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Inhibition of ATP-sensitive K^+-channels by a sulfonylurea analogue with a phosphate group

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Abstract

Hypoglycaemic sulfonylureas initiate insulin secretion by direct inhibition of ATP-sensitive K^+-channels in the pancreatic β-cells. These channels are composed of two proteins, a pore-forming subunit ($K_{IR}6.2$ in the case of β-cells) and a regulatory subunit, the sulfonylurea receptor (SUR). In the present study we characterised the interaction with SURs of the new sulfonylurea analogues 5-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]-2-methoxybenzamide (compound I) and {4-[2-(5-chloro-2-methoxybenzamido)ethyl]phenyl}phosphate (compound II). Compounds I and II differ from the sulfonylurea analogue meglitinide only in so far as the carboxylic group of meglitinide is replaced by a hydroxyl group or a phosphate group, respectively. The binding affinities of compound II for the SUR subtypes SUR1 (identified in β-cells) and SUR2A (identified in heart and skeletal muscle) were higher by 55 or 21-fold, respectively, than the corresponding affinities for compound I. In inside-out patch–clamp experiments compound II inhibited ATP-sensitive K^+-channels of the SUR1/$K_{IR}6.2$-type (characteristic of β-cells) with an IC_{50} value of 0.16 μM which is 6-fold lower than the corresponding value for meglitinide. These findings strongly support the conclusion that the interaction of sulfonylureas and acidic analogues with SURs is favoured by the anionic group of these drugs and that a phosphate group allows more efficient ligand interaction with SUR1 than a carboxylic group.

Keywords: K_{ATP}-channel; Sulfonylurea receptor subtypes; Sulfonylureas; Sulfonylurea analogue with a phosphate group; Structure–activity relationship; Pancreatic β-cell

1. Introduction

K^+-channels inhibited by cytosolic ATP (K_{ATP}-channels) have been identified in the plasma membranes of pancreatic β-cells, many neurons, cardiac myocytes, skeletal muscle and vascular and non-vascular smooth muscle [1]. Hypoglycaemic sulfonylureas (e.g. glibenclamide), too, inhibit K_{ATP}-channels and thereby initiate insulin release in the pancreatic β-cells [2]. K_{ATP}-channels are composed of two proteins, an inwardly rectifying K_{ATP}-channel subunit ($K_{IR}6.1$ or $K_{IR}6.2$) and the SUR, a member of the ATP-binding cassette protein superfamily [3]. Three SUR isoforms have been cloned, SUR1, and two splice products of a single gene differing only in their carboxyterminal 42–45 amino acids, SUR2A and SUR2B [3]. While SUR1 acts as the regulatory subunit of the K_{ATP}-channels in β-cells and many neurons, SUR2A has been suggested to represent the SUR in heart and skeletal muscle and SUR2B in smooth muscle [3].

Several derivatives of 3-phenylpropionic acid (e.g. nateglinide) or benzoic acid (e.g. meglitinide and the more lipophilic repaglinide) inhibit K_{ATP}-channels by binding to the same receptor site that mediates the responses to sulfonylureas [2,4–6]. Two of these sulfonylurea analogues (repaglinide and nateglinide) have recently been introduced into the therapy of type 2 diabetes mellitus [7]. The receptor site for sulfonylureas and their analogues is located at the cytoplasmic face of the plasma membrane [8]. Interaction with this site is favoured by the anionic group (sulfonamide or carboxylic group) characteristic of the sulfonylureas and analogues which are used in the antidiabetic therapy [5]. However, it is unknown whether the ligand interaction with the receptor site can be improved by an anionic group containing more than one negative charge. To test this possibility the effects of a

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Abbreviations: K_{ATP}-channel, ATP-sensitive K^+-channel; K_{IR}, inwardly-rectifying K_{ATP}-channel subunit; SUR, sulfonylurea receptor.

