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RESEARCH PAPER

Actions of ZD0947, a novel ATP-sensitive K^+ channel opener, on membrane currents in human detrusor myocytes

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Background and purpose: ATP-sensitive K^+ channels (K_{ATP} channels) play important roles in regulating the resting membrane potential of detrusor smooth muscle. Actions of ZD0947, a novel K_{ATP} channel opener, on both carbachol (CCh)-induced detrusor contractions and membrane currents in human urinary bladder myocytes were investigated.
Experimental approach: Tension measurements and patch-clamp techniques were utilized to study the effects of ZD0947 in segments of human urinary bladder. Immunohistochemistry was also performed to detect the expression of the sulphonylurea receptor 1 (SUR1) and the SUR2B antigens in human detrusor muscle.
Key results: ZD0947 ($\geq$0.1 μM) caused a concentration-dependent relaxation of the CCh-induced contraction of human detrusor, which was reversed by glibenclamide. The rank order of the potency to relax the CCh-induced contraction was pinacidil > ZD0947 > diazoxide. In conventional whole-cell configuration, ZD0947 ($\geq$1 μM) caused a concentration-dependent inward K^+ current which was suppressed by glibenclamide at -60 mV. When 1 mM ATP was included in the pipette solution, application of pinacidil or ZD0947 caused no inward K^+ current at -60 mV. Gliclazide ($\leq$1 μM), a selective SUR1 blocker, inhibited the ZD0947-induced currents (K_i = 4.0 μM) and the diazoxide-induced currents (high-affinity site, K_{i1} = 42.4 nM; low-affinity site, K_{i2} = 84.5 μM) at -60 mV. Immunohistochemical studies indicated the presence of SUR1 and SUR2B proteins, which are constituents of K_{ATP} channels, in the bundles of human detrusor smooth muscle.
Conclusions and Implications: These results suggest that ZD0947 caused a glibenclamide-sensitive detrusor relaxation through activation of glibenclamide-sensitive K_{ATP} channels in human urinary bladder.
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Keywords: ATP-sensitive K^+ channels; human urinary bladder; K^+ channel opener; overactive bladder; SUR1; SUR2; ZD0947

Abbreviations: BP, blocking peptide; BSA, bovine serum albumin; CCh, carbachol; DHP, dihydropyridine; DMSO, dimethylsulphoxide; E_K, theoretical equilibrium potential of K^+; FITC, fluorescein isothiocyanate; K_{ATP} channels, ATP-sensitive K^+ channels; Kir, inwardly rectifying K^+ channel; NDP, nucleoside diphosphate; OAB, overactive bladder; OCT, optimal cutting temperature; PBS, phosphate-buffered saline; PE, phycoerythrin; RT–PCR, reverse transcriptase–polymerase chain reaction; SUR, sulphonylurea receptor; ZD0947, 3-[(4*S*)-5-oxo-2-(trifluoromethyl)-1,4,5,6,7,8-hexahydroquinolin-4-yl]benzonitrile

Introduction

The overactive bladder (OAB) is a syndrome based on the presence of the symptoms of urgency, with or without urge incontinence, accompanied with frequency and nocturia in the absence of pathologic or metabolic diseases. Initial treatment of the condition normally involves an integrated approach using both behavioural methods and pharmacotherapy (Andersson, 2004).

Antimuscarinic agents are currently available for the clinical treatment for OAB syndrome. However, antimuscarinic agents are somewhat limited by certain side effects (such as dry mouth, constipation and blurred vision, etc.).

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Thus, the development of new compounds with novel mechanisms of action for the treatment of OAB is essential. Although several alternative pharmacotherapies have been tried in the clinical field, more urinary bladder-selective agents should be synthesized and developed (Andersson, 2004).

In the smooth muscle cells of the lower urinary tract, K^+ channels play an important role in regulating membrane potential and excitability (Brading, 1987; Andersson, 1993). In the condition of detrusor overactivity in which the smooth muscle becomes hyperexcitable, K^+ channel openers potently decrease excitability and may be useful in the treatment of OAB (Foster *et al*., 1989a, b). It is well-known that K^+ channel openers hyperpolarize the membrane potential of smooth muscle cells by increasing K^+ permeability, followed by a significant detrusor relaxation.

