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K_{ATP}-channel on the somata of spiny neurones in rat caudate nucleus: regulation by drugs and nucleotides

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1 The aim of the present study was to characterize the pharmacological properties of the adenosine 5′-triphosphate(ATP)-sensitive K^+ channel (K_{ATP}-channel) on the somata of spiny neurones in rat caudate nucleus and to compare them with those of β-cells. For that purpose we tested the effects of several K_{ATP}-channel-inhibiting and -activating drugs on the opening activity of the K_{ATP}-channel in caudate nucleus by use of the patch-clamp technique. In addition, the modulation of drug responses by cytosolic nucleotides was examined.

2 When K_{ATP}-channels in caudate nucleus were activated in cell-attached patches by inhibition of mitochondrial energy production, meglitinide (a benzoic acid derivative), Hoe36320 (a sulphonylurea of low lipophilicity) and glipizide reduced K_{ATP}-channel activity half-maximally at 0.4 μM, 0.4 μM and about 0.5 nM, respectively.

3 In inside-out patches (presence of 0.7 mM free Mg^{2+} at the cytoplasmic membrane side), tolbutamide (0.1 mM) caused only partial inhibition of K_{ATP}-channels in the absence of cytosolic nucleotides but complete inhibition in the simultaneous presence of the channel-activating nucleotide guanosine 5′-diphosphate (GDP; 1 mM) and the channel-inhibiting nucleotide adenylyl-imidodiphosphate (AMP-PNP; 0.2 mM).

4 Diazoxide (0.3 mM) strongly increased channel activity in the presence of ATP (0.1 mM) or GDP (0.03 mM), but was ineffective in the presence of AMP-PNP (0.1 mM). In the absence of cytosolic nucleotides diazoxide even decreased channel activity.

5 In the presence of 0.1 mM ATP, diazoxide activated K_{ATP}-channels half-maximally at 38 μM.

6 When K_{ATP}-channel activity was inhibited by 0.1 mM ATP, (−)-pinacidil (0.5 mM) elicited a slight activation of K_{ATP}-channels in caudate nucleus, whereas (+)-pinacidil (0.5 mM) and lemakalim (0.3 mM) were ineffective.

7 Since our data indicate similar control by drugs and nucleotides of K_{ATP}-channels in the somata of spiny neurones and pancreatic β-cells, we conclude that the high affinity sulphonylurea receptors of these tissues are probably closely related.

Keywords: K_{ATP}-channel; caudate nucleus; sulphonylureas; diazoxide; lemakalim; pinacidil; cytosolic nucleotides

Introduction

By use of single-channel recordings, sulphonylurea- and adenosine 5′-triphosphate(ATP)-sensitive K^+ channels (K_{ATP}-channels) with properties characteristic of the K_{ATP}-channel in pancreatic β-cells have been identified on demyelinated fibres of frog sciatic nerve, on presynaptic terminals of rat motor cortex and on the somata of neurones in hippocampus, neocortex, substantia nigra pars reticulata and caudate nucleus (Jonas *et al*., 1991; Politi & Rogawski, 1991; Ohno-Shosaku & Yamamoto, 1992; Schwanstecher & Panten, 1993; 1994; Lee *et al*., 1995). The K_{ATP}-channel in caudate nucleus which was detected on the somata of γ-aminobutyric acid (GABA)ergic spiny neurones could be opened by diazoxide or inhibition of ATP production and then showed a tolbutamide-sensitivity similar to that of β-cells (Schwanstecher & Panten, 1993; 1994). However, in neurones, but not in β-cells K_{ATP}-channels might be more sensitive to the opening drugs lemakalim and pinacidil than to diazoxide (Schmid-Antomarchi *et al*., 1990).

In pancreatic β-cells the sensitivity of the K_{ATP}-channel to sulphonylureas and diazoxide depends on the concentration of cytosolic nucleotides. In the absence of any nucleotides at the cytoplasmic face of excised membrane patches, the effectiveness of sulphonylureas is weak, but it is strongly enhanced in the simultaneous presence of appropriate concentrations of channel-activating (e.g. adenosine 5′-diphosphate (ADP), guanosine 5′-diphosphate (GDP)) and channel-inhibiting nucleotides (e.g. ATP, adenylyl-imidodiphosphate (AMP-PNP)) (Schwanstecher *et al*., 1992b; 1994a,b). Diazoxide, on the other hand, is ineffective in the absence of any nucleotides at the cytoplasmic membrane side, weakly effective in the presence of ATP (presence of Mg^{2+}) and strongly effective in the presence of channel-activating nucleoside diphosphates (e.g. ADP, GDP; Schwanstecher *et al*., 1992a).