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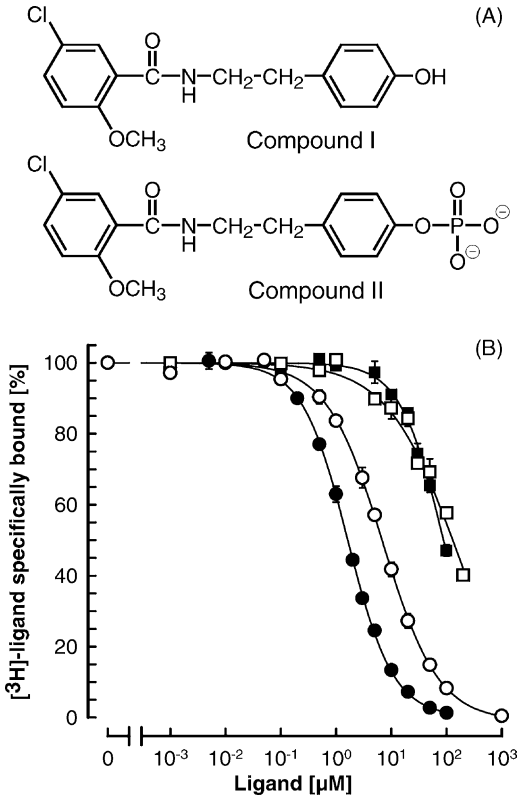

Fig. 1. Structures (A) and SUR binding (B) of compounds I and II. (A) The anionic form of compound II is shown. (B) In membranes from COS-1 cells transiently expressing hamster SUR1 or rat SUR2A, the effects of compounds I (squares) and II (circles) on specific [^{3}H]-ligand binding were measured. For binding to recombinant SUR1 (filled symbols) the [^{3}H]-ligand was [^{3}H]-glibenclamide (0.3 nM), for binding to recombinant SUR2A (open symbols) the [^{3}H]-ligand was [^{3}H]-P1075 (3 nM). Nonspecific binding was defined by incubations in the additional presence of 100 nM glibenclamide or 100 μM pinacidil, respectively. Results are expressed as percentage of control (absence of displacing drug). Symbols are means (with SEM shown when larger than symbols) from three to six separate binding experiments.

sulfonylurea analogue with a phosphate group (compound II, Fig. 1A) on SUR binding and K_{ATP}-channel activity were examined. Compound II differs from meglitinide only in so far as the carboxylic group of meglitinide is replaced by a phosphate group.

2. Materials and methods

2.1. Chemicals and solutions

Chemicals for organic syntheses were purchased from Aldrich Chemical Co. and Fluka Chemie AG. All other chemicals and radioactively labelled compounds were obtained from sources described elsewhere [5].

Unless stated otherwise, chemicals for syntheses (pure grade) were used as received. Solvents were dried and distilled before use. Using a minor modification of the method described by Brown and Foubister [9], compound I (5-chloro-*N*-[2-(4-hydroxyphenyl)ethyl]-2-methoxybenzamide) was prepared by the addition of 5-chloro-2-methoxybenzoyl chloride to tyramine and purified by recrystallisation from ethanol to water. Using a minor modification of a previously described method [10], compound II ({4-[2-(5-chloro-2-methoxybenzamido)ethyl]-phenyl}phosphate) was prepared by the addition of $POCl_3$ to compound I and purified by column chromatography on silica gel 60 (Merck). Melting points (uncorrected values) were 127° for compound I and 197° for compound II. Elementary analyses of compounds I and II were within +0.1, ±0.7 and −5% of the theoretical values (100%) for C, H and N, respectively. The structures of compounds I and II (Fig. 1A) were confirmed by ^{1}H-NMR (DMSO, 400 MHz), ^{13}C-NMR (DMSO, 100 MHz) and ^{31}P-NMR (DMSO, 81 MHz) spectroscopy and infrared spectroscopy, supported by electron-impact and fast atom bombardment mass spectrometry.

A solution (100 mM) of compound I was prepared daily in DMSO and diluted 1/100 in 50 mM KOH to give a 1 mM stock solution. A 10 mM stock solution of compound II was prepared daily in KOH (50 mM). Appropriate amounts of the stock solutions were added slowly, while stirring, to Tris buffer (binding experiments) or intracellular solution supplemented with 0.3 mM ADP (patch–clamp experiments). The pH of all test solutions was determined after addition of test substances and was readjusted if necessary. Compounds I and II were completely dissolved at the applied concentrations.

