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Blocking actions of glibenclamide on ATP-sensitive K^+ channels in pig urethral myocytes

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Abstract

The inhibitory effects of glibenclamide on the levcromakalim-induced ATP-sensitive K^+ (K_{ATP}) channels were investigated with cell-attached configuration in pig proximal urethra. Application of 10 μM glibenclamide reversibly inhibited the activity of the 100 μM levcromakalim-induced K_{ATP} channel, decreasing not only the channel open probability but also the amplitude of unitary current. The inhibitory concentration–response curve of the glibenclamide-induced sublevel conductance of K_{ATP} channel was shifted to the right (IC50 = 4.7 μM), compared with the levcromakalim-induced K_{ATP} channel (full conductance, IC50 = 0.5 μM). Glibenclamide is the first reported sulphonylurea to selectively block K_{ATP} channel, not only by decreasing the channel activity but also by reducing the unitary amplitude in smooth muscle.

Introduction

Sulphonylureas, hypoglycaemic agents, are a class of drugs used in clinical treatment of non-insulin-dependent diabetes mellitus (Ashcroft & Ashcroft 1990). It is well established that molecules in this family, especially glibenclamide ($\leq 10\,\mu$M), are specific inhibitors of ATP-sensitive K^+ (K_{ATP}) channels which have been observed in a variety of tissues (reviewed by Ashcroft & Ashcroft (1990)). Since Inagaki et al (1995) succeeded in co-expressing a new member of the inwardly-rectifying K^+ channel (Kir) family (Kir6.2), which forms functional K_{ATP} channels with the sulphonylurea receptor (SUR), the sulphonylurea (glibenclamide)-sensitivity for K_{ATP} channels has become significant pharmacologically. The sulphonylurea receptor itself does not possess intrinsic ion channel activity but seems to regulate several inwardly rectifying K^+ channels (Ämmälä et al 1996). Classically, binding sites for sulphonylureas have been investigated by binding assays and by autoradiography of tissue sections exposed to ^{3}H- or ^{125}I-labelled sulphonylureas. High and low affinity binding sites for sulphonylureas have been identified in a variety of tissues (reviewed by Ashcroft & Ashcroft (1992)). Glibenclamide was thought to reduce the flow of K^+ through the pore of K_{ATP} channels by decreasing the channel open probability and two different sensitivities for sulphonylureas in single channel recordings have not been identified (such as high and low affinity binding sites). Moreover, neither sulphonylureas nor K_{ATP} channel blockers have been reported to induce sublevel conductance of channel openings by reducing the unitary amplitude of the K_{ATP} current or by decreasing the channel activity (open probability) in smooth muscle. In this study, we have investigated the glibenclamide-induced sublevel conductance of the levcromakalim-induced K_{ATP} channels. The possibilities of different binding affinity to sulphonylurea receptors in pig urethra have been discussed.

Materials and Methods

Cell dispersion

Fresh female pig proximal urethras were obtained from an abattoir. The cell preparation was essentially the same as described by Teramoto et al (1997) (the gentle tapping method).

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Solution and drugs

The composition of the pipette and bath solution was the same (mM): K^+ 140, Cl^- 140, EGTA 5, glucose 5, HEPES 10/Tris (pH 7.35–7.40) i.e. symmetrical K^+ conditions at room temperature (21–23 °C). All chemicals were purchased from Sigma (Sigma Chemical K.K., Tokyo, Japan). Levcromakalim (SmithKline Beecham, Harlow, UK) and glibenclamide were prepared daily as a 100 mM stock solution in dimethyl sulfoxide (DMSO). The final concentration of DMSO was less than 0.3% and this concentration did not affect K^+ channels.

Recording procedure

The experimental system used was essentially the same as described by Teramoto et al (1997). The single-channel stored data were low-pass filtered at 2 kHz (−3 dB) and sampled into the computer with a digital interval of 80 μs (digital sampling time) using the 'PAT' program (kindly provided by Dr Dempster, University of Strathclyde, UK). However, events briefer than 500 μs were not included in the evaluation and no correction was made for missed events. Data points were fitted using a least-squares fitting. The all-point amplitude histogram was obtained from a continuous recording and fitted with the Gaussian equation with a least-squares fitting. Continuous traces in the figures were obtained from records filtered at 500 Hz for presentation (digital sampling time, 250 μs) although shorter traces demonstrated the original recordings. We did not define the total number of channels present in each patch membrane, so the channel activity was calculated using the following equation from an all-point amplitude histogram and expressed as an NP_o value (number of channels (N) × open state probability (P_o)):

$$NP_o = (\sum_{j=1}^{N} t_j \cdot j)/T$$

where t_j was the time spent at each current level corresponding to $j = 0, 1, 2 \ldots N$ and T was the indicated duration of the recording.