The aim of the present study was to characterize further the pharmacological properties of the K_{ATP}-channel on the somata of spiny neurones in caudate nucleus and to compare them with those of β-cells. For that purpose we tested the effects of several K_{ATP}-channel-inhibiting and -activating drugs on the opening activity of the K_{ATP}-channel in caudate nucleus. In addition, the modulation of drug responses by cytosolic nucleotides was examined.

Methods

Thin slice preparation of rat caudate nucleus

Coronal slices (200 μm) from brain of 10–15 days old rats of either sex were prepared as described previously (Schwanstecher & Panten, 1993). Electrophysiological recordings were obtained from spiny neurones of the caudate nucleus. The spiny neurones which make up 90–95% of the neurone population in rat caudate nucleus were identified on the basis of their size and their shape with an upright microscope (Zeiss Axioskop FS equipped with Nomarski differential interference contrast optics, a 40 × water immersion lens Achroplan and an

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ocular micrometer; for further details see Schwanstecher & Panten (1994)).

Electrophysiological recording and analysis

Single-channel currents were recorded in cell-attached and inside-out patches of neurones at room temperature (20–22°C) in a chamber (1.5 ml) continuously perfused at 3 ml min^{-1} with oxygenated physiological saline solution (cell-attached configuration) or with intracellular solution (inside-out configuration) (for details see Schwanstecher & Panten, 1993; 1994). Unless stated otherwise in the Results section, the pipette potential was held at 0 mV in cell-attached and inside-out patch experiments. This corresponded to patch potentials of −72 mV (see Schwanstecher & Panten, 1994) and 0 mV in the cell-attached and the inside-out configuration, respectively. Inward and outward membrane K^+-currents are indicated as downward and upward deflections, respectively, in all current traces.

In cell-attached experiments inward K_{ATP}-channel currents (up to four open channels in a patch; single channel current amplitude −5 pA at a membrane potential of −72 mV; see for example Figure 1b) were recorded as previously described (Schwanstecher & Panten, 1993; 1994).

In inside-out experiments outward currents characteristic of the K_{ATP}-channel and characteristic of a channel of unknown identity were observed. Under our recording conditions (5.6 mM K^+ in the pipette and 161 mM K^+ in the bath solution) the single-channel slope conductance of the K_{ATP}-channel amounted to 18 ± 1 pS ($n = 3$; data not shown in a figure) when measured at membrane potentials of −30 mV to +10 mV. In this potential range current flow was outward and the single-channel current amplitudes of the K_{ATP}-channel ranged between 1.5–1.8 pA at 0 mV. We observed up to four open K_{ATP}-channels in a single patch (see for example Figure 3a). The channel of unknown identity (amplitudes 0.7–1 pA at 0 mV) exhibited a rather low opening activity in most of our inside-out patches. None of our various test substances had a clear effect on the opening activity of the latter channel.

Amplitude histograms of sampled cell-attached or inside-out currents were formed to calculate the single-channel current amplitudes. Channel activity was defined as the product of N, the number of functional channels and P_o, the open state probability, and was determined by dividing the total time the channels spent in the open state by the total sample time. In the case of superpositions of channels (see for example Figures 1b, 2a–c, 3a, 4a, b) the channel activity was calculated by summing the activity for each different current level. In addition, the mean of the channel activity during the control periods (0.4 mM cyanide in cell-attached experiments, intracellular solution in inside-out experiments) before and after application of test substances (control activity) was set to 100% and the channel activity during the test period (presence of test substances) was normalized to this value. Data were sampled during the last min of a test period (3–20 min duration in cell-attached experiments, 1.5–4 min duration in inside-out experiments). Data sampling during the control periods was started 1–2 min before and 3–30 min or 1–3 min after the test period in cell-attached and inside-out experiments, respectively. Data samples of at least 30 s duration were analysed with the half-amplitude threshold technique by use of an interactive graphics-based analysis programme (pCLAMP 5.5.1, FETCH series).

Recordings were made with an LM-EPC 7 patch clamp amplifier (List Electronic, Darmstadt, Germany). Current signals were low-pass filtered at 2 kHz with a 4-pole Bessel filter (AF 173, Thomatronik Rosenheim, FRG), digitized with an A/D converter (Instrutech, New York, U.S.A.) and stored on video tape. For analysis, taped data were replayed, digitized at 10 kHz by an Axolab 1100 computer interface (Axon Instruments, Foster City, CA, U.S.A.) and stored by a microcomputer. For Figures 1a, 1b, 2a–c, 3a, 4a and 4b taped data were replayed into a chart recorder (WR7400, Graphtec, Solingen).