2.2. Binding experiments

Culture and transient transfections of COS-1 cells (obtained from the German Collection of Microorganisms and Cell Cultures), membrane preparation and measurement of ligand binding to the membranes were performed as described previously [5].

2.3. Electrophysiological experiments

Transient cotransfection of COS-1 cells with pECE-SUR1 and pECE-KIR6.2 complementary DNA and patch–clamp experiments using inside-out patches of these cells were performed as previously described by Dörschner *et al.* [11] and Meyer *et al.* [5], respectively. The membrane potential was clamped at −50 mV, and inward membrane currents flowing through K_{ATP}-channels were measured at room temperature. The solution at the cytoplasmic side of the inside-out membrane (intracellular solution) contained (in mM): KCl 140, $CaCl_2$ 2, $MgCl_2$ 1, EGTA 10 and HEPES 5 (titrated to pH 7.3 with KOH) (free $[Ca^{2+}] = 0.05$ μM). The bath was perfused continuously at 2 mL/min with intracellular solution containing 1 mM ATP, 0.3 mM ADP or 0.3 mM ADP + compound II (0.03–1 μM). The free Mg^{2+} concentration was held close to 0.7 mM by adding appropriate amounts of $MgCl_2$ to nucleotide-containing solutions. The pipette solution contained (in mM): KCl

146, $CaCl_2$ 2.6, $MgCl_2$ 1.2, HEPES 10 (titrated to pH 7.4 with KOH). The mean K_{ATP}-channel current during the last minute of application of 0.3 mM ADP (control solution) or 0.3 mM ADP + compound II (test solution) was measured and used to express the K_{ATP}-channel activity in the presence of compound II as percentage of channel activity in control solution before and after application of compound II. The single channel current amplitudes of the K_{ATP}-channels were not changed by the applied concentrations of nucleotides and compound II.

2.4. Treatment of results

Values are presented as mean ± SEM. Relations between drug concentration and specific binding or channel activity and calculation of K_d values were performed as described [5]. Significances were calculated by the two-tailed *U*-test of Wilcoxon and of Mann and Whitney. $P < 0.05$ was considered significant.

3. Results and discussion

Competitive inhibition assays (Fig. 1B) revealed that compounds I and II inhibited specific [^{3}H]-glibenclamide binding to SUR1 half-maximally at 89.6 ± 5.7 μM (Hill coefficient = −1.11) and 1.63 ± 0.07 μM (Hill coefficient = −1.06), respectively. From these IC_{50} values dissociation constants (K_d) of 58.0 ± 3.7 μM for compound I and 1.06 ± 0.04 μM for compound II were calculated. Compounds I and II inhibited specific [^{3}H]-P1075 binding to SUR2A half-maximally at 148.7 ± 2.9 μM (Hill coefficient = −0.82) and 7.01 ± 0.54 μM (Hill coefficient = −0.87), respectively. These IC_{50} values corresponded to K_d values of 139.6 ± 2.7 μM for compound I and 6.58 ± 0.51 μM for compound II.

As shown in Fig. 2A, compound II applied at the cytoplasmic membrane face rapidly inhibited K_{ATP}-channels of the SUR1/K_{IR}6.2-type which is characteristic of β-cells and many neurons. The periods of current recording in the sole presence of 0.3 mM ADP revealed run-down of channel activity. Inhibition by 0.03 or 1 μM of compound II amounted to 9.5 or 83.5%, respectively (Fig. 2A). Inhibition was rapidly and completely reversible. Analysis of the dependence of channel inhibition on the concentration of compound II (Fig. 2B) yielded an IC_{50} value of 0.16 μM (Hill coefficient = −1.33). This IC_{50} value was 6.6-fold lower than the corresponding K_d value for binding of compound II to SUR1. Similar differences (2–10-fold) have been regularly observed for sulfonylureas and their analogues [4,5,8]. These differences reflect the fact that occupation of one of the four SUR binding sites per channel complex is sufficient for K_{ATP}-channel closure [11].

