektronix 565 oscilloscope. Photographs were taken of the oscilloscope screen.

The Tyrode's solution had the following composition (in mM) 130 NaCl, 5.6 KCl, 20 $NaHCO_3$, 0.6 NaH_2PO_4, 2.15 $CaCl_2$, 1.1 $MgCl_2$. It was gassed with 95% O_2/5% CO_2. Stock solutions of tolbutamide, glibenclamide (Hoechst, Hounslow, U.K.), and diazoxide (Glaxo Group Research, Ware, U.K.) were prepared daily in 20 mM NaOH and added to Tyrode's solution to provide the desired final concentrations. No adjustment of the pH of the Tyrode's solution was necessary. All experiments were conducted at room temperature (20–23° C).

Single-channel recording

Individual myocytes were obtained from rat hearts by standard methods which have been described previously [12]. All experiments were performed at room temperature (20–23° C). Single-channel currents [13] were recorded with a Biologic RK300 patch and cell clamp amplifier, low-pass filtered at 1 kHz (5-pole Tchebyscheff filter), and stored on video cassette (Biologic PCM instrumentation recorder and Schneider VHS video recorder). Experimental results were subsequently replayed onto a Gould 2400 pen recorder (frequency response approx. 150 Hz).

These experiments used excised membrane patches in the inside-out membrane configuration. The external surface of the excised membrane patch was bathed in an Na-rich solution and the internal surface was bathed in a K-rich solution. Each excised membrane patch was voltage-clamped at 0 mV membrane potential. The Na-rich solution contained (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, 10 glucose; the pH was adjusted to 7.4 with NaOH. The K-rich solution contained (in mM) 140 KCl, 10 Hepes, 10 glucose then either 5 EDTA or 5 EGTA and 1.4 $MgCl_2$; the pH was adjusted to 7.4 with KOH. Stock solutions of tolbutamide and diazoxide were prepared in KOH (20 mM) and added to K-rich solutions to provide the desired final concentrations. ATP ((Na-salt), Sigma, St. Louis, MO, U.S.A.) was added to K-rich solution.

Excised membrane patches were continuously perfused by a stream of solution from one of a series of piped outlets. Changes of solution were performed manually under visual control and identified vocally upon the video recording. In this and in other studies we have occasionally observed shifts in the base-line current of single-channel recordings when solutions were changed (e.g., the application of diazoxide in Fig. 4C). Since these shifts were not consistently observed in any particular membrane patch or with any particular experimental solution, we assume that they are artifactual. At the present time we can provide no other explanation.

Results

The membrane potential recorded under control conditions in the 106 individual cells which make up this study was -75.5 ± 0.5 mV (mean ± S.E.). The peak of the evoked action potentials was $+15.6 \pm 0.4$ mV, and their duration measured at 50% of their repolarization (APD_{50}) was 12.1 ± 0.2 ms. All of these values were typical for action potentials recorded from rat ventricle under these experimental conditions [14].

Fig. 1A illustrates that in this experiment under control conditions the membrane potential was −79 mV and the action potential peaked at +13 mV. The APD_{50} was 13 ms. When this heart was perfused with a solution which contained 2 mM tolbutamide, neither the membrane potential nor the peak of the action potential was changed. But the duration of the action potential increased; after 3 min the APD_{50} was 17 ms. This effect was fully reversible. The effect of tolbutamide upon the duration of the action potentials recorded from intact rat ventricular muscle was dose-dependent (Fig. 2). The application of less than 0.5 mM tolbutamide had no significant effect upon the APD_{50}, 0.5 mM tolbutamide significantly increased the recorded APD_{50}, and higher concentrations of tolbutamide further increased the APD_{50}. The difficulty of maintaining more than 2 mM tolbutamide in solution at physiological pH prevented any extension of this dose–response relationship.

Glibenclamide had similar dose-dependent effects upon the action potentials, recorded from intact rat ventricular muscle. At concentrations less than 0.01 mM, glibenclamide had no effect; with 0.01 mM

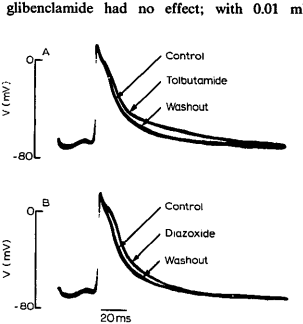

Fig. 1. Effects of tolbutamide and diazoxide upon action potentials recorded from intact ventricular muscle of the rat. The addition of 2 mM tolbutamide (A) or 0.5 mM diazoxide (B) to the Tyrode solution which perfused the heart resulted in an increase in the duration of the action potential within 2–5 min of application of the drugs. These effects were fully reversible (washout).

glibenclamide, the values for the APD_{50} were 12.3 ± 0.5 ms in control and 13.1 ± 0.6 ms in glibenclamide ($n = 14$). At 0.1 mM, glibenclamide increased the APD_{50} from 13.9 ± 0.5 ms in control to 25.8 ± 1.6 ms ($n = 7$, $P < 0.05$, paired t-test). These effects were only slowly and usually only partially reversible.