Chemicals and solutions

(−)-Pinacidil and (+)-pinacidil were from Leo Pharmaceutical Products (Ballerup, Denmark). Lemakalim was from Smith Kline Beecham (Worthing, West Sussex, U.K.). All other chemicals were obtained from sources described elsewhere (Panten *et al*., 1989; Schwanstecher & Panten, 1993; Schwanstecher *et al*., 1994a,b).

Stock solutions of tolbutamide, meglitinide, [[p-[2-(5-chloro-o-anisamido)ethyl]phenyl]sulphonyl]-urea (Hoe36320), diazoxide, (−)-pinacidil and (+)-pinacidil were prepared daily in 50–150 mM NaOH (cell-attached experiments) or in 50–150 mM KOH (inside-out experiments). A stock solution of lemakalim was prepared daily in DMSO and protected from light. Cyanide solution was freshly prepared for each experiment and was used for one hour. Test substances were applied via the solution perfusing the bath (physiological saline in cell-attached experiments, intracellular solution in inside-out experiments).

The physiological saline used for preparing slices and for bath perfusion in cell-attached experiments contained (in mM): NaCl 125, KCl 5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2, $MgCl_2$ 1, D-glucose 25 and 5, respectively (pH 7.4 when equilibrated with 95% O_2 + 5% CO_2). Unless stated otherwise the solution at the cytoplasmic side of the membrane (intracellular solution) contained (in mM): KCl 135, $CaCl_2$ 2, $MgCl_2$ 1, ethyleneglycol-bis-(2-aminoethylether)-N,N,N′N′-tetraacetic acid (EGTA) 10 and 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) 5 (titrated to pH 7.30 with KOH; total K^+ concentration 161 mM) (free $[Ca^{2+}] = 0.05$ μM). The free Mg^{2+} concentration of the intracellular solution was held close to 0.7 mM by adding appropriate amounts of $MgCl_2$ to the nucleotide-containing solutions. The required amounts of $MgCl_2$ were calculated as described in Schwanstecher *et al*. (1992b). In some experiments (see Results) the intracellular solution contained no $MgCl_2$ and was supplemented with 1 mM ethylenediamine tetraacetic acid (EDTA) (Mg-free intracellular solution). The addition of 1 mM EDTA reduced free $[Ca^{2+}]$ to 0.04 μM.

The pipette solution contained (in mM): KCl 150, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10 (titrated to pH 7.40 with KOH) in cell-attached experiments and NaCl 140, KCl 5.6, $CaCl_2$ 2.6, $MgCl_2$ 1.2, HEPES 10 (titrated to pH 7.40 with NaOH) in inside-out experiments.

The pH of all solutions was determined after addition of test substances and was readjusted if necessary.

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 with the Bonferroni correction for multiple comparisons (Wallenstein *et al*., 1980). $P < 0.05$ was considered significant. Relations between drug concentration and effects were analysed by fitting the function

$$E = \frac{a - b}{1 + (EC_{50}/[A])^{n_H}} + b$$

to the experimental data by a non-linear least-squares routine with E = channel activity, EC_{50} = half-maximally effective drug concentration, [A] = concentration of test substance, n_H = slope parameter (Hill coefficient), a = maximum value of channel activity and b = additive constant.

Results

Inhibition by sulphonylureas and benzoic acid derivatives

Spiny neurones in rat caudate nucleus were continuously exposed to 5 mM D-glucose. As described previously (Schwanstecher & Panten, 1994) in cell-attached patches of these

