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On the mechanism of ADP-induced alteration of sulphonylurea sensitivity in cardiac ATP-sensitive K^+ channels

[1]Akihiro Miyamura, *,[1]Masafumi Kakei, [1]Kotaro Ichinari, [1]Midori Okamura, [1]Naoya Oketani & [1]Chuwa Tei

[1]First Department of Internal Medicine, Faculty of Medicine, Kagoshima University 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan

1 To study the mechanism of regulation of sulphonylurea sensitivity in ATP-sensitive K^+ (K_{ATP}) channels, we used the inside-out patch clamp technique in guinea-pig ventricular myocytes.

2 In the absence of nucleotides, the half maximal concentration of tolbutamide inhibition of K_{ATP} channels (IC_{50}) was 0.4 mM, and it decreased to 0.1 mM when 0.1 mM ATP was added.

3 Increasing the ADP concentration from 0 to 0.1 and 0.3 mM in the absence of ATP shifted the IC_{50} from 0.4 to 5.3 and 11.4 mM, respectively. Increasing the ADP concentration further to 1 and 3 mM conversely reduced the IC_{50} to 9.5 and 4.4 mM, respectively.

4 In the absence of Mg^{2+} and ADP, the IC_{50} was calculated to 16.6 mM which was found to be less, 12.3, 5.1 and 2.5 mM, respectively, when the ADP concentration was increased to 0.1, 0.3 and 1 mM.

5 The IC_{50}s for tolbutamide obtained at various concentrations of ADP in the presence of Mg^{2+} were best fitted by equations reflecting a model that assumed two binding sites for ADP; one is a high affinity site that reduces the sensitivity to the sulphonylurea, while the other is a low affinity site that increases such sensitivity. Dissociation constants calculated for ADP to sites 1 and 2 were 2.6 μM and 46.7 mM, respectively. In the absence of Mg^{2+}, data were fitted by equations corresponding to a single site model (site 2); the dissociation constant for ADP was 25.0 mM.

6 It is concluded that ADP modifies tolbutamide sensitivity by binding to two sites. The high affinity site is strongly Mg^{2+}-dependent, whereas the low affinity site is Mg^{2+}-independent.
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Abbreviations: EGTA, ethylene glycol bis (β-aminoethylether)-N,N,N′,N′-tetraacetic acid; K_{ATP} channel, ATP-sensitive K^+ channel; NBF, nucleotide binding fold; PIP_2, phosphatidyl inositol-4,5-bisphosphate; SUR, sulphonylurea receptor

Introduction

Cardiac ATP-sensitive K^+ (K_{ATP}) channels are believed to exert a cardioprotective effect on the ischaemic heart (Yokoshiki *et al*., 1998). Opening of these channels reduced the myocardial damage induced by metabolic inhibition as a consequence of the shortening of the action potential duration, which decreases Ca^{2+} influx and increases Ca^{2+} efflux, thereby minimizing the Ca^{2+} overload in the myocytes. This concept has been accepted as a major pathophysiological role of the K_{ATP} channel since its discovery (Noma, 1983).

The β-cell type K_{ATP} channels are composed of a heteromultimer containing at least two subunits, an inward rectifier potassium channel pore subunit (Kir6.2), and a regulatory subunit as a sulphonylurea receptor (SUR1), which regulates the channel activity *via* sulphonylurea-induced inhibition (Inagaki *et al*., 1995). SUR, which is a high affinity receptor for the sulphonylureas, had been cloned (Aguilar-Bryan *et al*., 1995) and found to be a member of ATP binding cassette (ABC) superfamily in which two nucleotide binding folds (NBF1 and NBF2) have been identified (Aguilar-Bryan *et al*., 1995). Cytoplasmic ADP activates the channel by binding to NBF2 in a M^{2+}-dependent manner (Nichols *et al*., 1996; Gribble *et al*., 1997a). In pancreatic β cells, a hereditary structural deficiency of NBF2, to which ADP binds, has been reported to cause excessive inhibition of activity of the channels due to cytosolic ATP; there is consequently an inappropriate secretion of insulin in newborns associated with persistent hyperinsulinaemic hypoglycaemia (Thomas *et al*., 1995). These findings suggest that opening of K_{ATP} channels is induced not only by a reduction in cytosolic ATP levels but also by an increase in MgADP levels (Kakei *et al*., 1986; Dunne & Petersen, 1986). Cardiac-type K_{ATP} channels are composed of Kir6.2 and SUR2A, an isoform of SUR1 (Inagaki *et al*., 1996). Recently smooth muscle-type SUR has been identified as SUR2B (Isomoto *et al*., 1996; Yamada *et al*., 1997). The site for channel inhibition by ATP is reportedly located on Kir6.2, and at least two sites at the N- and C-termini may allosterically influence the channel inhibition by ATP (Tucker *et al*., 1997; Proks *et al*., 1999).