Fig. 1B illustrates the effect of the sulfilamide drug diazoxide. Under control conditions the membrane potential was -71 mV, the peak of the action potential was $+12$ mV, and the APD_{50} was 14 ms. After 3 min of perfusion of the heart with 0.5 mM diazoxide, the membrane potential and peak potential were unchanged, but the APD_{50} was increased to 20 ms. This effect was fully reversible. The effect of diazoxide was dose-dependent: 0.1 mM diazoxide had no significant effect upon the APD_{50} whereas 0.5 mM diazoxide provoked a strong effect (Fig. 2).

The effect of the application of tolbutamide and diazoxide at the same time to the same heart was additive (Fig. 3). 0.5 mM diazoxide increased the APD_{50} by $23 \pm 2\%$ ($n = 6$) when it was applied in the presence of 1 mM tolbutamide. The same concentration of diazoxide had little effect (an increase of $4 \pm 3\%$, $n = 4$) when it was applied in the presence of 2 mM tolbutamide.

Close observation of the action potentials shown in Figs. 1 and 3 seemed to indicate that tolbutamide and diazoxide might be preferentially acting upon different phases of the repolarization of the action potential. To examine this point, a detailed analysis of the action potentials recorded in the presence of 2 mM tolbutamide ($n = 16$) and 0.5 mM diazoxide ($n = 12$) was performed. For each experiment the duration of the action potential recorded in the presence of the drugs was measured at 30% (APD_{30}), 50% (APD_{50}) and 80% (APD_{80}) of the repolarization. In the presence of 0.5 mM diazoxide there were no significant differences between the increase in action potential duration measured at these three points. The APD_{30} had been increased by $36 \pm 5\%$, the APD_{50} by $35 \pm 4\%$, and the APD_{80} by $42 \pm 7\%$. On the other hand, the effect of 2 mM tolbutamide progressively increased with repolarization. The APD_{30} was increased by $17 \pm 2\%$, the APD_{50} by $25 \pm 2\%$ and the APD_{80} by $74 \pm 11\%$. These values were significantly different from each other ($P < 0.05$, t-test).

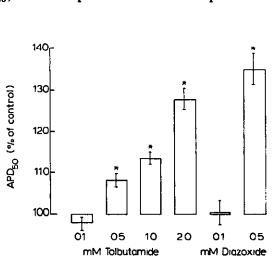

Fig. 2. Effects of tolbutamide and diazoxide upon the duration of action potentials, recorded from intact rat ventricle. Action potential duration was measured at 50% of repolarisation (APD_{50}). ★, $P < 0.05$, paired t-test, values were compared with those recorded in normal Tyrode solution. Columns and bars represent means ± S.E. for 5–19 different experiments.

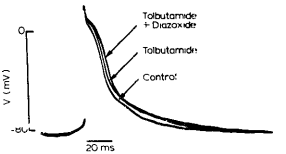

Fig. 3. Effects of tolbutamide and diazoxide upon action potentials recorded from intact rat heart are additive. The heart was first perfused with 2 mM tolbutamide, which increased APD_{50} from 15 ms in Tyrode solution to 17.5 ms. The heart was then perfused with a solution which contained 2 mM tolbutamide and 0.5 mM diazoxide, and APD_{50} was further increased to 19.5 ms. Both these effects were fully reversible (not shown).

Since we had not expected diazoxide and tolbutamide to have similar effects on the action potentials recorded from intact rat ventricle, this led us to examine the effects of these drugs upon single ATP-sensitive K^+ channels in membrane patches excised from rat ventricular myocytes. In these single-channel current recording experiments, the currents were identified as flowing through ATP-sensitive K^+ channels by the closure of the channel when ATP was applied to the internal surface of each excised membrane patch (not shown). Other ion channels were not encountered in this study.

Fig. 4A illustrates the effect of tolbutamide applied to the internal surface of an excised inside-out membrane patch. When the patch was bathed in control K-rich solution, simultaneous openings of up to ten single-channel current levels could be recorded. The application of 0.5 mM tolbutamide inhibited channel openings such that a maximum of five simultaneous single-channel current levels were seen. The inhibition was lifted when the tolbutamide was washed away from the patch. This result was typical of experiments conducted upon eight different inside-out membrane patches when either 0.5 or 2.0 mM tolbutamide was applied to the internal surface of the membrane.