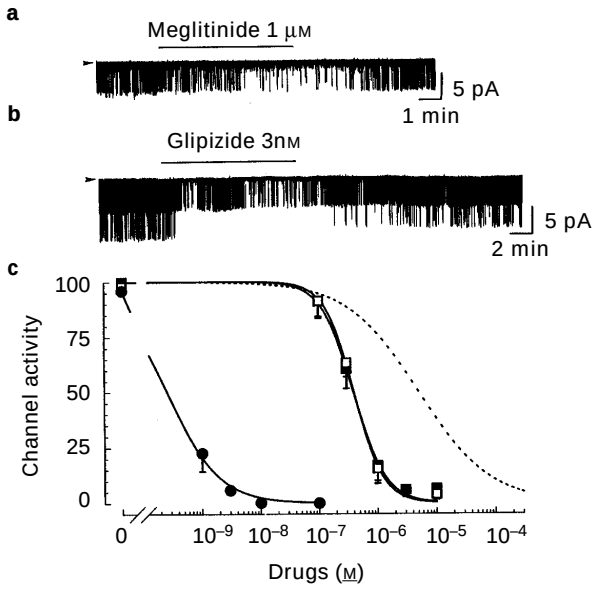

Figure 1 Inhibition of K_{ATP}-channels in cell-attached patches of spiny neurones in caudate nucleus by meglitinide, Hoe36320 and glipizide. The pipette potential was 0 mV. The K^+-concentration was 154 mM in the pipette and 5 mM in the bath solution. All experiments were performed with bath solution containing 5 mM D-glucose and 0.4 mM cyanide. (a, b) Continuous chart recorder traces demonstrating the responses of single K_{ATP}-channel currents when the solution was changed to one with 1 μM meglitinide (a) or 3 nM glipizide (b) and then back to control solution. Drugs were present during the periods indicated by horizontal lines. The arrowheads denote the zero-current level (all channels closed). (c) Relationship between normalized K_{ATP}-channel activity and concentration (logarithmic scale) of meglitinide (■), Hoe36320 (□), glipizide (●) and tolbutamide (dashed line, data taken from Figure 1 in Schwanstecher & Panten, 1994). By use of the experimental design shown in (a, b), channel activity during drug application was normalized to the mean channel activity during the control periods (drug-free) before and after drug application in each single experiment. Data analysis was performed as described in the Methods section. Symbols indicate the mean of 4–8 experiments (with s.e.mean shown by vertical lines when larger than symbols).

neurones single K_{ATP}-channel currents were induced by addition of cyanide to the bath solution. Figure 1a shows a typical chart recorder trace demonstrating the effects of the benzoic acid derivative meglitinide on single K_{ATP}-channel currents in the presence of 5 mM D-glucose and 0.4 mM cyanide. On changing to a bath solution containing 1 μM meglitinide, channel activity decreased from 0.07 (last 1 min interval of the control period before start of bath exchange) to 0.002 (last 1 min interval of the test period). After the solution had been changed back to meglitinide-free bath solution, it took 3.5 min until a steady state was reached again (channel activity 0.05). Single-channel current amplitudes were -5.1 pA and -5.0 pA during the test period and the control periods, respectively. The concentration-response curves for meglitinide or Hoe36320 (a sulphonylurea analogue representing glibenclamide devoid of its cyclohexyl group; Schwanstecher *et al.*, 1994b) in the presence of 5 mM D-glucose and 0.4 mM cyanide showed half-maximal channel inhibition at 0.4 μM for both drugs (Hill coefficients $= -1.6$ and -1.8, respectively; Figure 1c).

Figure 1b shows a representative experiment with glipizide. In this experiment channel activity decreased from 0.28 (last 1 min interval of the control period before start of bath exchange) to 0.006 within 10 min after switching to 3 nM glipizide. Reversal of channel activity to control values took about 10 min after the solution had been changed back to glipizide-free solution (channel activity 0.20). Glipizide 3 nM and 1 nM reduced channel activity by $94 \pm 2\%$ ($n = 5$) and $78 \pm 8\%$ ($n = 5$), respectively (Figure 1c). Usually, the channel blocking effect of 1 nM glipizide reached a stable value 15–20 min after drug application and reversal of channel activity to control values took at least 20 min after the solution had been changed back to glipizide-free solution. However, at concentrations < 1 nM, appearance and disappearance of the effects of glipizide were too slow to allow a clear discrimination between the blocking effects of glipizide and spontaneously occurring fluctuations in channel activity. Therefore, we can only roughly estimate the half-maximally effective glipizide concentration to be in the range of 0.3–0.6 nM.