Phosphatidylinositol metabolism has recently been reported to regulate the activity of K_{ATP} channels (Hilgemann & Ball, 1996; Fan & Makielski, 1997; Xie *et al*., 1999). Phosphatidyl-inositol-4,5-bisphosphate (PIP_2) lowered the ATP sensitivity upon exposure to the membrane patches (Okamura *et al*., 1998). PIP_2, which is negatively charged, may antagonize ATP binding to Kir6.2 (Shyng & Nichols, 1998; Baukrowitz *et al*., 1998). Thus, positively charged C terminal of Kir6.2 may be a likely site for the binding of ATP which inhibits the channel (Fan & Makielski, 1997; Shyng & Nichols, 1998; Baukrowitz *et al*., 1998).

*Author for correspondence;
E-mail: mkakei@med4.kufm.kagoshima-u.ac.jp

The sensitivity of the K_{ATP} channels to sulphonylureas is modulated by cytosolic nucleotide diphosphates (Zünkler *et al*., 1988; Venkatesh *et al*., 1991; Schwanstecher *et al*., 1992; 1994; Virag *et al*., 1993). ADP lowers the sensitivity of cardiac-type K_{ATP} channels to the drugs (Venkatesh *et al*., 1991), whereas it increases the sensitivity of pancreatic β-cell type K_{ATP} channels (Zünkler *et al*., 1988; Schwanstecher *et al*., 1992; 1994; Virag *et al*., 1993). In a study of the expression of K_{ATP} channels, MgADP enhanced the blocking efficacy of tolbutamide, against wild-type K_{ATP} channels (Kir6.2/SUR1), while it failed to do so against channels containing a mutated Walker A and/or B motif in the NBFs (Gribble *et al*., 1997b). Thus, the stimulation of either or both of the NBFs seems to modulate the K_{ATP} channels by altering their sensitivity to sulphonylureas.

The present study evaluated the effects of both ATP and ADP on the sensitivity of K_{ATP} channels to tolbutamide, a sulphonylurea, to determine how such nucleotides regulate inhibitory processes from the sulphonylurea binding to the K_{ATP} channel gating in guinea-pig ventricular cells.

Methods

Isolation of single ventricular myocytes

Single ventricular myocytes were isolated from the hearts of guinea-pigs (200–300 g) as has been previously described (Taniguchi *et al*., 1981). Briefly, the animals were anaesthetized with sodium pentobarbital (25 mg kg^{-1}, i.p.) and artificially ventilated. The chest was opened, the aorta was cannulated, and the coronary artery was perfused with Tyrode's solution. The heart was removed, suspended on a Langendorff-type apparatus and perfused with Tyrode's solution. The perfusate was switched to a nominally Ca^{2+}-free Tyrode's solution (100 ml). A digesting solution that contained 0.01% collagenase (Worthington, type II) was then perfused for 10 min. Next, the heart was perfused with 100 ml of K-B (Kraftbrühe) solution (Isenberg & Klöchner, 1982) to wash out the collagenase. The left ventricle was removed and cut into small pieces (5 mm) in K-B solution and dispersed mechanically into single cells. The dispersed myocytes were stored in K-B solution at 4°C for at least 1 h before use. Single ventricular myocytes were then placed in a recording chamber (500 μl) that was filled with Tyrode's solution. Experiments were started after the cells had settled to the bottom of the chamber.

Single channel current recordings

Standard patch-clamp techniques (Hamill *et al*., 1981) were used to record single K_{ATP} channel currents from inside-out membrane patches. Patch pipettes were pulled from hard glass tubing (Narishige, Tokyo, Japan), coated with silicon resin to reduce the electrical capacitance, and fire-polished immediately before use. The pipettes had resistances between 5 and 10 MΩ when filled with the pipette solution (140 mM KCl). After a GΩ had been established on a cell exposed to a standard internal solution containing 0.3 mM ATP, the patch membrane was excised.

The K_{ATP} channel current was recorded with an amplifier (AXOPATCH 200A, Axon Instruments, Foster City, CA, U.S.A.) and stored in a PCM digital data recorder (RD 101T, TEAC, Tokyo). Replayed data were then low-pass filtered (24 db $octave^{-1}$, E 3201A NF, Tokyo, Japan) at the cut off frequency of 2 kHz and digitized at 5 kHz by PCALMP6 software using a computer (IBM, New York, U.S.A.). On-line recordings of the single channel current were performed in some experiments.

