Br. J. Pharmacol. (1994), **111**, 302–310

Interaction of tolbutamide and cytosolic nucleotides in controlling the ATP-sensitive K^+ channel in mouse β-cells

[1]Christina Schwanstecher, Corinna Dickel & U. Panten

Institute of Pharmacology & Toxicology, University of Göttingen, Robert-Koch-Strasse 40, D-37075 Göttingen, Germany

1 In mouse pancreatic β-cells the role of cytosolic nucleotides in the regulation of the sulphonylurea sensitivity of the adenosine 5′-triphosphate-sensitive K^+ channel (K_{ATP}-channel) was examined. Patch-clamp experiments with excised inside-out membrane patches were carried out using an experimental protocol favouring phosphorylation of membrane proteins.

2 In the absence of Mg^{2+}, the K_{ATP}-channel-inhibiting potency of cytosolic nucleotides decreased in the order ATP = adenosine 5′-O-(3-thiotriphosphate) (ATPγS) > adenosine 5′-diphosphate (ADP) > adenosine 5′-O-(2-thiodiphosphate) (ADPβS) = adenylyl-imidodiphosphate (AMP-PNP) > 2′-deoxyadenosine 5′-triphosphate (dATP) > uridine 5′-triphosphate (UTP) > 2′-deoxyadenosine 5′-diphosphate (dADP) > guanosine 5′-triphosphate (GTP) > guanosine 5′-diphosphate (GDP) > uridine 5′-diphosphate (UDP).

3 In the presence of Mg^{2+}, the inhibitory potency of cytosolic nucleotides decreased in the order ATPγS > ATP > AMP-PNP > ADPβS > dATP > UTP. In the presence of Mg^{2+}, the K_{ATP}-channels were activated by dADP, GTP, GDP and UDP.

4 Tolbutamide inhibited the K_{ATP}-channels not only in the presence but also in the prolonged absence of Mg^{2+}. In nucleotide-free solutions, the potency of tolbutamide was very low. When about half of the K_{ATP}-channel activity was inhibited by ATP, AMP-PNP, ADPβS or ADP (absence of Mg^{2+}), the potency of tolbutamide was increased.

5 Tolbutamide (100 μM) slightly enhanced the channel-inhibiting potency of AMP-PNP and inhibited the channel-activating effect of MgGDP in a non-competitive manner.

6 Channel activation by MgGDP (0.5 mM) competitively antagonized the inhibitory responses to AMP-PNP (1 μM–1 mM). This effect of GDP was neutralized by tolbutamide (100 μM).

7 The stimulatory effect of 0.5 mM MgGDP was neutralized by 200 μM AMP-PNP. Under these conditions the potency of tolbutamide was much higher than in the presence of 0.5 mM MgGDP alone or in the absence of any nucleotides.

8 dADP (0.3–1 mM) increased the potency of tolbutamide. Additional application of 200 μM AMP-PNP caused a further increase in the potency of tolbutamide.

9 In conclusion, in the simultaneous presence of inhibitory and stimulatory nucleotides, binding of sulphonylureas to their receptor causes direct inhibition of channel activity, non-competitive inhibition of the action of stimulatory nucleotides and interruption of the competitive interaction between stimulatory and inhibitory nucleotides. The latter effect increases the proportion of K_{ATP}- channels staying in the nucleotide-blocked state. In addition, this state potentiates the direct effect of sulphonylureas.

Keywords: ATP-sensitive K^+ channel; pancreatic β-cell; tolbutamide; ATP; AMP-PNP; MgADP; MgGDP

Introduction

Initiation of insulin release requires increase in the cytosolic Ca^{2+} concentration of the pancreatic β-cell. Enhancement of Ca^{2+} influx across the plasma membrane is induced by depolarization of the β-cell to the threshold potential at which voltage-dependent Ca^{2+} channels are activated (for reviews see Henquin & Meissner, 1984; Matthews, 1985). Glucose and other insulin-releasing fuels depolarize the β-cell by raising the adenosine 5′-triphosphate (ATP) and lowering the adenosine 5′-diphosphate (ADP) concentration at the cytosolic face of the β-cell membrane and thereby inhibiting a distinct K^+ channel (K_{ATP}-channel) (Ashcroft *et al.*, 1984; Cook & Hales, 1984; for reviews see Ashcroft & Rorsman, 1991; Dunne & Petersen, 1991). The K_{ATP}-channel is controlled by at least three separate sites for cytosolic nucleotides: (1) ATP and some structurally related nucleotides inhibit the K_{ATP}-channel both in the absence and presence of Mg^{2+}. (2) The Mg complexes of ADP (MgADP) and some other nucleoside diphosphates increase the activity of the K_{ATP}-channel. (3) The Mg complex of ATP (MgATP) slows considerably the rapid decline of K_{ATP}-channel activity (channel run-down) observed in excised membrane patches in the absence of ATP (Ohno-Shosaku *et al.*, 1987). As run-down is also reduced in Mg^{2+}-free cytosol-like solution (Kozlowski & Ashford, 1990), it may involve a Mg^{2+}-dependent protein dephosphorylation and MgATP might act by serving as substrate for one or several protein kinases closely associated with the β-cell membrane.

Sulphonylureas (e.g. tolbutamide) inhibit and diazoxide activates the K_{ATP}-channel by interaction with separate binding sites in the β-cell plasma membrane (Sturgess *et al.*, 1985; Trube *et al.*, 1986; for reviews see Ashford, 1990; Ashcroft & Rorsman, 1991; Dunne & Petersen, 1991; Panten *et al.*, 1992; Ashcroft & Ashcroft, 1992). Binding studies and patch-clamp experiments suggest that the binding sites for these drugs are not identical with the sites mediating regulation of the K_{ATP}-channel by cytosolic nucleotides (Schwanstecher *et al.*, 1992b,c,e). Phosphorylation of the K_{ATP}-channel and/or regulatory proteins appears to be involved in the mechanism of action of diazoxide (Kozlowski *et al.*, 1989; Dunne, 1989). Moreover, diazoxide is only effective when the

[1] Author for correspondence.

site for stimulatory nucleoside diphosphates is occupied (Schwanstecher *et al.*, 1992c; Larsson *et al.*, 1993). There is also evidence that an intracellular regulatory protein, which binds Mg-purine nucleotides, controls the effect of diazoxide (Kozlowski & Ashford, 1992). The potency of sulphonylureas is much lower in excised membrane patches than in intact β-cells, unless the internal side of the plasma membrane is exposed to ADP in the presence of Mg^{2+} (Zünkler *et al.*, 1988; Panten *et al.*, 1990). Test solutions supplemented with ADP and Mg^{2+} contain both MgADP which activates the channel and free ADP which inhibits the channel, but less effectively than free ATP. Studies with nucleotides structurally related to ADP suggest that enhancement of the sulphonylurea sensitivity is due to the combined actions of cytosolic nucleotides at the aforementioned inhibitory and stimulatory nucleotide receptors (Schwanstecher *et al.*, 1992d). However, it is unclear why these nucleotide actions enhance the sulphonylurea sensitivity. Moreover, the situation has been complicated by the finding that even 5 mM tolbutamide did not induce complete block of K_{ATP}-channels in the absence of any nucleotides (Schwanstecher *et al.*, 1992c).

We have therefore performed patch-clamp experiments with excised inside-out membrane patches in order to elucidate further the role of cytosolic nucleotides in the regulation of the sulphonylurea sensitivity of the K_{ATP}-channel.

Methods

Isolation and culture of pancreatic β-cells

Pancreatic β-cells were isolated from male albino mice (NMRI, 11–15 weeks old, fed *ad libitum*) and cultured for 1–4 days as previously described (Panten *et al.*, 1990). RPMI 1640 tissue culture medium was supplemented with 10 mM D-glucose.