The finding that the K_d values for binding of compound I were 21–55-fold higher than the K_d values for binding of compound II strongly supports the previous conclusion

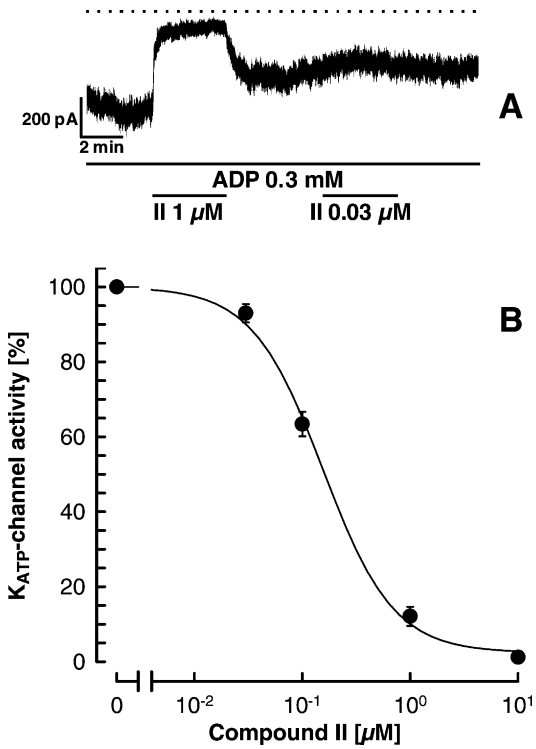

Fig. 2. Effect of compound II on K_{ATP}-channel activity in inside-out patches of COS-1 cells transiently expressing SUR1 and K_{IR}6.2. (A) Current trace obtained from an inside-out patch. Free Mg^{2+} (0.7 mM) was always present in the solutions applied at the cytoplasmic membrane side. The dotted line denotes the current level when all K_{ATP}-channels are closed by 1 mM ATP (90 sec periods of ATP application 4 min before and 12 min after the indicated current trace). The horizontal bars below the current trace indicate application of intracellular solution containing 0.3 mM ADP (with or without 0.03–1 μM compound II) by the bath. (B) Relationship between normalised K_{ATP}-channel activity and concentration of compound II in the presence of ADP. By use of the experimental design shown in (A), K_{ATP}-channel activity was normalised to K_{ATP}-channel activity during the control periods (presence of ADP, absence of drug) before and after application of each drug concentration. Symbols are means (with SEM shown when larger than symbols) from six experiments.

that the interaction of sulfonylureas and acidic analogues with SURs is favoured by the anionic group of these drugs [5].

The structure of compound II (Fig. 1A) differs from the structure of the sulfonylurea analogue meglitinide only in so far as the carboxylic group of meglitinide is replaced by a phosphate group. The binding affinity ($1/K_d$) for SUR1 and the K_{ATP}-channel-inhibitory potency ($1/IC_{50}$) of compound II were higher by 6.2 and 7.5-fold, respectively, than the corresponding values for meglitinide [5,8]. Thus, as compared with a carboxylic group a phosphate group allows more efficient ligand interaction with SUR1. The reason could be the greater negative charge of the phosphate group and/or the different position of the negative charge relative to the neighbouring benzene ring of the ligand.

The binding affinities of meglitinide for SUR1 and SUR2A did not differ significantly [5], in contrast to the observation that the binding affinity of compound II for

SUR1 was 6.2-fold higher ($P < 0.01$) than the binding affinity of compound II for SUR2A. Thus, replacement of the carboxylic group of meglitinide by a phosphate group produces a SUR1-selective ligand. This information might help to characterise the biochemical properties of the binding sites of the SURs.

The insulin releasing property of compound II has not been tested up to now. The receptor site for sulfonylureas and analogues is located at the cytoplasmic side of the β-cell plasma membrane [8]. Therefore, extracellular application of the polar compound II probably requires concentrations higher than the concentrations effective in the present inside-out patch–clamp experiments (Fig. 2).

It is unknown whether endogenous ligands for the SUR sites exist. The possibility that endogenous ligands might be found among intracellular phosphorylated peptides and proteins is stressed by the structure of compound II which includes the tyrosine phosphate side chain occurring in peptides and proteins.

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