To construct the concentration-response relationships between channel activity and tolbutamide concentration, we calculated the mean K_{ATP} channel current during superfusion with each test solution using the software CLAMPFIT. We usually calculated the average current during a 10 to 20 s period under steady-state conditions. The concentration-response curve was drawn according to the following equation:

$$\text{relative channel activity} = \{1 + ([L]IC_{50}^{-1})^{h}\}^{-1}, \qquad (1)$$

where [L] is the ligand (tolbutamide) concentration. Relative channel activity indicates the ratio of the mean channel current during the exposure of the membrane patches to the test solution containing the required concentration of tolbutamide to that in the control solution. IC_{50} was the half maximal concentration of tolbutamide on the channel inhibition; h was the Hill coefficient. All the curves illustrated in figures were drawn according to equation (1) with the Hill coefficient of 1 using the software DeltaGraph (DeltaPoint Inc. Tokyo, Japan). IC_{50} values with 95% confidence intervals in parentheses are given. The concentration-inhibition relationship was determined by best fitting to the data of three to 12 patches by using simple regression analysis of the software StatView. The data were plotted as the mean ± s.e.mean when error bars were greater than symbols. Patch membrane potential was held at −60 mV and low-pass filtered at 800 Hz to plot the current traces in the figures.

Solutions

Tyrode's solution contained (in mM): NaCl 136.9; KCl 5.4; $CaCl_2$ 1.8; $MgCl_2$ 0.5; $NaHPO_4$ 0.33; and glucose 5.5; the pH was adjusted to 7.4 with 5 mM NaOH. The K-B solution contained (in mM): KCl 25; taurine 10; glutamic acid 70; oxalic acid 10; HEPES 10; KH_2PO_4 10; ethylene glycol bis (β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) 0.5 and glucose 11; pH was adjusted to 7.4 with KOH. The composition (mM) of the pipette solution was as follows; KCl 140; $CaCl_2$ 2; HEPES 5 (pH 7.4 adjusted with NaOH). The standard internal solution contained (mM): KCl 134.5; KH_2PO_4 0.5; $MgCl_2$ 2; EGTA 1; HEPES 5 (pH 7.2 with KOH; final concentration 140 mM; free Ca^{2+} concentration <0.1 nM; free Mg^{2+} concentration 1.3 mM). The Mg^{2+}-free internal solution was nominally Mg^{2+}-free and contained 1 mM ethylenediamine tetraacetic acid (EDTA) instead of equimolar EGTA (pH 7.2 adjusted with KOH; free Mg^{2+} concentration <1 nM). Adenosine-5′-triphosphate (ATP-2Na) and adenosine-5′-diphosphate (ADP-2Na) were obtained from Boehringer (Mannheim, Germany). Tolbutamide was obtained from Sigma (St. Louis, MO, U.S.A.). We prepared a stock solution of 100 or 300 mM tolbutamide, diluting it to the final concentration required for use. All experiments were performed at room temperature (22–25°C). Data were expressed as mean ± s.e.mean from *n* membrane patches.

Results

Effects of nucleotides on tolbutamide-induced inhibition of K_{ATP} channels in the presence of Mg^{2+}

In Figure 1A, the single K_{ATP} channel current is shown in inside-out membrane patches. The openings of the channel