Electrophysiological recording and analysis

The inside-out configuration of the patch-clamp technique (Hamill *et al.*, 1981) was used to record currents flowing through K_{ATP}-channels as previously described (Panten *et al.*, 1990). All experiments were performed at room temperature (20–22°C). The bath was perfused continuously at 2 ml min^{-1}. Pipettes were pulled from borosilicate glass and had resistances between 3 and 8 MΩ when filled with pipette solution. Unless stated otherwise in the Results section, the membrane potential was clamped at − 50 mV. Inward membrane K^+ currents are indicated as downward deflections in all current traces.

With the exception of the experiments in Figure 7, the cytosplasmic face of the membrane patch was exposed for 45 s periods to intracellular solution which contained Mg^{2+} and 1 mM ATP and was applied directly to the patch from an U-shaped polythene capillary (Fenwick *et al.*, 1982; Ohno-Shosaku *et al.*, 1987). These 45 s periods alternated with 15 s periods of intracellular solution (with or without Mg^{2+}) which was supplemented with or without test substances (various nucleotides and/or drugs) and was applied to the patch by bath perfusion (experimental designs shown in Figures 1 and 4). Before and after application of test substance-containing bath solution, there were periods during which the same solution was applied except that the test substances were omitted. The mean of the amplitudes of the current responses (current amplitudes) during application of test substances was normalized to the mean current amplitude during nucleotide- and drug-free control periods in each single experiment. The single-channel current amplitudes of the K_{ATP}-channels were not changed by tolbutamide (1 μM–20 mM), diazoxide (300 μM) or the tested nucleotide concentrations.

Recordings were made with an LM-EPC 7 patch clamp amplifier (List Electronic, Darmstadt, Germany). Current signals were stored on magnetic tape (Store 4, Racal Recorders, Hythe, UK) at 1 7/8 in s^{-1} (bandwidth 0.5 kHz, − 3 dB point). For analysis, taped data were digitized at 2 kHz using an Axolab 1100 computer interface (Axon Instruments, Foster City, CA, U.S.A.) and stored in a microcomputer. Analysis of the data was performed with the computer programme, pCLAMP 5.5.1 (Axon Instruments). For Figures 1, 4 and 7 taped data were replayed into a chart recorder (220, Gould, Cleveland, OH, U.S.A.).

Chemicals and solutions

Tolbutamide, Na_2-2′-deoxyadenosine 5′-diphosphate (dADP) and Na_2-2′-deoxyadenosine 5′-triphosphate (dATP) were obtained from Sigma (St. Louis, MO, U.S.A.). Li_4-adenosine 5′-O-(2-thiodiphosphate) (ADPβS), Na_2-ATP, Li_4-adenylyl-imidodiphosphate (AMP-PNP), Li_4-adenosine 5′-O-(3-thiotriphosphate) (ATPγS), Li_2-guanosine 5′-diphosphate (GDP), Li_2-guanosine 5′-triphosphate (GTP), K_2-uridine 5′-diphosphate (UDP) and Na_3-uridine 5′-triphosphate (UTP) were from Boehringer (Mannheim, Germany). All other chemicals were obtained from the sources described elsewhere (Panten *et al.*, 1989). Stock solutions of tolbutamide and diazoxide were prepared daily in 50–100 mM KOH.

Unless stated otherwise, the solution at the cytoplasmic side of the membrane (intracellular solution) contained (concentrations in mM): KCl 140, $CaCl_2$ 2, $MgCl_2$ 1, ethylene glycol *bis*(β-aminoethyl ether)-*N,N,N′N′*-tetraacetic acid (EGTA) 10 and 4-(2-hydroxyethyl)-1-piperazineethane-sulphonic acid (HEPES) 5 (titrated to pH 7.15 with KOH) (free $[Ca^{2+}] = 0.05$ μM). The free Mg^{2+} concentration was held close to 0.7 mM by adding appropriate amounts of $MgCl_2$ to the nucleotide-containing solutions. The required amounts of $MgCl_2$ and the composition of the solutions for pH 7.15 were calculated with a computer programme (Fabiato, 1988) except that some stability constants of the programme were exchanged as detailed previously (Schwanstecher *et al.*, 1992d). In addition, the stability constants for ADP were also used for UDP and those for ATP were also used for dATP, UTP and GTP. Addition of total concentrations of nucleoside triphosphates as high as 100 μM to the intracellular solution caused only negligible reduction in calculated free Mg^{2+} concentration. Therefore, we assumed that the applied ATPγS concentrations (up to 100 μM) did not alter the free Mg^{2+} concentration, particularly since the stability constants for Mg^{2+} complexes of ATP and ADP decrease when sulphur replaces oxygen (Pecoraro *et al.*, 1984; Schwanstecher *et al.*, 1992d). All experiments of Figures 1, 2 and 6 began with application of normal intracellular solution (with 0.7 mM free Mg^{2+}) during the 15 s periods. Subsequently the intracellular solution applied during the 15 s periods contained no $MgCl_2$ and was supplemented with 1 mM EDTA (Mg-free intracellular solution). The addition of 1 mM EDTA reduced free $[Ca^{2+}]$ to 0.04 μM. After addition of 1 mM Na_2-ATP, the intracellular solution was also used for filling the U-shaped polythene capillary of the aforementioned microflow system (Ohno-Shosaku *et al.*, 1987). In the experiments of Figure 7 the capillary of the microflow system contained intracellular solution from which $MgCl_2$ was omitted and which was supplemented with 1 mM EDTA and 1 mM Na_2-ATP. Unless stated otherwise in the Results section, the pipette solution contained (in mM): KCl 146, $CaCl_2$ 2.6, $MgCl_2$ 1.2, HEPES 10 (titrated to pH 7.40 with KOH).

The pH of all solutions was determined after adding EDTA, tolbutamide, diazoxide or any nucleotides and was readjusted if necessary. Tolbutamide and diazoxide were completely dissolved at the highest concentrations used (20 mM and 0.3 mM, respectively).

Treatment of results

Values are presented as mean ± s.e.mean. Significances were calculated by the two-tailed U-test of Wilcoxon and of Mann and Whitney. $P<0.05$ was considered significant. The concentration-inhibition relationships for tolbutamide or nucleotides (Figures 2, 3, 5–9, 11) were analysed by fitting the function

$$(1) \qquad E = a\left(1 - \frac{[A]^n}{EC_{50}^n + [A]^n}\right) + b$$

to the experimental data by a non-linear least-squares routine where a = maximal current amplitude (normalized) in the absence of test substance, b = normalized current amplitude in the presence of maximally effective concentrations of test substance, E = normalized current amplitude in the presence of test substance, [A] = concentrations of test substance, EC_{50} = half-maximally effective concentration and n = slope parameter (Hill coefficient). Relations between nucleotide concentrations and stimulatory effects (Figures 3, 10) were analysed by fitting the function

$$(2) \qquad E = a\left(\frac{[A]^n}{EC_{50}^n + [A]^n}\right) + b$$

to the experimental data where a = normalized current amplitude in the presence of a maximally effective concentration of stimulatory nucleotide, b = normalized current amplitude in bath solution free of stimulatory nucleotide, E = normalized current amplitude in the presence of stimulatory nucleotide, [A] = concentration of stimulatory nucleotide, EC_{50} = half-maximally effective concentration and n = slope parameter (Hill coefficient).