In the urological field, recent molecular identification of ATP-sensitive K^+ channels (K_{ATP} channels) has renewed interest in the development of detrusor-selective K_{ATP} channel openers for the treatment of OAB and urinary incontinence. Several detrusor-selective K_{ATP} channel openers, such as YM-934, (Uchida *et al*., 1994), ZD6169, (Trivedi *et al*., 1995) and WAY-133537 (Wojdan *et al*., 1999), have been newly synthesized, targeting glibenclamide-sensitive K_{ATP} channels in smooth muscle of the urinary bladder.

ZD0947 (3-[(4*S*)-5-oxo-2-(trifluoromethyl)-1,4,5,6,7,8-hexahydroquinolin-4-yl]benzonitrile; Abdel-Karim *et al*., 2002) is a newly developed dihydropyridine (DHP) derivative K_{ATP} channel opener. Although the effects of ZD0947 in rats with spinal cord injury and with detrusor hyperreflexia have been reported (Abdel-Karim *et al*., 2002), the effects of ZD0947 on the muscle tone and the membrane currents have not been investigated as yet in detrusor smooth muscle. Furthermore, there has been no report concerning the effects of K_{ATP} channel openers on the glibenclamide-sensitive K_{ATP} currents in human detrusor myocytes. Recent studies have revealed that recombinant K_{ATP} channels are heteromeric complexes composed of at least two subunits; one from a family of inwardly rectifying K^+ channels (Kir6.x) and the other from a family of sulphonylurea receptors (SUR.x) (Inagaki *et al*., 1995). It is also considered that K_{ATP} channel openers interact with SUR.x subunits, and not with Kir6.x subunits of K_{ATP} channels (Schwanstecher *et al*., 1998). However, the nature of the SUR.x proteins in human detrusor K_{ATP} channels still remain to be established.

In the present study, we have firstly investigated the effects of ZD0947 on the muscle tone of isolated human detrusor smooth muscle by use of tension measurement. Secondly, we have studied the effects of ZD0947 on the membrane currents in human detrusor myocytes using whole-cell recordings. Thirdly, we have localized the SUR1 (Aguilar-Bryan *et al*., 1995) and SUR2B (Isomoto *et al*., 1996) proteins, by immunohistochemical methods, in the smooth muscle bundles of human urinary bladder.

Methods

Tension measurement and data analysis

Small segments of human detrusor was obtained from patients with a stable urinary bladder who were generally undergoing cystectomy for bladder cancer after informed patient consent and approved of ethical consent of the Kyushu University Faculty of Medicine Ethical Committee (Fukuoka, Japan) had been obtained. A segment of detrusor was excised and quickly transferred into modified physiological salt solution. For isometric tension recording, fine strips ($1 \times 1 \times 3$ mm) were prepared as described previously (Tomoda *et al*., 2005). Modified Krebs solution was used (mM): Na^+ 137, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 133.7, HCO_3^- 15.4, $H_2PO_4^-$ 1.2 and glucose 11.5 which was bubbled with 97% O_2 and 3% CO_2 (pH 7.35–7.45 at 37°C). An initial tension equivalent to 0.5 g weight was applied to each human detrusor strip, which was then allowed to equilibrate for approximately 1–1.5 h until the basal urethral tone became stable (36–37°C). Data were recorded on a Macintosh computer (Macintosh G4, Apple Computer Japan, Tokyo, Japan), through 'MacLab 3.5.6' (ADInstruments Pty Ltd, Castle Hill, Australia). The tension was expressed as mN mg^{-1} of tissue.

Cell preparation and patch-clamp experiment recording procedure

We used freshly dispersed single detrusor myocytes prepared from human urinary bladder as described previously (Tomoda *et al*., 2005). We employed the smooth cell dispersion method previously described (the gentle tapping method; Teramoto and Brading, 1996). The set-up of the patch-clamp experimental system used was essentially the same as described previously (Tomoda *et al*., 2005). All experiments were performed at room temperature (21–23°C).

Data analysis

The whole-cell current data were low-pass filtered at 500 Hz (−3 dB) by an 8 pole Bessel filter (3611 multifunction filter, NF Electronic Instruments, Yokohama, Japan), sampled at 1 ms and analysed on a computer (Macintosh G4, Apple Computer Japan, Tokyo, Japan) by use of the commercial software 'Mac Lab 3.5.6' (ADInstruments Pty Ltd, Castle Hill, Australia).