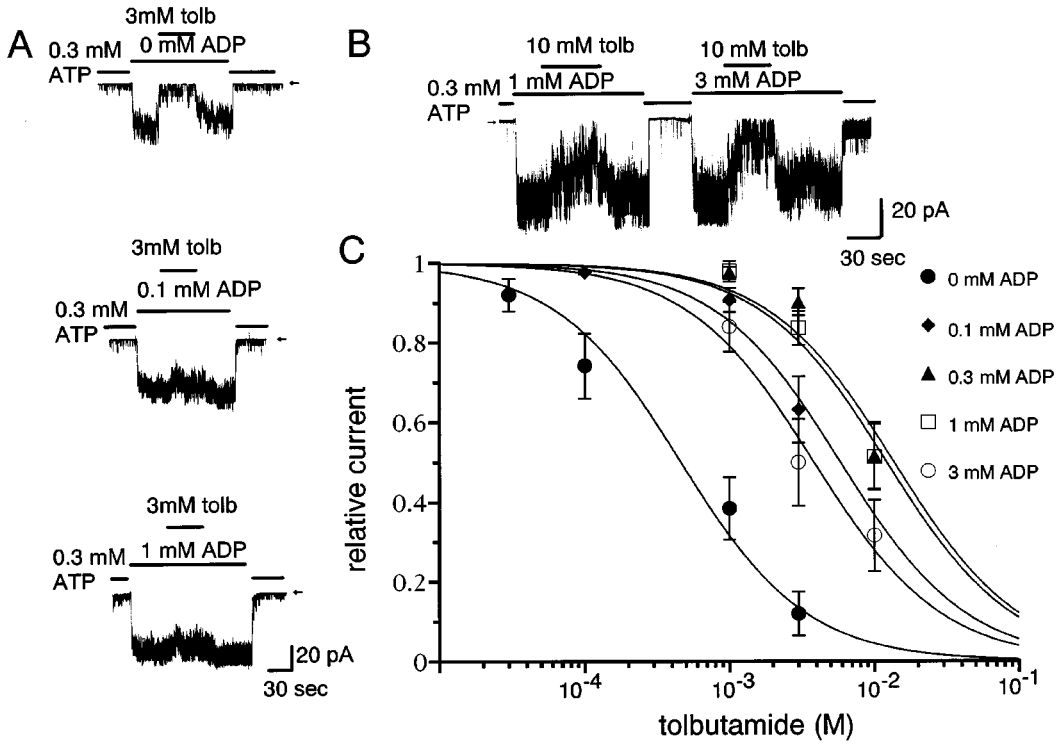


Figure 1 Effects of ADP on the tolbutamide-induced inhibition of K_{ATP} channels. (A) ADP lowers the efficacy of tolbutamide blocking of the channel. The fractional K_{ATP} channel current recorded from the inside-out membrane patch is illustrated. Arrows indicate the zero current levels. The bars shown above each trace indicate the period of exposure of membrane patches to the test solution containing tolbutamide (tolb) or ADP in the absence of ATP. The solution containing 0.3 mM ATP was superfused at the beginning and the end of traces to prevent the rundown of the channel activity. The activity of K_{ATP} channel is immediately increased and decreased at the time when ATP in the solution was removed and resuperfused, respectively. (B) Continuous recordings were made to test the effects of different concentrations of ADP (1 and 3 mM) on the blocking efficacy of 10 mM tolbutamide. (C) Concentration-inhibition relationship of activity of K_{ATP} channels for tolbutamide obtained during superfusion of various concentrations of ADP.

was increased by exchanging the solution to ATP- and ADP-free solution, indicating ATP-inhibited channel properties. In Figure 1A, 3 mM tolbutamide was superfused to test whether the drug-induced channel inhibition was modified by the presence of various concentrations of ADP. Exposure to 3 mM tolbutamide inhibited the opening of the channel. After removal of tolbutamide, the channel activity was reversed but its activity was less than that before the tolbutamide exposure. This may have been due to the rundown of the K_{ATP} channel activity observed in the ATP-free solution. When the rundown of the K_{ATP} channel was observed despite intermittent superfusion with 0.3 mM MgATP to prevent the rundown (Figure 1A, top trace), the averaged current before and after exposure to tolbutamide was taken as the control. Tolbutamide inhibited the activity of the K_{ATP} channel by 90% in the absence of ADP (Figure 1A, top trace), whereas it inhibited the opening of the channel by only $\sim$5% during superfusion of 0.1 mM ADP (middle trace) and hardly inhibited the channel in the presence of 1 mM ADP (bottom trace). The continuous recordings in Figure 1B show that the channel inhibition induced by 10 mM tolbutamide was stronger in the presence of 3 mM ADP than of 1 mM ADP (Figure 1B). The concentration-inhibition relationship between tolbutamide and the K_{ATP} channel activity was determined by normalizing the channel activity (mean patch current) obtained in test solutions to the control current in which tolbutamide was absent (Figure 1C). The curves were fitted by equation (1) with the Hill coefficient of 1. Because the tolbutamide was difficult to dissolve in the solution at concentrations greater than 10 mM, we performed the concentration-inhibition study at concentrations up to 10 mM and assumed that 100% inhibition of the channel was attained at high doses of tolbutamide. That is, we extrapolated the curve of the concentration-inhibition relationship from the data points obtained at concentrations less than 10 mM. Increasing the ADP concentration from 0 (closed circles) to 0.1 mM (diamonds) and 0.3 mM (triangles) shifted the IC_{50} from 0.4 (95% confidence interval: 0.34, 0.52) mM to 5.3 (3.6, 9.9) mM and 11.4 (7.0, 27.0) mM respectively. However, a further increase in the ADP concentration to 1 mM (squares) and 3 mM (open circles) reduced the IC_{50} to 9.5 (6.5, 17.5) mM and 4.4 (3.0, 7.7) mM, respectively, (Figure 1B, C). Thus, the modulatory effect of ADP on tolbutamide sensitivity was biphasic, showing rightward and then leftward shifts.

Effects of ADP on the tolbutamide inhibition of K_{ATP} channels in the absence of Mg^{2+}

In Figure 2A, the illustrated traces, fractions of current recordings obtained from the same membrane patches, showed that 0.1 mM ADP enhanced the inhibitory effect of 3 mM tolbutamide on the channel in the absence of Mg^{2+}. Increasing ADP to 0.1 and 0.3 mM further enhanced the tolbutamide-induced channel inhibition. Concentration-inhibition curves showed an ADP concentration-dependent decrease in IC_{50} (Figure 2B). In the absence of ADP, the IC_{50} was calculated to 16.6 mM (closed circles; 95% confidence interval, 13.7, 20.8),