Effects of nucleotides on sulphonylurea-sensitivity

Experiments with inside-out patches of pancreatic β-cells suggest that occupation of all specific receptor sites by sulphonylureas or their analogues appears to cause only partial inhibition of the K_{ATP}-channels in the absence of nucleotides but complete channel-block in the simultaneous presence of appropriate concentrations of channel-activating and channel-inhibiting nucleotides (Schwanstecher *et al.*, 1992b; 1994a,b). Therefore, the K_{ATP}-channel inhibiting effect of tolbutamide (0.1 mM) applied to the cytoplasmic membrane side of spiny neurones was examined both, in the absence of nucleotides and in the presence of cytosolic GDP and AMP-PNP (Figure 2). All experiments were carried out in the presence of 0.7 mM free Mg^{2+} at the cytoplasmic membrane side. GDP alone (1 mM) increased channel activity to $718 \pm 221\%$ of control activity ($n = 6$; $P < 0.01$; Figure 2d). When GDP (1 mM) was applied in conjunction with the channel-inhibiting nucleotide AMP-PNP (200 μM), channel activity amounted to $252 \pm 130\%$ of control activity (Figure 2d). The maximally inhibitory concentration of AMP-PNP is 200 μM when tested in the absence of any other test compound (Schwanstecher & Panten, 1994). In the absence of cytosolic nucleotides or in the presence of GDP, 0.1 mM tolbutamide inhibited channel activity to $28 \pm 7\%$ ($n = 7$) or $187 \pm 84\%$ ($n = 6$) of control activity, respectively (Figure 2d). Normalized to channel activity before the addition of the drug, these effects corresponded to an inhibition of $72 \pm 7\%$ ($n = 7$) or $74 \pm 4\%$ ($n = 6$), respectively (Figure 2d). However, a much stronger tolbutamide-induced inhibition of channel activity was observed in the simultaneous presence of GDP and AMP-PNP ($99 \pm 0.3\%$ inhibition of the channel activity before addition of tolbutamide; $n = 6$; $P < 0.02$; Figure 2d).

Effects of nucleotides on diazoxide-sensitivity

Figure 3a shows the typical design of our inside-out experiments with diazoxide. Unless stated otherwise the free Mg^{2+} concentration at the cytoplasmic membrane side was held close to 0.7 mM. Consistent with previous studies on β-cells a high concentration of diazoxide (0.3 mM) decreased channel activity in nucleotide-free solution ($48 \pm 11\%$ of control activity; $n = 5$; Figure 3b) (Kozlowski *et al.*, 1989). Addition of 0.1 mM ATP decreased channel activity by $89 \pm 2.7\%$ of control activity ($n = 14$). Further addition of 0.3 mM diazoxide not only attenuated ATP-induced channel inhibition but increased channel activity to $389 \pm 79\%$ of control activity ($n = 14$). In the presence of 0.1 mM ATP, diazoxide was half-maximally effective at 38 μM (Hill coefficient 1.4; $n = 8$; Figure 3c).

Consistent with previous findings in pancreatic β-cells, the stimulating effect of diazoxide was not observed in the presence of AMP-PNP (0.1 mM; $n = 4$; data not shown in a figure) or in the presence of ATP (0.1 mM) but absence of Mg^{2+} ($n = 5$; data not shown in a figure) (Kozlowski *et al.*, 1989; Dunne, 1989; Schwanstecher *et al.*, 1992a).

In inside-out patches of pancreatic β-cells the K_{ATP}-channel activating potency of diazoxide was not only increased by ATP but also by activating nucleoside diphosphates (Schwanstecher *et al.*, 1992a; Larsson *et al.*, 1993). We therefore tested diazoxide in the presence of a low concentration of GDP. When K_{ATP}-channels were activated by 0.03 mM GDP, channel ac-