Results

It has been previously observed that AMP-PNP is less potent at the inhibitory site of the K_{ATP}-channel in the absence than in the presence of Mg^{2+} (Ashcroft & Kakei, 1989; Schwanstecher *et al.*, 1992d). To test whether this effect of Mg^{2+} reflects a general phenomenon being valid for all inhibitory nucleotides, we examined the concentration-dependent effects of several nucleotides both in the absence and in the presence of Mg^{2+}. Figure 1 shows the typical design of our inside-out experiments. In order to slow the rundown of channel activity, the cytoplasmic face of the membrane patch was exposed for 45 s periods to an intracellular solution containing 0.7 mM free Mg^{2+} and 1 mM ATP, alternating with 15 s periods serving as test or control periods. The example in Figure 1 demonstrates that addition of 0.1 or 0.3 mM dATP inhibited the K_{ATP}-channel activity in the absence of Mg^{2+} by 30 or 66%, respectively. Similar experiments in the absence (Figure 2, Table 1) and in the presence of Mg^{2+} (Figure 3, Table 1) revealed that ATPγS, ADPβS and dATP were less potent channel inhibitors in the absence than in the presence of Mg^{2+} whereas ATP was slightly less inhibitory in the presence than in the absence of Mg^{2+}. The presence of Mg^{2+} caused a great decrease in potency of UTP and channel activation by GDP, UDP and GTP (Figure 3, Table 1).

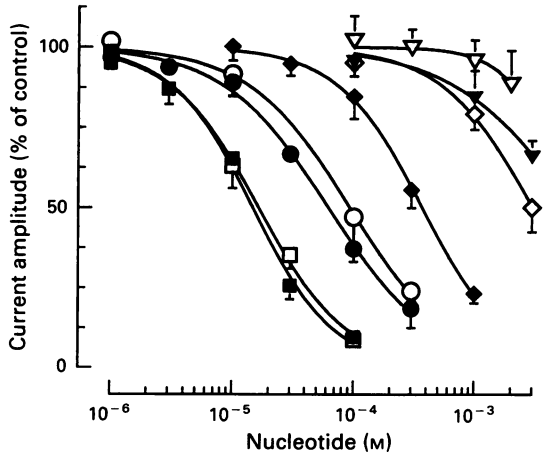

Figure 2 Inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells by nucleotides in the absence of Mg^{2+}. Using the experimental design shown in Figure 1, the mean current amplitude during 15 s periods of exposure to Mg-free intracellular solution containing the indicated concentrations of nucleotide (logarithmic scale) was normalized to the mean current amplitude during 15 s periods of exposure to control solution (Mg-free intracellular solution) before and after application of nucleotide in each single experiment. The curves through the current amplitude data points were fitted as described in the Methods section. (■) ATP; (□) ATPγS; (●) ADPβS; (○) dATP; (◆) UTP; (◇) GTP; (▼) GDP; (▽) UDP. Symbols indicate the mean with s.e.mean (when larger than symbols). For abbreviations, see text.

We then examined the effects of channel-inhibiting nucleotides on the potency of tolbutamide in an intracellular solution with 0.7 mM free Mg^{2+}. Figure 4 shows a typical experiment with some channel run-down which was observed in most of the experiments of this series. AMP-PNP (30 μM) inhibited the activity of the K_{ATP}-channels by 50%. Further addition of 100 μM tolbutamide decreased the channel activity to 17% of the activity in control solution (nucleotide- and tolbutamide-free). When the K_{ATP} channels were inhibited by 30 μM AMP-PNP, 50 μM ADPβS or 20 μM ATP, 20 mM tolbutamide caused a reduction of channel activity to 1.4 ± 0.2, 3.7 ± 2.4 or 3.2 ± 0.7%, respectively, of the activity in control solution ($n = 3-5$; Figure 5). These effects of 20 mM tolbutamide were not significantly different from its effect in the absence of any nucleotides (reduction to 1.4 ± 0.6% of control activity, $n = 5$; Figure 5), suggesting that maximally effective tolbutamide concentrations induce complete channel block under these conditions. However, the half-maximally effective concentrations (EC_{50}) of tolbutamide

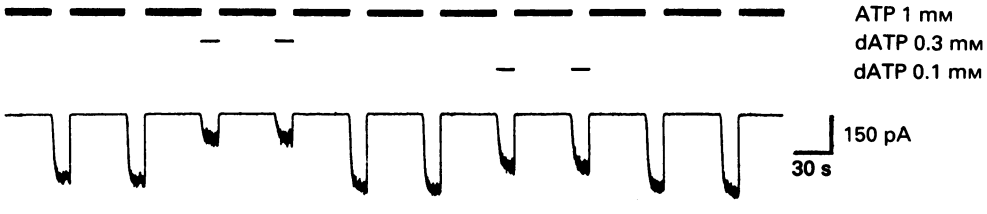

Figure 1 Inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells by dATP in the absence of Mg^{2+}. The uppermost horizontal bars indicate application of intracellular solution containing 0.7 mM free Mg^{2+} and 1 mM ATP for 45 s by the capillary of the microflow system. The other horizontal bars above the current trace indicate application of Mg-free intracellular solution with dATP for 15 s by the bath. The 15 s periods of application of nucleotide- and Mg-free intracellular solution to the bath represent the control periods.

Table 1 Effects of nucleotides on opening activity of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells

Nucleotide	EC_{50} (μM) absence of Mg^{2+}	Hill coefficient absence of Mg^{2+}	n	EC_{50} (μM) presence of Mg^{2+}	Hill coefficient presence of Mg^{2+}	n
ATP	14.8	1.3	(10)	18.8	1.2	(4–16)
ATPγS	16.5	1.2	(5–6)	11.6	1.1	(4–5)
ADPβS	61.9	1.0	(3–4)	43.6	1.0	(6)
dATP	92.8	1.0	(3–4)	85.9	1.1	(3–6)
UTP	369.0	1.2	(3–9)	1684.0	1.3	(4–12)
GTP	3048.0	1.2	(5–6)	423.0[a]	3.1[a]	(3–4)
GDP	>3000.0		(3–4)	34.0[a]	1.2[a]	(8–21)
UDP	>2000.0		(4–7)	114.0[a]	1.4[a]	(3–16)

Data were obtained in the experiments shown in Figures 2 and 3. Unless stated otherwise data refer to inhibitory effects. Number of experiments (n) in parentheses. [a]Channel activation. For abbreviations, see text.

were lower in the presence than in the absence of these inhibitory nucleotides (Figure 5, Table 2).

In accordance with previous results (Ashcroft & Rorsman, 1991), absence of Mg^{2+} in the intracellular solution induced activation of the K_{ATP}-channels (Figure 6). When the K_{ATP}-channels were inhibited by 100 μM ADP under these conditions, 20 mM tolbutamide caused a reduction of channel activity to 3.6 ± 0.9% ($n = 3$) of the activity in control solution (nucleotide-and tolbutamide-free intracellular solution with 0.7 mM free Mg^{2+}) (Figure 6), suggesting complete channel block by maximally effective tolbutamide concentrations in the absence of Mg^{2+}. In a Mg-free intracellular solution, the EC_{50} value for tolbutamide was lower in the presence than in the absence of 100 μM ADP (Figure 6, Table 2).

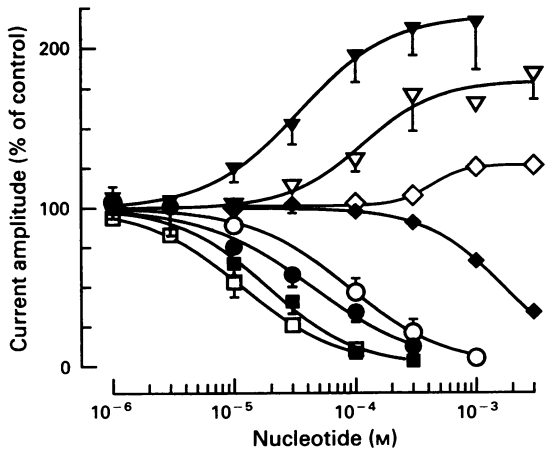

Figure 3 Activation and inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells by nucleotides in the presence of Mg^{2+}. Using an experimental design similar to that in Figure 1, the mean current amplitude during 15 s periods of exposure to intracellular solution (with 0.7 mM free Mg^{2+}) containing the indicated total concentrations of nucleotide (logarithmic scale) was normalized to the mean current amplitude during 15 s periods of exposure to control solution (intracellular solution with 0.7 mM free Mg^{2+}) before and after application of nucleotide in each single experiment. The curves through the current amplitude data points were fitted as described in the Methods section. (■) ATP; (□) ATPγS; (●) ADPβS; (○) dATP; (◆) UTP; (◇) GTP; (▼) GDP; (▽) UDP. Symbols indicate the mean and the vertical lines the s.e.mean (when larger than symbols). For abbreviations, see text.