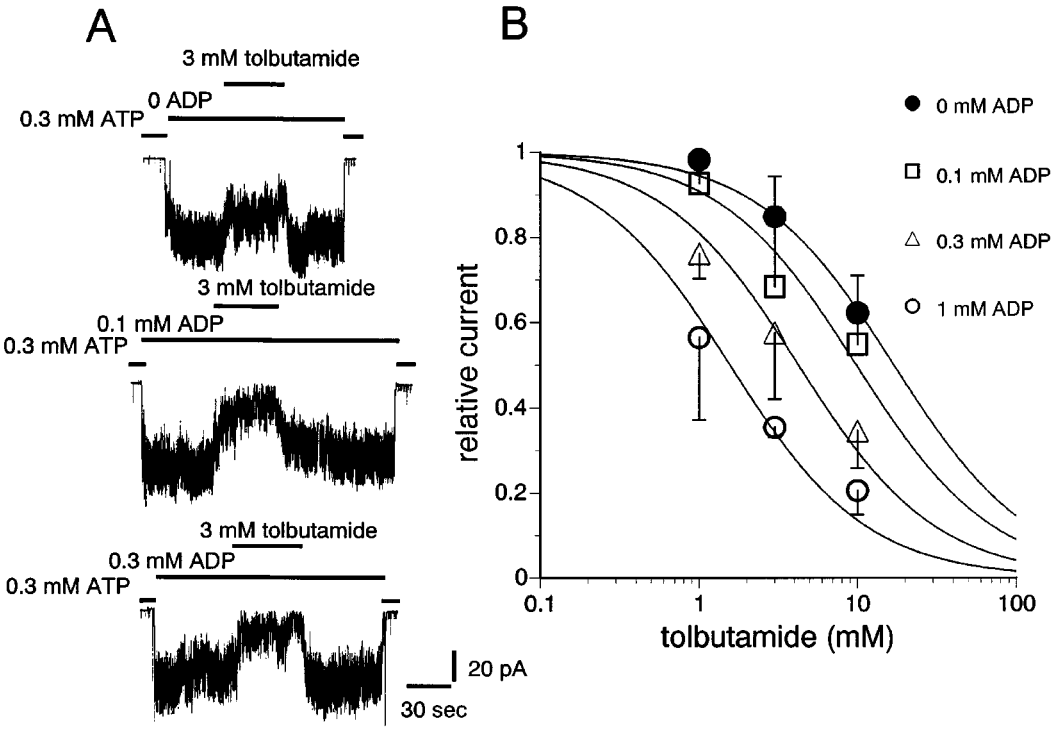


Figure 2 Effects of ADP on the tolbutamide-induced inhibition of K_{ATP} channels in the absence of Mg^{2+}. (A) ADP enhanced the efficacy of tolbutamide blocking of the channel. The current fractions illustrated were recorded from the same membrane patch and filtered at 800 Hz. The bars shown above each trace indicate the period of exposure of the membrane patch to the test solution containing tolbutamide or ADP in the absence of ATP. A solution containing 0.3 mM ATP was superfused at the beginning and end of traces to protect the channel activity from rundown. (B) Concentration-inhibition relationships obtained during superfusion with various concentrations of ADP.

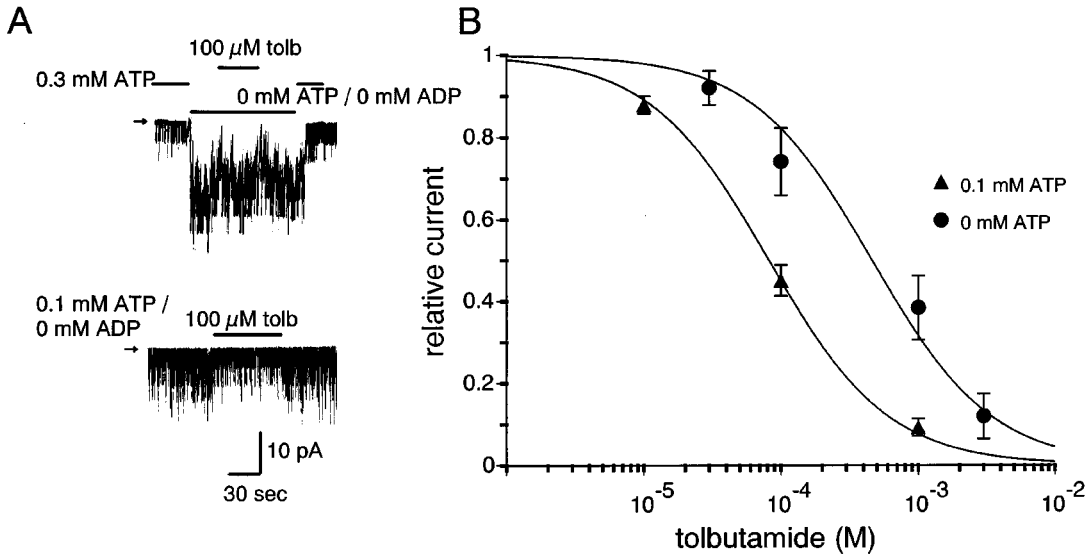


Figure 3 Effects of ATP on the tolbutamide sensitivity of K_{ATP} channels in the presence of Mg^{2+}. (A) The bars above each trace indicate the period of exposure of membrane patches to the test solution. In the lower trace, 100 μM tolbutamide applied during the period indicated by the bar in the absence of ATP showed little effect on the channel activity, whereas it decreased the activity in the presence of 0.1 mM ATP (lower trace). (B) Concentration-inhibition relationship between tolbutamide concentration and channel activity is shown in the absence and presence of 0.1 mM ATP.

suggesting that Mg^{2+} also influenced the channel's sensitivity to tolbutamide. As the ADP concentration was increased to 0.1 mM (open squares), 0.3 mM (open triangles) and 1 mM (open circles), the IC_{50} shifted to 12.3 (11.1, 14.9) mM, 5.1 (3.9, 7.3) mM and 2.5 (1.6, 5.1) mM, respectively. Thus, Mg-free forms of ADP enhanced the tolbutamide sensitivity.