Diazoxide also inhibited ATP-sensitive K^+ channels when it was applied to the internal surface of excised inside-out membrane patches (Fig. 4B). In this example, which is typical of results obtained from eight different membrane patches, 0.5 mM diazoxide reduced channel

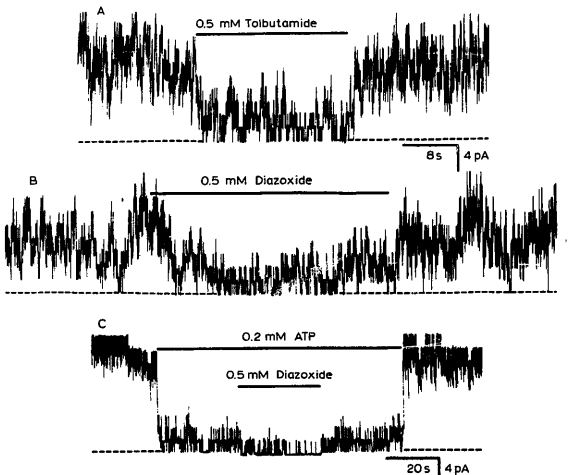

Fig. 4. Tolbutamide (A) and diazoxide (B) close ATP-sensitive K^+ channels. Both drugs were applied to the internal surface of excised membrane patches when indicated by the bars above the current traces. Diazoxide enhances closure of ATP-sensitive K^+ channels evoked by ATP (C). The bars above this current trace indicate the periods for which the internal surface of the excised inside-out membrane patch was perfused with solutions which contained ATP or ATP and diazoxide. A, B and C were obtained from different membrane patches. Currents flowing through open channels passed outwards through the excised membrane (from the bath and into the pipette) and are shown as upwards deflections of the current traces. The dotted lines indicate the patch current levels which were recorded when all channels were closed by the application of ATP (not shown).

openings from a maximum of nine simultaneously open channel current levels to a maximum of five simultaneously open levels. This effect was fully reversed when the diazoxide was washed away from the patch. Diazoxide was found to enhance the inhibition of opening of ATP-sensitive K^+ channels when it was applied in the presence of ATP (Fig. 4C). Fig. 4C is typical of the results obtained from eight different inside-out membrane patches where 0.5 mM diazoxide was applied to the exposed internal surface of the membrane in the presence of ATP. In this patch there were nine ATP-sensitive K^+ channels. The application of 0.2 mM ATP reduced channel openings to a maximum of 2–3 simultaneous current levels. In the continued presence of ATP 0.5 mM diazoxide further reduced channel openings to one, infrequent, level. These effects were fully reversible.

Discussion

This report provides two new pieces of information. The first is that tolbutamide, glibenclamide and diazoxide evoke an elongation of the action potential recorded from epicardial cells of the intact rat heart. The second is that the sulfilamide drug, diazoxide, inhibits openings of ATP-sensitive K^+ channels in rat ventricular cells.

Previous studies of the effects of sulfonylurea drugs upon cardiac muscle cells have shown that both glibenclamide and tolbutamide reverse the shortening of the action potential induced by either the internal perfusion of isolated cells with a low concentration of ATP, or the application of a metabolic poison [10,11]. These results have been interpreted as resulting from the sulfonylureas inhibiting the opening of ATP-sensitive K^+ channels, since metabolic poisons evoke openings of ATP-sensitive K^+ channels [9,15] and ATP-sensitive K^+ channels from cardiac muscle cells can be inhibited by the application of tolbutamide or glibenclamide [10,11]. This evidence points to an almost pathological role for the ATP-sensitive K^+ channel in the heart, a channel which is activated only under adverse conditions [9]. We have shown that the sulfonylurea drugs and diazoxide evoke an elongation of the action poten-

tial recorded from ventricular cells of an intact and working heart. We have also shown that they inhibit openings of ATP-sensitive K^+ channels. It seems reasonable to apply the given interpretation [10,11] to our results, and to suggest that the increase in the duration of the action potential arises from the inhibition of ATP-sensitive K^+ channels. In this case, a current could be flowing through these channels in the intact and working heart. A similar increase in the duration of action potentials of rat ventricular myocytes has been recorded with the application of α_1-adrenergic agonists and protein kinase C activating phorbol esters [16]. Phorbol esters have been shown to close ATP-sensitive K^+ channels [17]. It is possible that the suppression of outward current which resulted from α_1-stimulation and phorbol esters in rat heart [16] could also have resulted from the inhibition of ATP-sensitive K^+ channels. Clearly this is a subject which will bear further investigation.

In insulin-secreting cells, tolbutamide and diazoxide have antagonistic effects [3–6,8,18,19]. We have found that they evoke the same effect in the intact rat heart, and that both drugs inhibit openings of ATP-sensitive K^+ channels in excised patches of ventricular membrane. Our observation that tolbutamide evokes inhibition of ATP-sensitive K^+ channels agrees with other studies in cardiac muscle where this effect has been fully quantified [10,11]. That diazoxide evokes inhibition of ATP-sensitive K^+ channels of cardiac membrane is a new observation. In insulin-secreting cells, diazoxide was found to have little effect when applied alone [5], but strong stimulatory effects when the channel had been closed by ATP [4,5,8]. We found that, alone, diazoxide markedly inhibited opening of ATP-sensitive K^+ channels, and in the presence of ATP it enhanced channel closure.

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