Evidence has been presented that the binding properties of the sulphonylurea receptor are modulated by protein phosphorylation (Schwanstecher *et al.*, 1991). Therefore, we wanted to see whether tolbutamide is effective during prolonged inhibition of protein kinase reactions. As these reactions require MgATP as substrate, we removed Mg^{2+} not only from the intracellular solution perfusing the bath but also from the intracellular solution applied to the inside of the patch membrane by the capillary of the microflow system. A typical experiment illustrated in Figure 7a shows that channel run-down was usually not observed in the experiments of this series. The base-line for the recording was set by exposing the patch membrane for 5 s periods to Mg-free intracellular solution with 1 mM ATP and thereby inducing complete block of the K_{ATP}-channels. The channel activity decreased within 5 s after the start of application of 100 μM tolbutamide and rose again within 25 s after changing back to tolbutamide-free solution. Channel activity in the new steady state induced by 100 μM tolbutamide amounted to 58.1% of the activity in the absence of tolbutamide. Similar kinetics as shown in Figure 7a were observed in each single experiment when testing 10 μM–5 mM tolbutamide. Assuming complete channel block at maximally effective tolbutamide concentrations, the EC_{50} value for tolbutamide was 45.3 μM (Hill coefficient = 0.34; $n = 10–17$; Figure 7b).

To test whether the potency of tolbutamide depends on the extracellular K^+ concentration or the direction of the ATP-sensitive K^+ currents, experiments with a pipette solution containing 5.6 mM K^+ (appropriate replacement of KCl by NaCl in the pipette solution, membrane potential clamped at 0 mV) were performed, using the experimental design applied for determination of the potency of tolbutamide in the

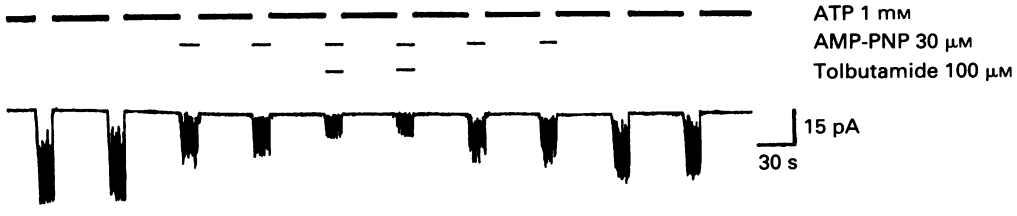

Figure 4 Effect of AMP-PNP on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. The uppermost horizontal bars indicate application of intracellular solution containing 0.7 mM free Mg^{2+} and 1 mM ATP for 45 s by the capillary of the microflow system. The other horizontal bars above the current trace indicate application of intracellular solution containing 0.7 mM free Mg^{2+} and 30 μM AMP-PNP (with or without 100 μM tolbutamide) for 15 s by the bath. The 15 s periods of application of nucleotide- and tolbutamide-free intracellular solution (with 0.7 mM free Mg^{2+}) by the bath represent the control periods. For abbreviations, see text.

absence of any nucleotides, but in the presence of 0.7 mM free Mg^{2+} (Figure 5). Under these conditions 5 mM tolbutamide caused a reduction of channel activity to 12.8 ± 4.2% ($n = 6$) of the activity in the control solution (nucleotide- and tolbutamide-free), and the EC_{50} value for tolbutamide was 123.2 μM (Hill coefficient = 0.39; $n = 6-9$; data not shown in a figure). This EC_{50} value was even higher than that observed in the presence of 150 mM K^+ in the pipette solution (78.9 μM; Table 2).

The role of channel-activating nucleotides in the control of the effectiveness of sulphonylureas was examined with GDP and dADP. When the K_{ATP}-channels were activated by 1 mM

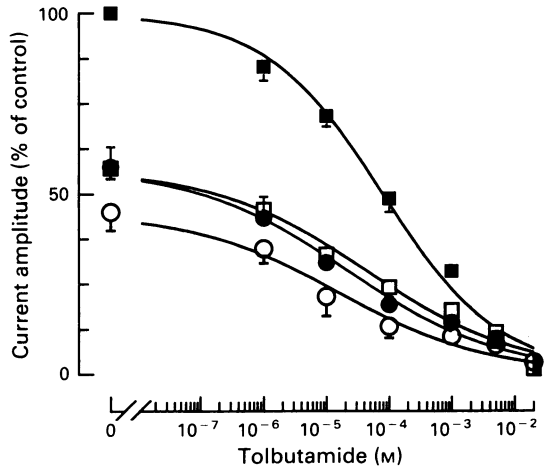

Figure 5 Effects of inhibitory nucleotides on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. The curves represent the relationships between normalized current amplitudes and tolbutamide concentration (logarithmic scale) during 15 s periods of intracellular solution (with 0.7 mM free Mg^{2+}) containing no nucleotide (■), 30 μM AMP-PNP (□), 50 μM ADPβS (●) or 20 μM ATP (○). Using the experimental design shown in Figure 4, the mean current amplitude during application of tolbutamide was normalized to the mean current amplitude during 15 s periods of exposure to control solution (intracellular solution with 0.7 mM free Mg^{2+}) before and after application of tolbutamide or nucleotide alone in each single experiment. The curves through the current amplitude data points were fitted as described in the Methods section. Symbols indicate the mean and the vertical lines the s.e.mean (when larger than symbols).

Table 2 Effects of nucleotides on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells

Nucleotide	*EC_{50} (μM) for tolbutamide*	*Hill coefficient*	*Number of experiments*
None	78.9	0.46	5–8
None[a]	167.6	0.43	3–18
AMP-PNP (30 μM)	44.2	0.35	4–13
ADPβS (50 μM)	21.7	0.36	3–9
ATP (20 μM)	15.9	0.36	3–18
ADP (100 μM)[a]	74.1	0.36	3–6
GDP (1 mM)	101.4	0.44	3–16
GDP (1 mM) + AMP-PNP (30 μM)	104.9	0.43	3–8
GDP (1 mM) + AMP-PNP (200 μM)	4.6	0.76	5–7
dADP (1.0 mM)	24.5	0.62	3–11
dADP (0.3 mM)	22.7	0.49	4–11
dADP (0.3 mM) + AMP-PNP (30 μM)	26.9	0.52	3–13
dADP (0.3 mM) + AMP-PNP (200 μM)	3.4	0.92	5–7

Data were obtained in the experiments shown in Figures 5, 6, 8 and 9. Unless stated otherwise, 0.7 mM free Mg^{2+} was present.[a] Mg-free intracellular solution was applied by bath perfusion. For abbreviations, see text.

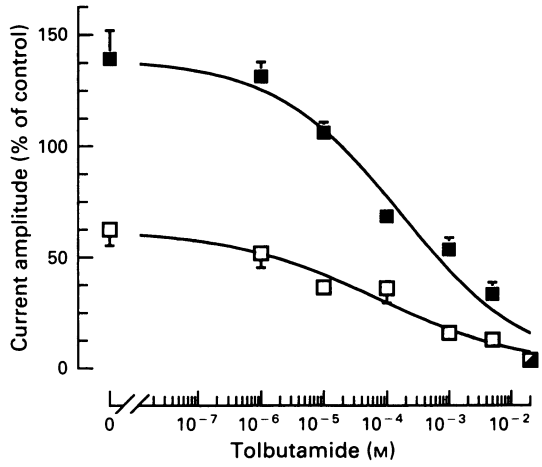

Figure 6 Effects of ADP in the absence of Mg^{2+} on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. The curves represent the relationships between normalized current amplitudes and tolbutamide concentration (logarithmic scale) during 15 s periods of exposure to Mg-free intracellular solution containing no nucleotide (■) or 100 μM ADP (□). Using an experimental design similar to that in Figure 4, the mean current amplitude during application of tolbutamide was normalized to the mean current amplitude during 15 s periods of exposure to control solution (intracellular solution with 0.7 mM free Mg^{2+}) before and after application of Mg-free solutions in each single experiment. The curves through the current amplitude data points were fitted as described in the Methods section. Symbols indicate the mean with s.e.mean (when larger than symbols).