The curve was drawn by fitting the equation using the least-squares method:

$$I/I_c = 1/\{1 + (D/K_i)^h\}$$

where I is the mean amplitude of the muscle contraction in the presence of K_{ATP} channel openers, I_c is the mean amplitude of carbachol (CCh)-induced contraction in control (in the absence of K_{ATP} channel openers), K_i, D and h are apparent dissociation constant, concentration of K_{ATP} channel openers (μM) and the Hill coefficient, respectively (Figure 3d). In Figure 6b, I is the mean amplitude of the diazoxide-induced K_{ATP} current in the presence of gliclazide, I_c is the mean amplitude of the diazoxide-induced K_{ATP} current in control (in the absence of gliclazide), K_i, D and h are the inhibitory dissociation constant, concentration of gliclazide (μM) and Hill's coefficient, respectively.

For curve fitting of the biphasic effects of gliclazide on the diazoxide-induced currents, following equation was used:

$$I/I_c = xy$$

where I and I_c are same as above, x is a term describing the high-affinity site and y a term describing the low-affinity site.

$$x = L + \{(1 - L)/(1 + ([D]/K_{i1})^{h1})\}$$

$$y = 1/(1 + ([D]/K_{i2})^{h2}$$

where [D] is the concentration of ZD0947 (μM), K_{i1}, K_{i2} are the ZD0947 concentrations at which relaxant effect is half maximum at high- and low-affinity sites, respectively; $h1$, $h2$ are the Hill coefficients for the high- and low-affinity sites, respectively; and L is the fractional current amplitude remaining when the high-affinity sites are fully occupied.

Solutions

For whole-cell recording, the following solutions were used: 140 mM K^+ solution containing (mM): Na^+ 5, K^+ 140, Mg^{2+} 1.2, Ca^{2+} 2, glucose 5, Cl^- 151.4, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES) 10, titrated to pH 7.35–7.40 with Tris base (sometimes 60 mM K^+ solution was used; Na^+ 85, K^+ 60, Mg^{2+} 1.2, Ca^{2+} 2, glucose 5, Cl^- 151.4, HEPES 10/Tris); high potassium pipette solution containing (mM): K^+ 140, Cl^- 140, glucose 5, ethylene glycol-bis-(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 5, HEPES 10 and ATP 0.1 (sometimes 1 mM ATP was included)/Tris (pH 7.35–7.40). The bath solution was superfused by gravity throughout the experiments at a rate of 2 ml min^{-1}.

Immunohistochemical studies

Tissue samples from human urinary bladder were embedded in optimal cutting temperature (OCT) compound (Tissues-Tek, SAKURA, Tokyo, Japan) in disposable plastic plates and rapidly frozen in hexane surrounded by liquid nitrogen. Frozen sections were cut with a cryostat (CM3050S, Leica, Tokyo, Japan) at a thickness of 6 μm and mounted on silane-precoated glass slides, then allowed to air dry at room temperature for approximately 30 min. Sections were fixed in cold acetone for 5 min and washed thoroughly in phosphate-buffered saline (PBS) before staining. The tissue sections were treated in 5% normal mouse serum (Santa Cruz Biotechnology, CA, USA), 3% bovine serum albumin (BSA, GIBCO, NY, USA) and 0.2% Triton-X 100 (Amersham Biosciences, Uppsala, Sweden) in PBS for 1 h to avoid nonspecific staining, and then primary antibodies were applied to sections at 4°C overnight at the following dilutions with 1.5% normal mouse serum and 1% BSA in PBS: purified goat anti-SUR1 antibody (sc-5789; Santa Cruz Biotechnology, CA, USA), 1:200; goat anti-SUR2B antibody (sc-5793; Santa Cruz Biotechnology, CA, USA), 1:200. As a negative control, the primary antibody was absorbed with the peptide against which it was made (blocking peptide). Following washing, three times (5 min duration) in PBS, sections were incubated with phycoerythrin (PE)-conjugated mouse anti-goat IgG (sc-3752; Santa Cruz Biotechnology, CA, USA) diluted to 1:50 in PBS with 1.5% normal mouse serum and 1% BSA, and fluorescein isothiocyanate (FITC)-conjugated mouse anti-goat IgG (sc-2356; Santa Cruz Biotechnology, CA, USA) diluted to 1:50 for 1 h at room temperature under dark conditions. Sections were then washed for three times (5 min duration) in PBS. Coverslips were mounted onto slides by use of fluorescence mounting medium and slides viewed by fluorescent microscopy (Biozero BZ-8000, KEYENCE, Osaka, Japan).