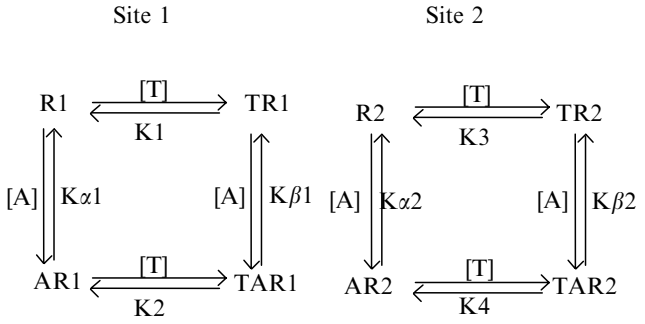
Enhancement of the tolbutamide inhibition of K_{ATP} channels by ATP

The presence of ATP at the cytoplasmic side increased the sensitivity of the K_{ATP} channels to tolbutamide in inside-out membrane patches (Figure 3). In the absence of ATP and

ADP, tolbutamide (100 μM) did not influence the activity of the channel, whereas the opening of the channel was inhibited in the presence of 0.1 mM ATP (Figure 3A, upper and lower traces). This finding indicates that the cytoplasmic ATP enhances the channel's sensitivity to tolbutamide. The nucleotide regulation of tolbutamide sensitivity is summarized in Figure 3B. The IC_{50} was 0.4 mM in the absence of nucleotides (circles) and shifted to 0.10 (0.09, 0.11) mM in the presence of 0.1 mM ATP (triangles).

Two-site model for ADP modulation of tolbutamide inhibition of K_{ATP} channels

The IC_{50}s for tolbutamide obtained at various concentrations of ADP in the presence and absence of Mg^{2+} are plotted in Figure 4. The IC_{50} increased according to the increase in the ADP concentration. The peak occurred around a concentration of 0.3 mM ADP, and there was a decline in IC_{50} at higher concentrations of ADP (closed circles). In contrast, the IC_{50} showed a monophasic decline in the absence of Mg^{2+} (open circles). The lines drawn on the data points in the presence of Mg^{2+} were obtained by assuming the following scheme, where the sulphonylurea receptor has two binding sites for ADP, one that reduces the sensitivity to the sulphonylurea (site 1), while the other enhances such sensitivity (site 2).



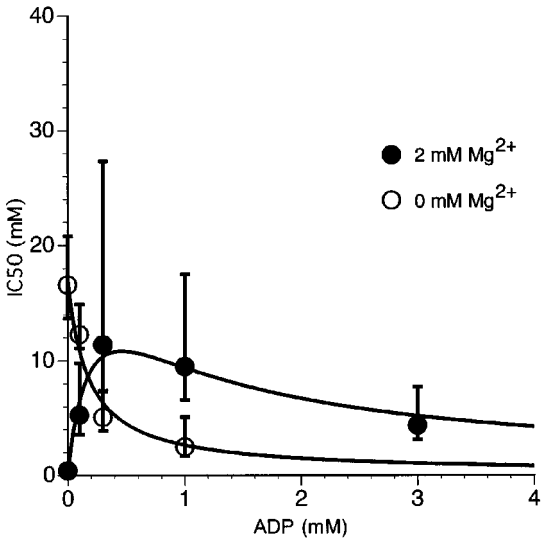


Figure 4 Changes in IC_{50} for tolbutamide inhibition obtained during exposure to various concentrations of ADP in the presence and absence of Mg^{2+}. The lines in the presence and absence of Mg^{2+} were plotted according to the equation (3) and equation (6), respectively. The error bars indicate the 95% confidence intervals of each data point.