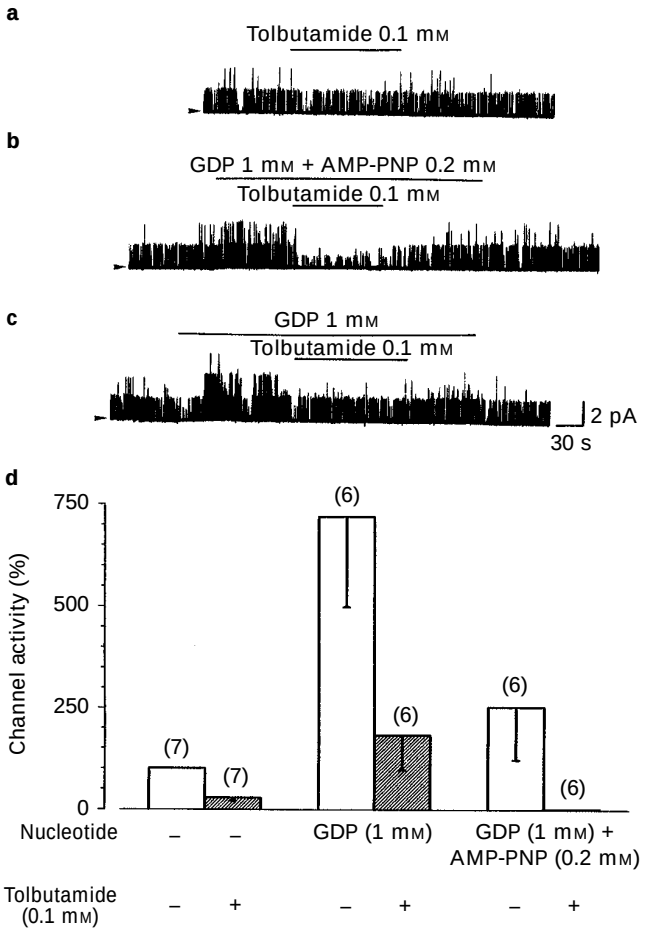

Figure 2 Effects of GDP and AMP-PNP on tolbutamide-induced inhibition of K_{ATP}-channels in inside-out patches of spiny neurones in caudate nucleus. The K^+ concentration was 5.6 mM in the pipette and 161 mM in the bath solution. The membrane potential was clamped at 0 mV. The Mg^{2+} concentration at the cytoplasmic membrane side was held close to 0.7 mM. (a–c) Continuous chart recorder traces demonstrating the responses of single K_{ATP}-channel currents to 0.1 mM tolbutamide alone (a), to 0.1 mM tolbutamide after addition of 1 mM GDP + 0.2 mM AMP-PNP (b) or to 0.1 mM tolbutamide after addition of 1 mM GDP (c). (d) Normalized activity of K_{ATP}-channels during treatment with different test substances. By use of the experimental design shown in (a–c), channel activity during application of test substances was normalized to the mean channel activity during the control periods (drug- and nucleotide-free) before and after application of test substances in each single experiment. The numbers above the columns indicate the corresponding numbers of experiments. For further details see legend of Figure 1.

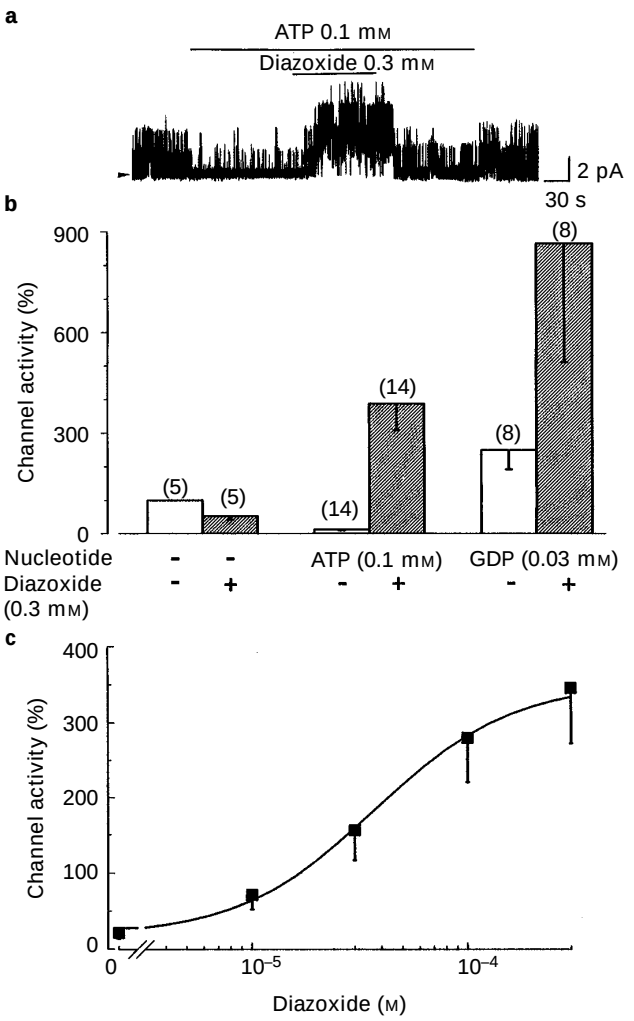

Figure 3 Activation of K_{ATP}-channels in inside-out patches of spiny neurones in caudate nucleus by diazoxide. (a) Current trace obtained from an inside-out patch. The horizontal lines above the current trace indicate application of 0.1 mM ATP alone or together with 0.3 mM diazoxide. (b) Normalized activity of K_{ATP}-channels during treatment with diazoxide alone or together with nucleotides as indicated. (c) Relationship between normalized channel activity and concentration of diazoxide in the presence of 0.1 mM ATP ($n=8$). For further details see legend of Figure 2.

tivity amounted to 250±58% of control activity. Diazoxide 0.3 mM increased this channel activity by 3.5 fold (860±350% of control activity ($n=8$; Figure 3b).

Effects of other potassium channel opening drugs

In an initial set of experiments we wanted to see whether lemakalim and pinacidil can open K_{ATP}-channels in cell-attached patches of rat spiny neurones. However, when spiny neurones were continuously exposed to 5 mM D-glucose, neither 0.3 mM lemakalim nor 0.5 mM (−)-pinacidil (the more active pinacidil isomer; Southerton *et al.*, 1988) were able to elicit single K_{ATP}-channel openings (data not shown in a figure).