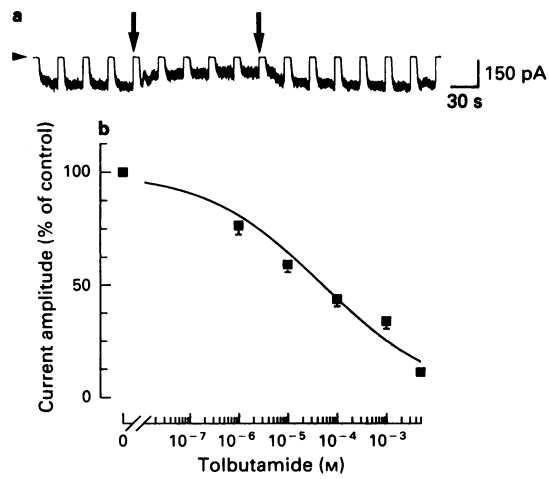

Figure 7 Effects of prolonged removal of Mg^{2+} on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. Mg-free intracellular solution was applied both during 20 s periods by bath perfusion and during 5 s periods by the capillary of the microflow system. (a) Current trace obtained from an inside-out patch exposed for 5 s periods to 1 mM ATP, alternating with 20 s periods with tolbutamide or without test substance. The trace starts about 15 min after switching to bath perfusion with Mg-free intracellular solution and 10 min after establishing the inside-out configuration. The arrows above the trace indicate the start of bath exchange when switching from control solution (no test substance) to solution with 100 μM tolbutamide and then back to control solution. The horizontal arrowhead indicates the base-line (all K_{ATP}-channels closed by 1 mM ATP). (b) Relationship between normalized current amplitude and tolbutamide concentration (logarithmic scale). Using the experimental design shown in (a), the mean current amplitude during the last three 20 s periods of exposure to tolbutamide was normalized to the mean current amplitude during 20 s periods of exposure to control solution (last three periods before the left arrow and third to fifth period after the right arrow). The curves through the current amplitude data points were fitted as described in the Methods section. Symbols indicate the mean with s.e.mean (when larger than symbols).

GDP, 20 mM tolbutamide caused a reduction of channel activity to 16.3 ± 1.1 ($n = 3$) of the activity in control solution (nucleotide- and tolbutamide-free) (Figure 8). Assuming complete channel block by maximally effective tolbutamide concentrations in the presence of 1 mM GDP, the EC_{50} value for tolbutamide was only slightly higher in the presence of 1 mM GDP than in its absence (Table 2). However, in conjunction with 0.3 mM diazoxide, 1 mM GDP strongly antagonized the channel-inhibiting effect of tolbutamide (Figure 8). On the other hand, neutralization of the stimulatory effect of 1 mM GDP by 200 μM AMP-PNP enhanced the potency of tolbutamide by about 17 fold as compared to its potency in the absence of any nucleotides

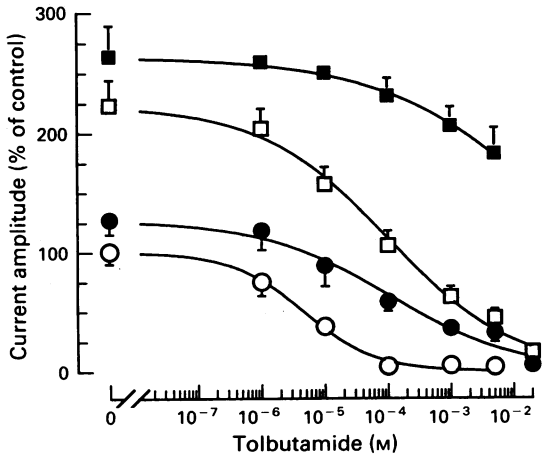

Figure 8 Effects of GDP on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. The curves represent the relationships between normalized current amplitudes and tolbutamide concentration (logarithmic scale) during 15 s periods of intracellular solution (with 0.7 mM free Mg^{2+}) containing 1 mM GDP (□), 1 mM GDP + 0.3 mM diazoxide (■), 1 mM GDP + 30 μM AMP-PNP (●) or 1 mM GDP + 200 μM AMP-PNP (○). Experimental design, normalization of the current amplitudes and curve fitting were similar to those in Figure 5, except that curve (■) was fitted by eye. Symbols indicate the mean with s.e.mean (when larger than symbols).

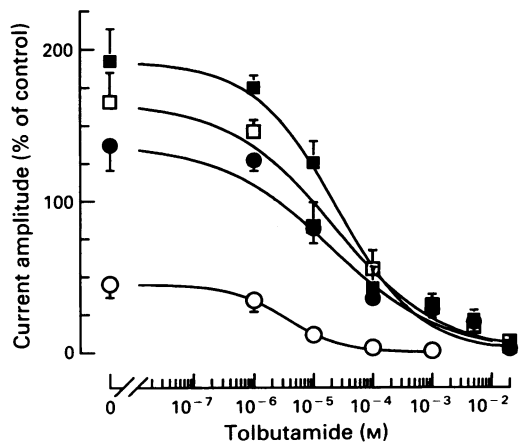

Figure 9 Effects of dADP on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. The curves represent the relationships between normalized current amplitudes and tolbutamide concentrations (logarithmic scale) during 15 s periods of intracellular solution (with 0.7 mM free Mg^{2+}) containing 1 mM dADP (■), 0.3 mM dADP (□), 0.3 mM dADP + 30 μM AMP-PNP (●), or 0.3 mM dADP + 200 μM AMP-PNP (○). Experimental design, normalization of the current amplitudes and curve fitting were similar to those in Figure 5. Symbols indicate the mean and the vertical lines the s.e.mean (when larger than symbols). For abbreviations, see text.

(Figure 8, Table 2). A 30 μM concentration of AMP-PNP was insufficient to affect the potency of tolbutamide observed in the presence of 1 mM GDP (Figure 8, Table 2).

When the K_{ATP}-channels were activated by 1 mM dADP, 20 mM tolbutamide caused a reduction of channel activity to 7.3 ± 2.5% ($n = 3$) of the activity in the control solution (nucleotide- and tolbutamide-free) (Figure 9), suggesting complete channel block by maximally effective tolbutamide concentrations in the presence of 1 mM dADP. The EC_{50} value for tolbutamide was much lower in the presence of 1 mM dADP than in its absence (Table 2). A 0.3 mM concentration of dADP induced less pronounced channel activation than 1 mM dADP, but was sufficient to produce a decrease in the EC_{50} value for tolbutamide (Figure 9, Table 2). The combined effects of 0.3 mM dADP and 200 μM AMP-PNP caused inhibition of channel activity and enhanced the potency of tolbutamide by about 23 fold as compared to its potency in the absence of any nucleotides (Figure 9, Table 2). A 30 μM concentration of AMP-PNP was insufficient to affect the potency of tolbutamide observed in the presence of 0.3 mM dADP (Figure 9, Table 2).

Figure 10 shows the concentration-dependent effects of GDP on K_{ATP}-channel activity in the presence of an AMP-PNP or tolbutamide concentration inducing about 50% channel inhibition in the absence of GDP. AMP-PNP (30 μM) and tolbutamide (100 μM) increased the EC_{50} value for GDP (compared to control; see Table 1) to 225 μM (Hill coefficient = 0.93; $n = 5-7$) and 232 μM (Hill coefficient = 2.69; $n = 4-6$), respectively. However, the maximum effect of GDP was reduced by tolbutamide (100 μM), but not by AMP-PNP (30 μM).