Statistical analysis

Statistical analyses were performed with analysis of variance (ANOVA) test (two-factor with replication). Changes were considered significant at $P<0.01$ (*). Data are expressed as mean with the standard deviation (s.d.).

Materials

ZD0947 (kindly provided by the AstraZeneca Pharmaceuticals, Wilmington, DE, USA) was prepared daily as 100 mM stock solutions in dimethylsulphoxide (DMSO). The final concentration of DMSO was less than 0.3%, not affecting membrane currents. The rest of the chemicals were purchased from Sigma (Sigma Chemical KK, Tokyo, Japan).

Results

The effects of ZD0947 on the CCh-induced contraction in human detrusor

Application of CCh ($\geqslant$30 nM, 7 min duration) caused a concentration-dependent contraction in the strips of human detrusor (Figure 1a). In the presence of 100 nM ZD0947, the CCh ($\leqslant$300 nM)-induced contraction was inhibited although the 1 μM CCh-induced detrusor contraction was not affected by 100 nM ZD0947. Similarly, 1 μM ZD0947 caused little effect on 1 μM CCh-induced detrusor contraction. In the presence of 10 μM ZD0947, the CCh-induced contractions were inhibited. Figure 1b summarizes the effects of ZD0947 on the CCh-induced contractions in human detrusor strips; the contractions induced by 1 μM CCh, in the absence of ZD0947, were normalized to a value of 1.0. The reduction produced by ZD0947 (10 μM) in the 300 nM CCh-induced contraction was reversed by additional application of 1 μM glibenclamide to the control level (Figure 2, $n=4$). Application of 300 nM CCh caused a phasic contraction following a tonic contraction in human detrusor. After the muscle tone level of the CCh-induced tonic contraction became stable, cumulative application of ZD0947 (0.1–10 μM) produced a concentration-dependent relaxation of the CCh-induced contraction (Figure 3a). After washing out of the drug, the detrusor tone recovered to the control level. Subsequently, 10 μM pinacidil was applied in order to obtain the maximum relaxation in the detrusor strips. Similar experiments were performed to use different types of K_{ATP} channel openers (pinacidil, Figure 3b; diazoxide, Figure 3c). Figure 3d shows the concentration–response curves for pinacidil ($K_i=0.8$ μM), ZD0947 ($K_i=3.5$ μM) and diazoxide ($K_i=51.3$ μM), expressed relative to the maximum relaxation to 10 μM pinacidil.

Effects of ZD0947 on membrane currents in human urinary bladder myocytes

In order to study further the actions of ZD0947 on human detrusor, the membrane currents were recorded from

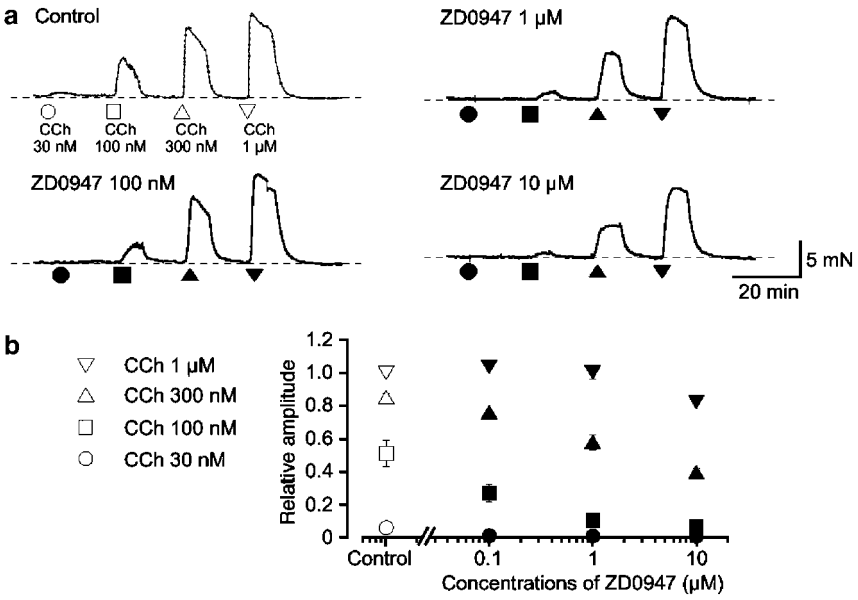

Figure 1 Effects of ZD0947 on CCh-induced contraction of human detrusor strips. (**a**) CCh-induced contraction in the absence (control) and presence of ZD0947. (**b**) Effects of ZD0947 on the peak amplitude of CCh-induced contraction of human detrusor strips, when the peak amplitude of 1 μM CCh-induced contraction in the absence of ZD0947 is normalized as unity. Each symbol indicates mean with s.d. shown by vertical lines. Some of s.d. bars are less than the size of the symbols.