These sites are assumed to be independent of each other in the modulation of sulphonylurea-induced channel inhibition and to be located on SUR. [A] and [T] are concentrations of ADP and tolbutamide. R1 and R2 are binding sites for ADP at site 1 and site 2. TR1 and TAR1 are the states to which tolbutamide bound in the absence and presence of ADP, respectively, in site 1, and TR2 and TAR2 are similarly related to site 2. K_n are the dissociation constants for tolbutamide and $K_{\alpha n}$ and $K_{\beta n}$ are the dissociation constants for ADP at each site. According to the above thermodynamic scheme, the following equation is obtained.

$$K_{\beta 1} = K_{\alpha 1} K_2 K_1^{-1} \quad (2)$$

The same correspondence should also be for site 2. In this two-site model, the IC_{50} for tolbutamide is assumed to be expressed by the following equation; Similar analysis has been performed in the high and low affinity model of the sulphonylureas in recombinant K_{ATP} channels (Gribble *et al*., 1997b).

$$IC_{50} = f_1 f_2. \quad (3)$$

f_1 and f_2 are the equations describing the steady state half-maximal concentration for the tolbutamide inhibition of the channel modified under the conditions of stimulation of site 1 and site 2, respectively. They are determined by the following equations:

$$f_1 = \frac{K\alpha 1 + [A]}{(K_{\alpha 1}/K_1 + [A]/K_2)} \quad (4)$$

$$f_2 = \frac{K_{\alpha 2} + [A]}{(K_{\alpha 2}/K_3 + [A]/K_4)} \quad (5)$$

In Figure 4, the ascending limb (closed circles) corresponds to f_1 and the descending limb f_2. When [A] is zero, f_1 and f_2 are identical to K_1 and K_3, respectively. When [A] is infinite, f_1 and f_2 become K_2 and K_4, respectively. The ADP concentration at the half-point of the ascending limb in the presence of Mg^{2+} is calculated to $K_{\alpha 1} K_2 K_1^{-1}$. Similar correspondences can also be derived in f_2 (descending limb). When Mg^{2+} is absent, only site 2 is functional. Thus, equation (3) is changed to:

$$IC_{50} = f_2. \quad (6)$$

The data obtained in the presence of Mg^{2+} were well fitted with equation (3) (Figure 4, closed circles, $r = 0.98$). $K_{\alpha 1}$ and $K_{\alpha 2}$ were 2.6 (1.1, 2.9) μM and 46.7 (28.3, 124.0) mM, K_1 and K_2 0.2 (0.1, 0.3) mM and 32.2 (22.6, 41.7) mM, and K_3 and K_4 1.3 (0.5, 2.1) mM and 12.9 (9.0, 14.0) μM, respectively. Similarly, the data obtained in the absence of Mg^{2+} were best fitted with equation (6) (Figure 4, open circles, $r = 0.99$); $K_{\alpha 2}$, K_3 and K_4 were 25.0 (15.0, 54.2) mM, 16.9 (14.2, 20.9) mM and 0.1 (0.05, 0.3) mM, respectively. Thus, site 1 is strongly Mg^{2+}-dependent, whereas site 2 is little dependent on the presence of Mg^{2+}. The affinity for ADP at site 1 was approximately 18,000 fold higher than that at site 2 in the presence of Mg^{2+}. The removal of Mg^{2+} increased the IC_{50} for tolbutamide in the absence of ADP. Therefore, Mg^{2+} may also influence SUR to modify the relationship between sulphonylurea binding and channel inhibition.

Discussion

The present study demonstrated that the inhibition of K_{ATP} channel is regulated by the levels of MgATP, ADP and Mg^{2+} on the cytoplasmic side. Exposure to MgATP enhanced the sensitivity to tolbutamide, whereas ADP exposure led to a

biphasic regulation with an increase followed by a decrease in the IC_{50}; the peak was observed with 0.3 mM ADP in the presence of Mg^{2+}. In contrast, when ADP was tested in the absence of Mg^{2+}, the change in IC_{50}s was monophasic. This indicates that Mg^{2+}-free forms of ADP (ADP^{3-}) only reduce IC_{50}. The IC_{50}s obtained in the presence and absence of Mg^{2+} were well fitted to a two-site model of SUR for nucleotide binding, in which one site was the MgADP binding site (site 1) and the other the ADP^{3-} binding site (site 2). The former reduced the sensitivity of the channel to tolbutamide, while the latter increased it. In the presence of Mg^{2+}, the affinity for MgADP of site 1 was approximately 18 000 fold greater than that of site 2, whereas site 1 did not function without Mg^{2+}. We conclude that MgADP and ADP^{3-} influence the tolbutamide sensitivity of K_{ATP} channels at different sites on SUR.

Because of the difficulty in dissolving tolbutamide at concentrations >10 mM, we extrapolated the data obtained with concentrations of the drug below 10 mM to construct a concentration-dependent inhibition curve for channel activity. This could have led us to err in our estimate of the IC_{50}s (Figure 4, closed circles), since the 95% confidence intervals in the IC_{50} at 0.3 and 1 mM ADP would become larger. Thus, the dissociation constant (K_2), which corresponds to the steady-state value at ADP concentration of infinity in the ascending limb of Figure 4, may include a large error as compared to the other parameters. On the other hand, the data points obtained in the absence of Mg^{2+} showed a good fit ($r=0.99$) and a narrow confidence interval. Despite relatively large confidence intervals in the data between 0.3 and 1 mM MgADP, the fitted curve shown in Figure 4 indicates that micromolar levels of MgADP are sufficient to influence the tolbutamide sensitivity. Similar MgADP-dependent changes in the sensitivity of the K_{ATP} channel opener (KCO) have been reported with respect to binding studies of KCOs ([^{3}H]-P1075) to the SUR2A (Hambrock *et al*., 1999).