In order to characterize further the mode of interaction between GDP on the one hand and AMP-PNP and tolbutamide on the other hand, concentration-response curves for AMP-PNP were obtained (Figure 11). In the absence of any other test-substance, AMP-PNP inhibited the K_{ATP}-channel activity half-maximally at 30 μM (dashed line in Figure 11; taken from Schwanstecher *et al.*, 1992d). A tolbutamide concentration (100 μM) decreasing the channel activity by 52 ± 5% enhanced the potency of AMP-PNP

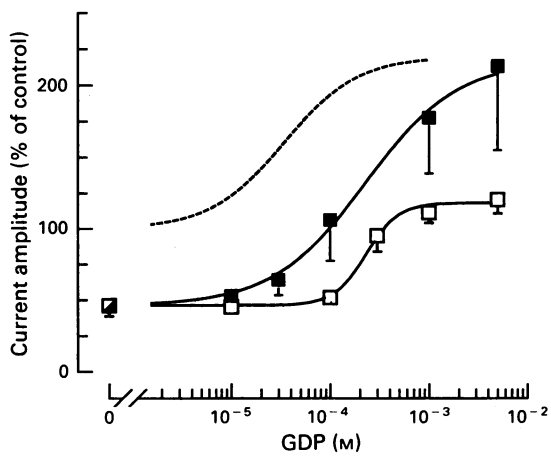

Figure 10 Effects of AMP-PNP and tolbutamide on GDP-induced activation of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. The curves represent the relationships between normalized current amplitudes and GDP concentrations (logarithmic scale) during 15 s periods of intracellular solution (with 0.7 mM free Mg^{2+}) containing 30 μM AMP-PNP (■) or 100 μM tolbutamide (□). Experimental design, normalization of the current amplitudes (control periods free of nucleotides and tolbutamide) and curve fitting were similar to those in Figure 5. Symbols indicate the mean with s.e.mean (when larger than symbols). To facilitate comparison, the corresponding concentration-dependent effects of GDP in the absence of tolbutamide and AMP-PNP are shown as a dashed line (taken from Figure 3). For abbreviations, see text.

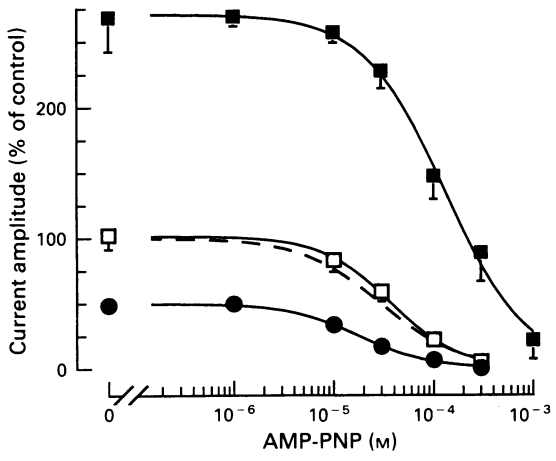

Figure 11 Effects of tolbutamide on AMP-PNP-induced inhibition of K_{ATP}-channels in inside-out patches of mouse pancreatic β-cells. The curves represent the relationships between normalized current amplitudes and AMP-PNP concentration (logarithmic scale) during 15 s periods of intracellular solution (with 0.7 mM free Mg^{2+}) containing 1 mM GDP (■), 1 mM GDP + 0.1 mM tolbutamide (□) or 0.1 mM tolbutamide (●). Experimental design, normalization of the current amplitudes (control periods free of nucleotides and tolbutamide) and curve fitting were similar to those in Figure 5. Symbols indicate the mean and the vertical lines the s.e.mean (when larger than symbols). To facilitate comparison, the corresponding concentration-dependent effects of AMP-PNP in the absence of GDP and tolbutamide are shown as a dashed line (data taken from Figure 3 in Schwanstecher *et al.*, 1992d). For abbreviations, see text.

(EC_{50} = 18 μM; Hill coefficient = 1.24; n = 3–4). Channel activation by 1 mM GDP strongly antagonized the responses to AMP-PNP over the whole range of tested concentrations; the EC_{50} value for AMP-PNP was 131 μM (Hill coefficient = 1.07; n = 5–16). This effect of 1 mM GDP was neutralized by 100 μM tolbutamide; the EC_{50} value for AMP-PNP was 36 μM (Hill coefficient = 1.26; n = 4–6).

Discussion

In the inside-out experiments of the present study, a maximally effective concentration of MgATP was usually present as substrate for protein kinases except for 15 s test periods. This experimental protocol favoured phosphorylation of the K_{ATP}-channels and/or regulatory proteins and strongly reduced channel run-down (Ohno-Shosaku *et al.*, 1987). Under these conditions the order of inhibitory effectiveness of cytosolic nucleotides in Mg^{2+}-free solution was ATP = ATPγS > ADP > ADPβS = AMP-PNP > dATP > UTP > dADP > GTP > GDP > UDP (Figure 2; for ADP, dADP and AMP-PNP see Panten *et al.*, 1990; Schwanstecher *et al.*, 1992d). Neglecting minor variations, this order is consistent with previous findings in β-cells, skeletal muscle and cardiac muscle (Spruce *et al.*, 1987; Lederer & Nichols, 1989; Ashcroft & Kakei, 1989). Variations in half-maximally inhibitory ATP concentrations (15 μM, Figure 2; 4 μM, Ashcroft & Kakei, 1989) probably reflect the different methods used to make allowance for channel run-down. Unlike non-hydrolysable ATP and ADP analogues (ATPγS, AMP-PNP, AMP-PCP, ADPβS), ATP was less inhibitory in the presence than in the absence of Mg^{2+} (see Results: Ashcroft & Kakei, 1989). It is therefore conceivable that enzymatic degradation of MgATP in the inside-out membrane and the attached bleb of cytoplasm led to underestimation of the potency of MgATP (Schwanstecher *et al.*, 1992c).

In contrast to a previous interpretation (Schwanstecher *et al.*, 1992d), there was no evidence for activation of K_{ATP}-channels by the Mg complex of the non-hydrolysable ADP analogue ADPβS. As has been reported for cardiac cells (Tung & Kurachi, 1991), MgUDP induced opening of K_{ATP}-channels in β-cells, though less effectively than MgGDP. Channel activation by MgGDP and MgdADP was less pronounced than in previous investigations (Schwanstecher *et al.*, 1992c,d). One possible explanation could be variation in cytoplasmic constituents involved in the mechanism of channel activation by nucleoside diphosphates (Bokvist *et al.*, 1991). The concentration-response curves for GTP and UTP in the presence of Mg^{2+} could reflect a combination of channel inhibition and relief of channel inhibition by enzymatically formed MgGDP and MgUDP.

In inside-out patches exposed to nucleotide-free solutions, tolbutamide concentrations higher than 20 mM were required for complete block of K_{ATP}-channels (Figure 5). In previous studies the tolbutamide concentrations were probably not high enough to induce complete channel closure (Zünkler *et al.*, 1988; Sturgess *et al.*, 1988; Schwanstecher *et al.*, 1992c). However, it is unclear whether all effects of millimolar tolbutamide concentrations resulted from occupation of specific sulphonylurea receptors. When inhibiting about half of the channel activity by ATP, AMP-PNP, ADPβS or ADP (in the absence of Mg^{2+}), the potency of tolbutamide was increased. This increase seems to reflect a reciprocal interaction between channel-inhibiting nucleotides and tolbutamide since tolbutamide slightly enhanced the potency of AMP-PNP. The previous observation that ADPβS (100 μM) enhanced the effectiveness of tolbutamide (100 μM) (Schwanstecher *et al.*, 1992d) is in accordance with the present observations. The failure to detect similar effects for ADP (50–100 μM in the absence of Mg^{2+}) and AMP-PNP (200 μM) (Panten *et al.*, 1990; Schwanstecher *et al.*, 1992d) has probably been due to the shapes of the concentration-response curves for tolbutamide (Figure 6) and the difficulty in determining the percentage of channel inhibition induced by a single tolbutamide concentration at rather low channel activity. Far too low channel activity also prevented reliable determination of the potency of tolbutamide in conjunction with inhibitory nucleotide concentrations higher than those in the presented experiments. Presumably the potency of tolbutamide is highest in the presence of near maximally inhibitory concentrations of nucleotides.