Statistics

Statistical analyses were performed with analysis of variance (two-factor with replication). Changes were considered significant at $P < 0.01$ and data were expressed as the mean ± s.d.

Results

Cell-attached patch was performed in dispersed cells of pig proximal urethra at a holding potential of −50 mV (Figure 1A). Levcromakalim (100 μM) was present in the bath solution throughout the experiments, activating the small amplitude channel (K_{ATP} channels, 2.15 pA; full conductance) at –50 mV. Although application of 10 μM glibenclamide reversibly blocked the channel opening of the full conductance, reducing the NP_o value to nearly zero, a small amount of channel openings were occasionally observed, even in the presence of 10 μM glibenclamide, showing a smaller unitary amplitude (1.15 pA; sublevel conductance) at −50 mV (Figure 1B, C). Similar 10 μM glibenclamide-induced sublevel conductance openings were observed in 10 of 63 membrane patches. On removal of 10 μM glibenclamide, the channel openings of K_{ATP} channels reappeared and the glibenclamide-induced sublevel conductance disappeared.

To investigate the sensitivity to glibenclamide of the sublevel conductance, higher concentrations of glibenclamide were applied to the bath in cell-attached mode. Glibenclamide (10 or 30 μM) inhibited the channel activity of K_{ATP} channel not only by decreasing the NP_o value but also by reducing the amplitude of the unitary current at a holding potential of −50 mV. Applying 100 μM glibenclamide, the channel activity of the sublevel conductance was completely suppressed (Figure 2). Figure 2 shows the relationships between the relative value of NP_o for the sublevel conductance and the concentration of glibenclamide at −50 mV (n = 5), compared with the previously described K_{ATP} channels in pig urethra (Teramoto et al 1997).

Discussion

Subconductance states of K_{ATP} channels

Subconductance states of K_{ATP} channels have been identified in cardiomyocytes (guinea-pig ventricular cell (Kakei et al 1985); human ventricular cell (Babenko et al 1992)). Furthermore, the reduction of an intracellular pH below 6.5 produced a distinct subconductance level in K_{ATP} channels of guinea-pig ventricular cells (Fan et al 1993). Blocker-induced subconductance has been widely observed in several types of K^+ channels (reviewed by Fox 1987). In single-channel recordings, glibenclamide (≤ 10 μM) selectively inhibited K_{ATP} channels, only reducing the open probability without modifying the unitary amplitude (all-or-none blocking action, reviewed by Babenko et al (1998)). However, recently, it was reported that subconductance states of K_{ATP} channels were observed in ventricular myocytes, revealed by partial block with glibenclamide (Ju & Saint 2001). In this study, although the number of the observations was small (10 of 63 patches), glibenclamide inhibited the activity of the levcromakalim-induced K_{ATP} channel, not only by decreasing the channel open-state probability but also by reducing the amplitude of the unitary current in pig urethra. The results provided the first observation concerning the glibenclamide-induced sublevel conductance openings of the K_{ATP} channel in smooth muscle. Furthermore, in this study, we were able to demonstrate that the two types of glibenclamide-sensitive K^+ channels possessed significantly different glibenclamide-sensitivity. The results suggested several possibilities. Firstly, two different types of sulphonylurea receptor with different glibenclamide-