dispersed human detrusor smooth muscle myocytes by use of a conventional whole-cell configuration. Cumulative application of ZD0947 (1–100 μM) caused a significant inward current in a concentration-dependent manner at a holding potential of -60 mV (Figure 4a and b). As shown in Figure 4a, the peak amplitude of the 100 μM ZD0947-induced inward current was inhibited by 5 μM glibenclamide. When 1 mM ATP was included in the pipette solution, little appreciable inward current was evoked by application of ZD0947 after the conventional whole-cell configuration had become established (Figure 4c). Similar results were observed on application of 100 μM pinacidil (Figure 4d). These results suggest that the ZD0947-induced inward current seems to possess not only a glibenclamide-sensitivity but also an intracellular ATP-sensitivity in human detrusor myocytes.

To make a rough estimate of the ion selectivity and the reversal potential of the ZD0947-induced glibenclamide-sensitive current, voltage ramps were applied and the extracellular K^+ concentration ($[K^+]_o$) was changed by iso-osmotic substitution of Na^+. Figure 5a shows the experimental protocol. ZD0947 (10 μM) caused a sustained inward current in 140 mM K^+ solution and then the voltage protocol was performed. In the presence of ZD0947 (control), current–voltage relationships were obtained by the application of four ramp pulses in solutions containing 140 mM K^+ solution followed by 60 mM K^+ solution. When $[K^+]_o$ was decreased from 140 to 60 mM, the ZD0947-induced membrane current at -60 mV was markedly decreased. Figure 5b shows the average of the four ramp currents before and during application of 5 μM glibenclamide for the cell shown in Figure 5a when ZD0947 was present. In each $[K^+]_o$ condition, the net membrane current inhibited by 5 μM glibenclamide was obtained by subtracting the averaged control current from the mean ZD0947-induced current. The current demonstrated an inwardly rectifying property. The reversal potential of the ZD0947-induced glibenclamide-sensitive membrane current in this cell was 4.4 mV in 140 mM K^+ solution and -21.6 mV in 60 mM K^+ solution (Figure 5c). Mean values from four cells were 2.8 ± 3.0 mV ($n=4$) for 140 mM K^+ solution and -23.5 ± 5.5 mV ($n=4$) for 60 mM K^+ solution. These values were close to the theoretical potassium equilibrium potential (E_K) in each $[K^+]_o$ condition (140 mM K^+; $E_K = 0$ mV, 60 mM K^+; $E_K = -21$ mV). These results suggest that in human detrusor myocytes, the ZD0947-induced glibenclamide-sensitive membrane currents are carried mainly by potassium ions.

The effects of gliclazide on K_{ATP} channel opener-induced currents

In order to further investigate whether the SUR1 subunit may be involved in the ZD0947-induced glibenclamide-sensitive membrane currents in these cells, gliclazide, a specific SUR1 blocker (Gribble and Ashcroft, 2000), was utilized. The nystatin-perforated patch configuration was utilized to maintain the basal amplitude of K_{ATP} channel opener-induced inward currents at a holding potential of -60 mV. As shown in Figure 6a, gliclazide (0.1–0.3 μM) showed little effect on the ZD0947-induced currents. When the concentrations of gliclazide were increased ($\leqslant 1$ μM), the ZD0947-induced membrane current at -60 mV was inhibited in a concentration-dependent manner ($K_i = 4.0$ μM) and gliclazide (100 μM) completely suppressed the ZD0947-induced current at -60 mV (Figure 6b). In contrast, 100 nM gliclazide reduced the current amplitude induced by 500 μM diazoxide by approximately 10% (to 0.89 ± 0.05 times the initial current, $n=4$, $P<0.01$) and higher concentrations