In test solutions supplemented with Mg^{2+} and GDP, dADP or ADP, about half of the nucleotide is present as Mg complex. As 0.5 mM free GDP inhibits the K_{ATP}-channel activity by less than 10% (Figure 2), the responses to GDP (up to 1 mM total concentration) in our Mg^{2+}-containing test solutions must have been produced almost exclusively by MgGDP. Channel activation by 0.5 mM MgGDP strongly reduces the inhibitory potency of AMP-PNP, but weakly reduces that of tolbutamide (Figures 8 and 11). On the other hand, both AMP-PNP and tolbutamide diminish the stimulatory potency of MgGDP (Figure 10). However, the maximum effect of MgGDP is decreased by tolbutamide, but not by AMP-PNP (Figure 10). These findings suggest competitive and non-competitive inhibition of the action of MgGDP by AMP-PNP and tolbutamide, respectively. Evidence has been presented that the K_{ATP}-channel is controlled via four separate binding sites for inhibitory nucleotides, stimulatory nucleotides, sulphonylureas and diazoxide (Schwanstecher *et al.*, 1992c). When the stimulatory effect of MgGDP is neutralized by applying an appropriate concentration of AMP-PNP, a proportion of the K_{ATP}-channels stays in the AMP-PNP-blocked state. In this situation tolbutamide decreases channel activity both directly and indirectly by non-competitive inhibition of the action of MgGDP. In addition, it is assumed that tolbutamide binding interrupts the competitive antagonism between MgGDP and AMP-PNP and thereby enhances the proportion of the AMP-PNP-blocked state. This view is consistent with the finding that sensitization of MgGDP-induced channel activation by diazoxide (0.3 mM) strongly reduces the potency of

tolbutamide (Figure 8). Occupation of the binding site for diazoxide inhibits binding of glibenclamide to both the membrane-bound and the solubilized sulphonylurea receptor (Schwanstecher *et al.*, 1992a).

At a concentration of 0.5 mM, MgdADP is probably as effective a channel activator as MgGDP, and both are maximally effective (see Results; Schwanstecher *et al.*, 1992d). However, the action of dADP at the inhibitory site is stronger than that of GDP. Free dADP (0.5 mM) inhibits the K_{ATP}-channel activity by 40% (Schwanstecher *et al.*, 1992d). This effect can explain that dADP (1 mM total concentration), but not GDP (1 mM total concentration) enhances the potency of tolbutamide (Figures 8 and 9; Schwanstecher *et al.*, 1992d). In conjunction with 200 μM AMP-PNP, dADP (0.3 mM total concentration) leads to an EC_{50} value for tolbutamide of 3.4 μM. This high potency is probably brought about by the additive effects of 200 μM AMP-PNP and 0.15 mM free dADP at the receptor site for inhibitory nucleotides. The action of ADP at the inhibitory site is even more potent than that of dADP. Free ADP causes 50 and 90% inhibition of K_{ATP}-channel activity at 0.05 and 0.5 mM, respectively (Panten *et al.*, 1990). In the presence of Mg^{2+} and 1 mM total ADP, the channel activity is similar to that observed in the absence of any nucleotides (Zünkler *et al.*, 1988; Schwanstecher *et al.*, 1992c), indicating neutralization of the inhibitory effect of free ADP (0.5 mM) by the stimulatory effect of MgADP (0.5 mM). Under these conditions the EC_{50} value for tolbutamide is 4.0 μM (Schwanstecher *et al.*, 1992c). A nearly identical EC_{50} value (4.6 μM) is obtained for tolbutamide in the presence of 200 μM AMP-PNP and 0.5 mM MgGDP (Figure 8). This AMP-PNP concentration induces the same percentage of channel inhibition as 0.5 mM free ADP (Schwanstecher *et al.*, 1992d). As the above EC_{50} values are similar to the EC_{50} values in intact β-cells (2.2–2.5 μM; Gillis *et al.*, 1989; Panten *et al.*, 1990), the potency of sulphonylureas in intact β-cells, too, appears to be controlled by stimulatory and inhibitory nucleotides.

The mechanisms discussed so far do not explain why the potency of tolbutamide for K_{ATP}-channel inhibition is so low in the absence of cytosolic nucleotides (EC_{50} = 79 μM). In microsomal membranes from pancreatic islets, the K_D value for tolbutamide binding is 12–15 μM in the absence of nucleotides and 32 μM in the presence of 0.1 mM MgATP (Panten *et al.*, 1989; Schwanstecher *et al.*, 1992e). These findings and additional evidence suggest that the binding affinity of the sulphonylurea receptor is decreased by protein phosphorylation (for reviews see Ashcroft & Ashcroft, 1992; Panten *et al.*, 1992). It is therefore conceivable that the low potency of tolbutamide for K_{ATP}-channel inhibition is due to phosphorylation of the sulphonylurea receptor. However, rapid alteration of phosphorylation of the sulphonylurea receptor does not appear to be necessary for the mechanism of action of sulphonylureas. Presumably prolonged removal of Mg^{2+} inhibits protein phosphorylation, but does not prevent strong and rapidly reversible responses to tolbutamide (see Results).

In conclusion, the present study reveals a complex mechanism leading to enhanced potency of sulphonylureas in the presence of certain cytosolic nucleotides. Firstly, channel-inhibiting nucleotides increase the potency of sulphonylureas in a reciprocal manner. Secondly, sulphonylureas inhibit the effect of channel-stimulatory nucleotides by a non-competitive mechanism, whereas inhibitory nucleotides competitively antagonize stimulatory nucleotides. This competitive interaction is interrupted by sulphonylureas. Therefore, in the simultaneous presence of stimulatory and inhibitory nucleotides, application of sulphonylureas enhances the proportion of K_{ATP}-channels staying in the nucleotide-blocked state.

This work was supported by the Deutsche Forschungsgemeinschaft (Pa 229/5-1).

References

ASHCROFT, F.M., HARRISON, D.E. & ASHCROFT, S.J.H. (1984). Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. *Nature*, **312**, 446–448.

ASHCROFT, F.M. & KAKEI, M. (1989). ATP-sensitive K^+ channels in rat pancreatic β-cells: Modulation by ATP and Mg^{2+} ions. *J. Physiol.*, **416**, 349–367.

ASHCROFT, F.M. & RORSMAN, P. (1991). Electrophysiology of the pancreatic β-cell. *Prog. Biophys. Mol. Biol.*, **54**, 87–143.

ASHCROFT, S.J.H. & ASHCROFT, F.M. (1992). The sulfonylurea receptor. *Biochim. Biophys. Acta*, **1175**, 45–59.

ASHFORD, M.L.J. (1990). Potassium channels and modulation of insulin secretion. In *Potassium Channels: Structure, Classification, Function and Therapeutic Potential.* ed. Cook, N.S. pp. 300–325. Chichester: Ellis Horwood.

BOKVIST, K., ÄMMÄLÄ, C., ASHCROFT, F.M., BERGGREN, P.-O., LARSSON, O. & RORSMAN, P. (1991). Separate processes mediate nucleotide-induced inhibition and stimulation of the ATP-regulated K^+-channels in mouse pancreatic β-cells. *Proc. R. Soc. B.*, **243**, 139–144.