We then examined the K_{ATP}-channel opening effects of lemakalim and pinacidil in inside-out patch experiments in the presence of a low concentration of ATP (0.1 mM) at the cytoplasmic membrane side (free Mg^{2+} concentration held close to 0.7 mM; Figure 4). Channel activity in the presence of 0.1 mM ATP amounted to 14±4% of the activity during ATP-free control periods ($n=14$; Figure 4c). Whereas lemakalim (0.3 mM) or (+)-pinacidil (0.5 mM) did not significantly increase channel activity in the presence of 0.1 mM ATP, a slight increase of channel activity was observed in the additional presence of (−)-pinacidil (0.5 mM) (40±18% of control activity; $n=5$; $P<0.05$; Figure 4a–c).

Discussion

In the present study, the meglitinide-, Hoe36320- and glipizide-concentrations giving 50% inhibition of the K_{ATP}-channel in spiny neurones of caudate nucleus were found to be 0.4 μM, 0.4 μM and about 0.5 nM, respectively (Figure 1c). In previous studies similar values were observed for the K_{ATP}-channel in pancreatic β-cells (Panten *et al.*, 1989; Schwanstecher *et al.*, 1994b).

Sulphonylurea concentrations occupying all specific receptor sites do not produce complete channel block in inside-out patches of pancreatic β-cells unless the internal side of the membrane is exposed to appropriate concentrations of channel-activating and channel-inhibiting nucleotides (Schwanstecher *et al.*, 1992b; 1994a,b). The present results demonstrate that this is also true for the interaction of tolbutamide and the

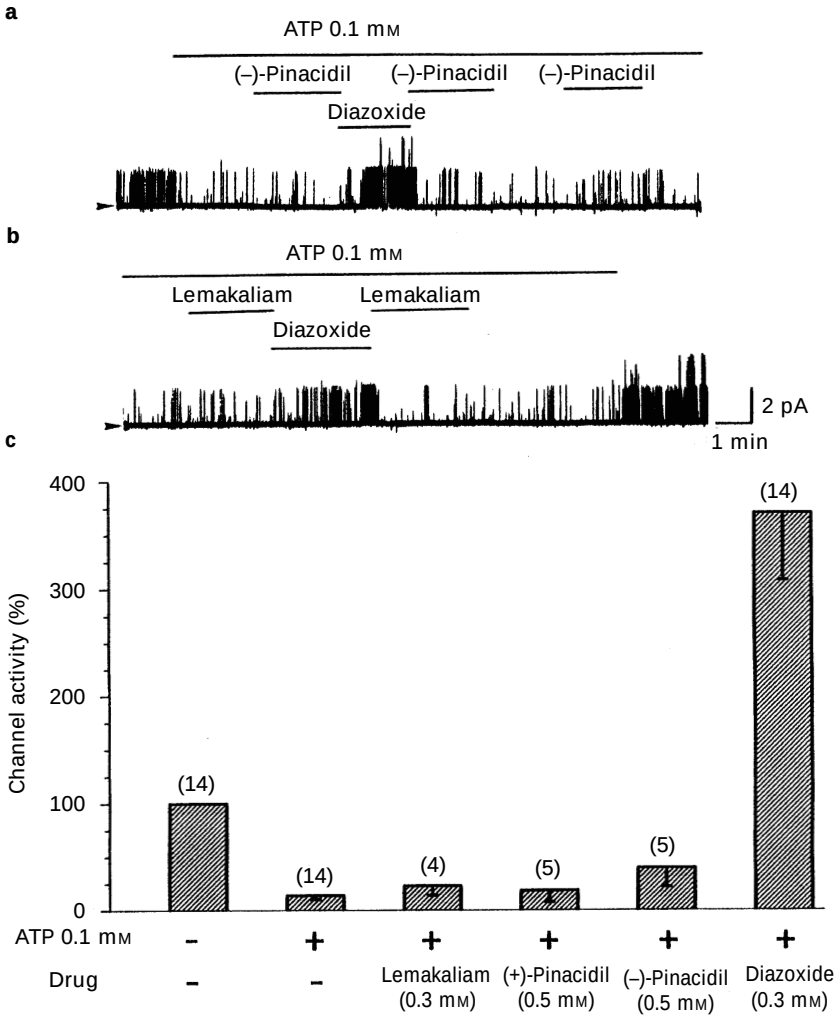

Figure 4 Activation of K_{ATP}-channels in inside-out patches of spiny neurones in caudate nucleus by lemakalim, (+)-pinacidil, (−)-pinacidil and diazoxide. (a, b) Current traces obtained from an inside-out patch. The horizontal lines above the current traces indicate application of 0.1 mM ATP alone or together with 0.5 mM (−)-pinacidil or 0.3 mM lemakalim or 0.3 mM diazoxide. (c) Normalized activity of K_{ATP}-channels during treatment with ATP alone or ATP together with lemakalim or (+)-pinacidil or (−)-pinacidil or diazoxide. For further details see legend of Figure 2.