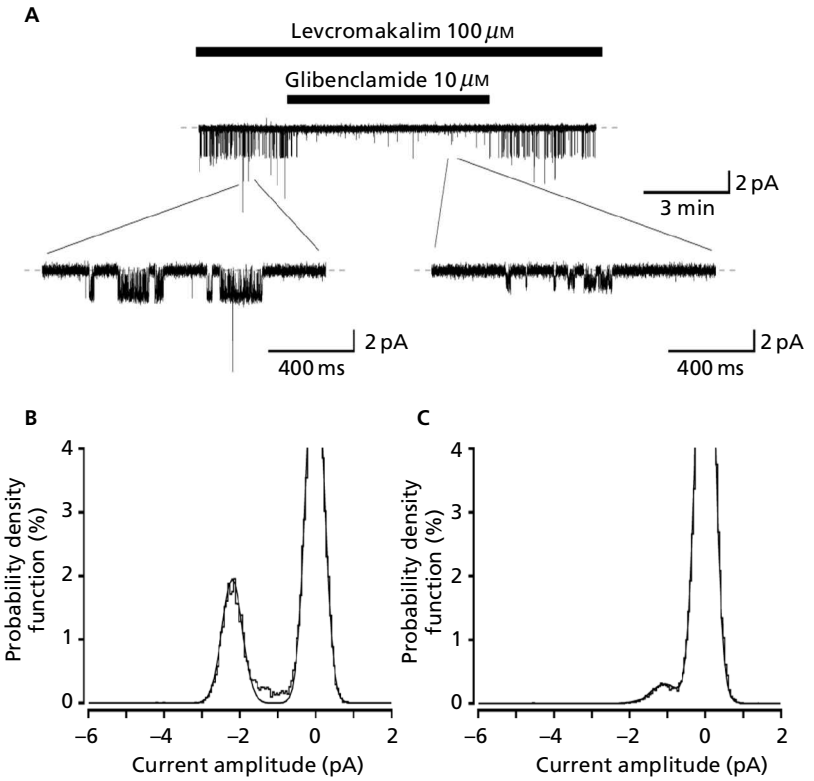

Figure 1 The inhibitory effects of 10 μM glibenclamide on a 100 μM levcromakalim-induced K^+ channel at a holding potential of −50 mV in cell-attached patch (the bath, 140 mM K^+ solution; the pipette, 140 mM K^+ solution). A. Glibenclamide-induced sublevel conductance in the presence of 100 μM levcromakalim and 10 μM glibenclamide. Lower traces show expansions of the upper trace. The dashed line indicates the current base line where the channel is not open. B and C. The all-point amplitude histogram in the (B) absence and (C) presence of 10 μM glibenclamide. Continuous lines in the histograms are theoretical curves fitted with the Gaussian distribution, by the least-squares method. The abscissa scale shows the amplitude of the current (pA) and the ordinate scale shows the percentage value of the probability density function (%) for the recording period (120 s).

sensitivity might be present in pig urethral K_{ATP} channels. Secondly, the different glibenclamide-sensitivity might be due to the stoichiometry of the sulphonylurea receptor with different types of Kir6.x. Thirdly, the shift of the glibenclamide-sensitivity might be related to allosteric changes of K_{ATP} channels.

Two different types of sulphonylurea receptor (SUR)

In functional expression experiments, pharmacological and electrophysiological studies have indicated that SUR1/Kir6.2 represents the pancreatic β-cell K_{ATP} channel, SUR2A/Kir6.2 is thought to represent the cardiac K_{ATP} channel, whereas SUR2B/Kir6.1 represents the smooth muscle-type K_{ATP} channel (reviewed by Babenko et al 1998; Fujita & Kurachi 2000). Recently, in pig urethra, Yunoki et al (2003) have shown the presence of SUR1 and SUR2B transcripts by use of reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Furthermore, by use of patch-clamp experiments, it has been demonstrated that SUR1 and SUR2B play a functional role in the activity of pig urethral K_{ATP} channels, suggesting that SUR1 and SUR2B do not combine, but rather form homomultimeric subunits (Yunoki et al 2003). Moreover, in binding studies, both high and low affinity binding sites for [^{3}H]glibenclamide have been identified in a variety of tissues (reviewed by Ashcroft & Ashcroft 1992). However, in recombinant K_{ATP} channel experiments, because the glibenclamide-sensitivity of SUR1 is much higher compared with SUR2B, it is difficult to explain the shift of the glibenclamide-sensitivity simply due to different types of sulphonylurea receptor in pig urethral K_{ATP} channels.

The stoichiometry between the sulphonylurea receptor and Kir6.x in the presence of glibenclamide and K_{ATP} channel openers

It is generally thought that a high affinity sulphonylurea receptor itself does not possess intrinsic ion channel activity, but seems to regulate several inwardly rectifying K^+ channels, giving the sulphonylurea-sensitivity (Ämmälä et al 1996). Glibenclamide had no significant effect on the channel activity of a carboxy terminus truncated Kir6.2 (Kir6.2ΔC26 or Kir6.2ΔC36) in inside-out configuration,