COOK, D.L. & HALES, C.N. (1984). Intracellular ATP directly blocks K^+ channels in pancreatic B-cells. *Nature*, **311**, 271–273.

DUNNE, M.J. (1989). Protein phosphorylation is required for diazoxide to open ATP-sensitive potassium channels in insulin (RINm5F) secreting cells. *FEBS Lett.*, **250**, 262–266.

DUNNE, M.J. & PETERSEN, O.H. (1991). Potassium selective ion channels in insulin-secreting cells: physiology, pharmacology and their role in stimulus-secretion coupling. *Biochim. Biophys. Acta*, **1071**, 67–82.

FABIATO, A. (1988). Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. In *Methods in Enzymology*, Vol. 157, Part Q. ed. Fleischer, S. & Fleischer, B. pp. 378–417. San Diego: Academic Press.

FENWICK, E.M., MARTY, A. & NEHER, E. (1982). A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J. Physiol.*, **331**, 577–597.

GILLIS, K.D., GEE, W.M., HAMMOND, A., MCDANIEL, M.L., FALKE, L.C. & MISLER, S. (1989). Effects of sulfonamides on a metabolite-regulated ATP_i-sensitive K^+ channel in rat pancreatic B-cells. *Am. J. Physiol.*, **257**, C1119–C1127.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.

HENQUIN, J.C. & MEISSNER, H.P. (1984). Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic B-cells. *Experientia*, **40**, 1043–1052.

KOZLOWSKI, R.Z. & ASHFORD, M.L.J. (1990). ATP-sensitive K^+ channel run-down is Mg^{2+} dependent. *Proc. R. Soc. B.*, **240**, 397–410.

KOZLOWSKI, R.Z. & ASHFORD, M.L.J. (1992). Nucleotide-dependent activation of K_{ATP} channels by diazoxide in CRI-G1 insulin-secreting cells. *Br. J. Pharmacol.*, **107**, 34–43.

KOZLOWSKI, R.Z., HALES, C.N. & ASHFORD, M.L.J. (1989). Dual effects of diazoxide on ATP-K^+ current recorded from an insulin-secreting cell line. *Br. J. Pharmacol.*, **97**, 1039–1050.

LARSSON, O., ÄMMÄLÄ, C., BOKVIST, K., FREDHOLM, B. & RORSMAN, P. (1993). Stimulation of the K_{ATP} channel by ADP and diazoxide requires nucleotide hydrolysis in mouse pancreatic β-cells. *J. Physiol.*, **463**, 349–365.

LEDERER, W.J. & NICHOLS, C.G. (1989). Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. *J. Physiol.*, **419**, 193–211.

MATTHEWS, E.K. (1985). Electrophysiology of pancreatic islet β-cells. In *The Electrophysiology of the Secretory Cell.* ed. Poisner, A.M. & Trifaro, J.M. pp. 93–112. Amsterdam: Elsevier.

OHNO-SHOSAKU, T., ZÜNKLER, B.J. & TRUBE, G. (1987). Dual effects of ATP on K^+ currents of mouse pancreatic β-cells. *Pflügers Arch.*, **408**, 133–138.

PANTEN, U., BURGFELD, J., GOERKE, F., RENNICKE, M., SCHWANSTECHER, M., WALLASCH, A., ZÜNKLER, B.J. & LENZEN, S. (1989). Control of insulin secretion by sulfonylureas, meglitinide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets. *Biochem. Pharmacol.*, **38**, 1217–1229.

PANTEN, U., HEIPEL, C., ROSENBERGER, F., SCHEFFER, K., ZÜNKLER, B.J. & SCHWANSTECHER, C. (1990). Tolbutamide-sensitivity of the adenosine 5′-triphosphate-dependent K^+ channel in mouse pancreatic B-cells. *Naunyn-Schmied. Arch. Pharmacol.*, **342**, 566–574.

PANTEN, U., SCHWANSTECHER, M. & SCHWANSTECHER, C. (1992). Pancreatic and extrapancreatic sulfonylurea receptors. *Horm. Metab. Res.*, **24**, 549–554.

PECORARO, V.L., HERMES, J.D. & CLELAND, W.W. (1984). Stability constants of Mg^{2+} and Cd^{2+} complexes of adenine nucleotides and thionucleotides and rare constants for formation and dissociation of MgATP and MgADP. *Biochemistry*, **23**, 5262–5271.

SCHWANSTECHER, M., LÖSER, S., RIETZE, I. & PANTEN, U. (1991). Phosphate and thiophosphate group donating adenine and guanine nucleotides inhibit glibenclamide binding to membranes from pancreatic islets. *Naunyn-Schmied. Arch. Pharmacol.*, **343**, 83–89.

SCHWANSTECHER, M., BEHRENDS, S., BRANDT, C. & PANTEN, U. (1992a). The binding properties of the solubilized sulfonylurea receptor from a pancreatic B-cell line are modulated by the Mg^{++}-complex of ATP. *J. Pharmacol. Exp. Ther.*, **262**, 495–502.

SCHWANSTECHER, M., BRANDT, C., BEHRENDS, S., SCHAUPP, U. & PANTEN, U. (1992b). Effects of MgATP on pinacidil-induced displacement of glibenclamide from the sulphonylurea receptor in a pancreatic β-cell line and rat cerebral cortex. *Br. J. Pharmacol.*, **106**, 295–301.

SCHWANSTECHER, C., DICKEL, C., EBERS, I., LINS, S., ZÜNKLER, B.J. & PANTEN, U. (1992c). Diazoxide-sensitivity of the adenosine 5′-triphosphate-dependent K^+ channel in mouse pancreatic β-cells. *Br. J. Pharmacol.*, **107**, 87–94.

SCHWANSTECHER, C., DICKEL, C. & PANTEN, U. (1992d). Cytosolic nucleotides enhance the tolbutamide-sensitivity of the ATP-dependent K^+ channel in mouse pancreatic B-cells by their combined actions at inhibitory and stimulatory receptors. *Mol. Pharmacol.*, **41**, 480–486.

SCHWANSTECHER, M., LÖSER, S., BRANDT, C., SCHEFFER, K., ROSENBERGER, F. & PANTEN, U. (1992e). Adenine nucleotide-induced inhibition of sulphonylureas to their receptor in pancreatic islets. *Br. J. Pharmacol.*, **105**, 531–534.

SPRUCE, A.E., STANDEN, N.B. & STANFIELD, P.R. (1987). Studies of the unitary properties of adenosine-5′-triphosphate-regulated potassium channels of frog skeletal muscle. *J. Physiol.*, **382**, 213–236.

STURGESS, N.C., ASHFORD, M.L.J., COOK, D.L. & HALES, C.N. (1985). The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet*, **ii**, 474–475.

STURGESS, N.C., KOZLOWSKI, R.Z., CARRINGTON, C.A., HALES, C.N. & ASHFORD, M.L.J. (1988). Effects of sulphonylureas and diazoxide on insulin secretion and nucleotide-sensitive channels in an insulin-secreting cell line. *Br. J. Pharmacol.*, **95**, 83–94.

TRUBE, G., RORSMAN, P. & OHNO-SHOSAKU, T. (1986). Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. *Pflügers Arch.*, **407**, 493–499.

TUNG, R.T. & KURACHI, Y. (1991). On the mechanism of nucleotide diphosphate activation of the ATP-sensitive K^+ channel in ventricular cell of guinea-pig. *J. Physiol.*, **437**, 239–256.

ZÜNKLER, B.J., LINS, S., OHNO-SHOSAKU, T., TRUBE, G. & PANTEN, U. (1988). Cytosolic ADP enhances the sensitivity to tolbutamide of ATP-dependent K^+ channels from pancreatic B-cells. *FEBS Lett.*, **239**, 241–244.

(Received February 19, 1993
Revised September 1, 1993
Accepted September 21, 1993)