K_{ATP}-channel in spiny neurones and thus suggest similar control of the sulphonylurea-sensitivity of the K_{ATP}-channel from β-cells and spiny neurones by cytosolic nucleotides.

In pancreatic β-cells diazoxide concentrations stimulating K_{ATP}-channel activity in inside-out patches by 50% range between 20 and 90 μM (Schwanstecher *et al.*, 1992a). In the present work a similar value (38 μM) was observed when recording the activity of the K_{ATP}-channels in inside-out patches of spiny neurones.

Experiments with pancreatic β-cells led to the conclusion that Mg complexes of nucleoside diphosphates induce a channel conformation most susceptible to diazoxide. The outcome of the experiments with inside-out patches of spiny neurones is consistent with this conclusion: whereas diazoxide was found to activate the channel strongly in the presence of GDP, it was ineffective in the absence of cytosolic nucleotides or presence of AMP-PNP. Consistent with previous findings in pancreatic β-cells, ATP was also effective at enhancing diazoxide-induced activation of the K_{ATP}-channel albeit its potency was weaker than that of GDP (Figure 3). However, the effect of ATP on diazoxide-induced channel activation was significantly stronger in spiny neurones than in pancreatic β-cells (390% or 50% of control activity, respectively; Figure 3, Schwanstecher *et al.*, 1992a; $P<0.01$). Still, whether this discrepancy reflects different properties of the K_{ATP}-channels in these tissues in unclear, since it might be due to enhanced enzymatic formation of MgADP from MgATP in inside-out patches of spiny neurones (Schwanstecher *et al.*, 1992a).

In the present study, (−)-pinacidil (0.5 mM) elicited a slight activation of K_{ATP}-channels in spiny neurones of caudate nucleus, whereas (+)-pinacidil and lemakalim (0.3 mM; the more active cromakalim isomer; Edwards & Weston, 1990) were ineffective (Figure 4). In β-cells the effects of the single stereoisomers of pinacidil and cromakalim have not been examined so far. However, in accordance with the findings in spiny neurones (±)-pinacidil slightly increased K_{ATP}-currents in pancreatic β-cells while cromakalim was ineffective (Garrino *et al.*, 1989; Schwanstecher & Bassen, unpublished observations). In one study with RINm5F cells cromakalim weakly increased K_{ATP}-currents (Dunne *et al.*, 1990) which might be due to special features of this cell-line.

Cromakalim (0.1 mM) has been shown to open a glibenclamide-sensitive K^+ channel in cell-attached patches of rat cultured hippocampal neurones (Politi & Rogawski, 1991). Moreover, lemakalim and pinacidil have been shown to stimulate potently sulphonylurea-sensitive ^{86}Rb-efflux from slices of substantia nigra (Schmid-Antomarchi *et al.*, 1990). Since these potassium channel openers were only weakly effective or ineffective in spiny neurones and β-cells, K_{ATP}-channels in these tissues on the one hand and neurones of hippocampus and substantia nigra on the other hand are probably differentially regulated by potassium channel openers. This phar-

macological difference might reflect structural differences of the K_{ATP}-channels in these tissues.

The K_{ATP}-channel in pancreatic β-cells is probably formed by a complex of the high affinity sulphonylurea receptor (SUR1) and Kir6.2, a member of the family of inwardly-rectifying K^+ channels in an as yet unknown stoichiometry (Aguilar-Bryan *et al*., 1995; Inagaki *et al*., 1995). Recently it was shown that mutations in SUR1 lead to an altered sensitivity of the K_{ATP}-channel to opening nucleotides and diazoxide suggesting that the binding sites for these nucleotides and potassium channel opening drugs are located at SUR1 (Nichols *et al*., 1996). Since our data indicate similar control by drugs and nucleotides of K_{ATP}-channels in the somata of spiny neurones and pancreatic β-cells, we conclude that the high affinity sulphonylurea receptors of these tissues are likely to be closely related.

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