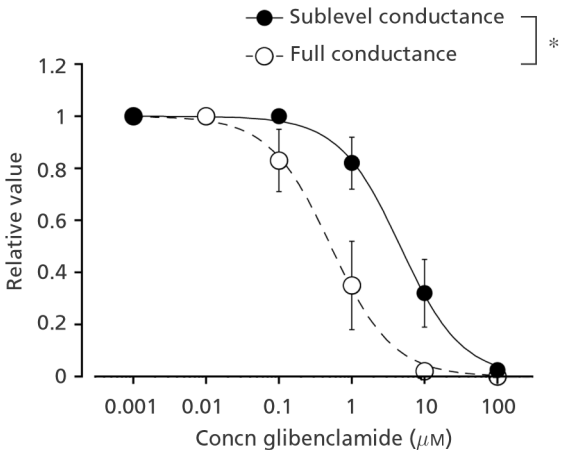

Figure 2 Relationships between relative inhibition of the NP_o value for the K^+ channels in the presence of 100 μM levcromakalim and the concentration of glibenclamide. The NP_o value just before applying glibenclamide was normalized as one. The curves were drawn by fitting the equation using the least-squares method: relative value of $NP_o = 1/(1 + (Ki/D)^{nH})$, where K_i, is the inhibitory dissociation constant, D is the concentration of glibenclamide (nM), and n_H is the Hill coefficient. The following values were used for the curve fitting: $K_i = 4.7\,\mu M$, $n_H = 1.0$ (sublevel conductance, $n = 5$). Each symbol indicates the mean with s.d. shown by vertical lines. The broken-line curve was obtained for original K_{ATP} channel (full conductance) from Teramoto et al (1997) i.e. $K_i = 0.5\,\mu M$, $n_H = 1.0$, $n = 4$. *The two curves were significantly different ($P < 0.01$).

suggesting that glibenclamide had no effect on Kir6.2-containing channels (Tucker et al 1997; Teramoto et al 2001). In contrast, the direct action of glibenclamide on the pore of Kir6.1 still remains elusive. Surprisingly, in recombinant K_{ATP} channel studies, it has been reported that the activity of SUR2B/Kir6.1 (IC50 = 43 nM) was more sensitive to glibenclamide than SUR2B/Kir6.2 (IC50 = 167 nM) (Hambrock et al 2001). These results suggested that the glibenclamide-sensitivity of K_{ATP} channels depended also on the Kir6.x subtype, although additional regulatory components of K_{ATP} channels (such as Mg ATP, phospholipids, nucleotides etc.) were poorly controlled in the recombinant K_{ATP} channels. In functional expression experiments, Cui et al (2001) reported that heteromultimerization readily occurred between Kir6.1 and Kir6.2, producing functional glibenclamide-sensitive K_{ATP} channels. Cui et al (2001) suggested the possibility that K_{ATP} channels in pig urethra might possess a heteromeric Kir6.1/Kir6.2 structure according to the size of the channel conductance. Other recent evidence supports this hypothesis. In pig urethra, it was found that RT-PCR analysis revealed the presence of Kir6.1 and Kir6.2 transcripts and that the subunit proteins of both Kir6.1 and Kir6.2 were detected in the membrane fraction of pig urethra using Western blotting (Teramoto et al 2003). However, in the absence of glibenclamide, the size of the conductance of pig urethral K_{ATP} channel was approximately 43 pS (Teramoto et al 1997). No multiple size of the unitary amplitude was observed in this study. Thus, it is difficult to explain that the glibenclamide-induced sublevel conductance was due to the multiple combinations of Kir6.1 and Kir6.2.

Allosteric change of the sulphonylurea receptor

The negative allosteric coupling of glibenclamide and K_{ATP} channel openers is complex. Recent binding studies suggested that co-operative sites between glibenclamide and K_{ATP} channel openers might exist, producing possibly mixed complexes (Hambrock et al 2001). Cui et al (2003) reported that the potency of U-37883A, a selective K_{ATP}-channel blocker, was sevenfold on Kir6.2ΔC26 current compared with Kir6.2ΔC26/SUR2B current. They suggested that interaction between the pore and the sulphonylurea receptor played an important role in determining the pharmacological actions of K_{ATP} channel agents. Thus, we suppose that allosteric changes of the sulphonylurea receptor by application of glibenclamide modified Kir subunits, followed by change in the size of unitary amplitude in K_{ATP} channels. We suggest that the shift of the glibenclamide-sensitivity might be related to the cross-action between the sulphonylurea receptor and Kir due to its reciprocation and complex modulated by the negative allosteric interaction between glibenclamide and levcromakalim. Ju & Saint (2001) suggested that partial block of ventricular K_{ATP} channels by glibenclamide might be due to occupancy of one of the sulphonylurea receptor binding sites, whereas complete channel closure requires occupancy of more than one site. However, we are not certain of the precise mechanisms of the glibenclamide-induced sublevel conductance. Further studies are necessary to investigate the allosteric nature of K_{ATP} channels in the urethra.

Conclusion

We have demonstrated that partial block of K_{ATP} channels by glibenclamide could be manifested as subconductance state in pig urethra and that glibenclamide selectively blocked urethral K_{ATP} channel, not only by decreasing the channel activity but also by reducing the unitary amplitude.

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