In Figure 1B, channel openings that were not inhibited by 0.3 mM ATP were observed at the end of the trace. This type of channel seemed to be the opening of the K_{ATP} channel, because the single channel amplitudes were identical to those of the K_{ATP} channels. The ATP sensitivity of the channel may be altered during exposure to a high dose of MgADP. Although the mechanism underlying the alteration in channel properties with a lower sensitivity to ATP is presently not clear, we speculate that the conversion of MgADP to MgATP may occur at the submembrane space (Schwanstecher *et al*., 1998). Because ATP is a phosphate donor for lipid kinase to phosphorylate phosphatidyl inositol, the concentration of PIP_2 in the membrane may be increased as ATP concentration increases (Xie *et al*., 1999). Consequently, PIP_2 may reduce the sensitivity of the channels to ATP (Shyng *et al*., 1998; Baukrowitz *et al*., 1998; Okamura *et al*., 1998).

The modulation by nucleotides of the inhibition of the K_{ATP} channels by tolbutamide has been described in rodent β-cell K_{ATP} channels as well as in the K_{ATP} channels expressed in *Xenopus* oocytes. ADP reportedly enhances the blocking efficacy of glibenclamide in β-cell type K_{ATP} channels (Kir6.2/SUR1) (Zünkler *et al*., 1988; Schwanstecher *et al*., 1992; Gribble *et al*., 1997b), whereas ADP decreases it in the cardiac-type K_{ATP} channels (Venkatesh *et al*., 1991; Gribble *et al*., 1998). The tolbutamide-induced block of the channel and [^{3}H]-glibenclamide binding are attenuated by mutation of a single amino acid, S123Y, in the intracellular loop between transmembrane segments 15 and 16 of SUR1 (Ashfield *et al*., 1999). Dörschner *et al*. (1999) and Russ *et al*. (1999) have shown that the binding for sulphonylureas to SUR closes the channels. We observed a reduction in the channel-blocking efficacy of tolbutamide by MgADP in guinea-pig ventricular cells, confirming the results of Venkatesh *et al*. (1991). We suggest that MgADP may bind to a site on SUR and thereby reduce the channel sensitivity to tolbutamide in cardiac cells. Nucleotide-induced changes in the channel-blocking efficacy by tolbutamide may not be a consequence of the interactions between nucleotide and tolbutamide bindings at the same time, but instead may be due to the allosteric modification of the binding of tolbutamide to its effective site. The binding of ATP to NBF1 is reportedly antagonized by MgASP (Ueda *et al*., 1997). Glibenclamide releases ATP at NBF1 in the presence of MgADP or MgATP (Ueda *et al*., 1999). Thus, the bindings of Mg^{2+}, sulphonylurea, MgADP, ADP^{3-} and MgATP to SUR may influence each other allosterically.

The KCO receptors are reportedly located on the SURs of K_{ATP} channels, and the binding of KCO to SURs requires MgATP (Schwanstecher *et al*., 1998). The hydrolysis of ATP at NBFs may induce a conformational change in SUR, and consequently, increases in the affinity for KCO (Schwanstecher *et al*., 1998). The presence of MgATP may lead to an increase in MgADP levels at a site close to SUR because of the ATPase activity of SUR. Conversely, MgADP may be converted to MgATP in the membrane. The production of MgATP at the submembrane space or an increase of ADP^{3-} in the presence of excessive ADP as compared to the Mg^{2+} concentrations would increase the sensitivity to tolbutamide. This may explain, in part, why the change in the affinity of tolbutamide was biphasic in the presence of Mg^{2+} (Figure 4). In the presence of MgATP, concentrations of MgADP in the μM range, which were formed by the ATPase activity of the membrane, inhibited the binding of KCO ([^{3}H]-P1075) to SUR2B (Hambrock *et al*., 1999). Binding studies of KCOs on recombinant SUR2B showed that MgATP-binding appeared to be required for the binding of KCO to SUR in the presence of micromolar levels, whereas increasing the Mg^{2+} concentration to levels greater than 10 μM conversely decreased the affinities for KCO and glibenclamide (Hambrock *et al*., 1998). Thus, free Mg^{2+} also influences the effects of these drugs.

Under physiological conditions and with normal intracellular metabolism, the shift in the sensitivity of K_{ATP} channels to tolbutamide, as demonstrated by the present results, seems unlikely to influence cardiac K_{ATP} channel activity, since the IC_{50} observed with millimolar-range shift exceeded the pharmacological level *in vivo*. However, assuming that the nucleotide and Mg^{2+} modulation of the sensitivity to tolbutamide as demonstrated in the present paper, reflects the properties of sulphonylureas in general, therapeutic use of these drugs in patients with severe ischaemic heart disease may produce a variety of action potential durations and thereby induce arrhythmia.

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