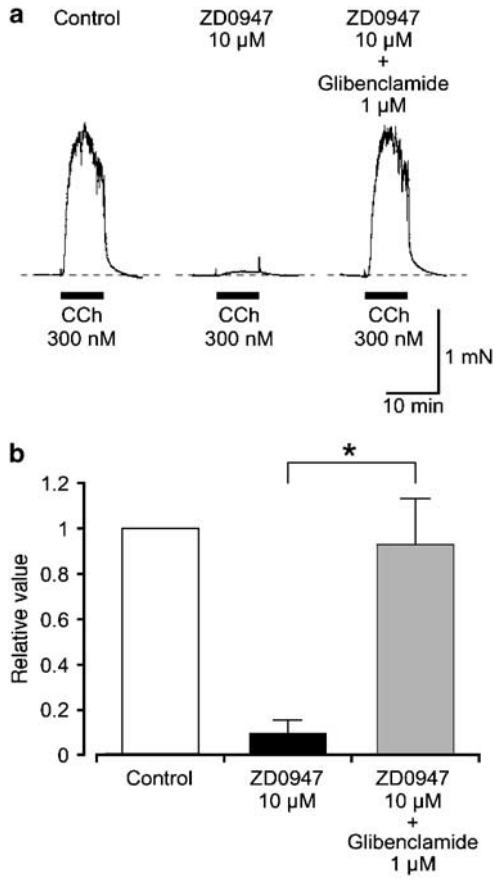

Figure 2 Effects of both ZD0947 and glibenclamide on the CCh-induced contraction of human detrusor strips. The dashed line indicates the zero tone level. (**a**) CCh (300 nM, 4 min application) caused a contraction. In the presence of 10 μM ZD0947, the CCh-induced contraction was suppressed. After treatment with 1 μM glibenclamide, the CCh-induced contraction recovered to the control level, even in the presence of ZD0947. The results illustrated were obtained from a single smooth muscle strip. (**b**) Relative amplitude of the CCh-induced contraction under the indicated conditions. The amplitude of the CCh-induced contraction was normalized as 1.0 (Control, open column). The solid column indicates the relative amplitude of 10 μM ZD0947-induced relaxation. The hatched column represents the relative amplitude of the CCh-induced contraction in the presence of ZD0947. Asterisks indicate a statistically significant difference, demonstrated using a paired *t*-test ($P<0.01$). Each column represents the relative mean value with + s.d. ($n=4$).

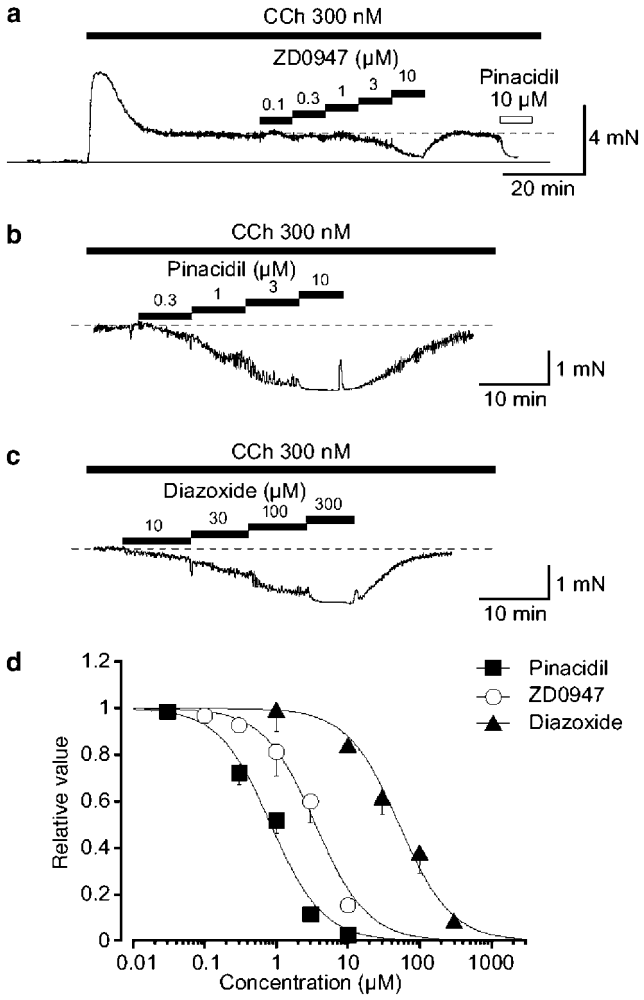

Figure 3 Relaxing effects of K_{ATP} channel openers on the 300 nM CCh-induced contraction of human detrusor strips. The dashed line indicates the stabilized muscle tone level and the line indicates the zero tone level. (**a**) The effects of cumulative addition of ZD0947 and after recovery, 10 μM pinacidil. (**b**, **c**) The effects of cumulative addition of pinacidil and diazoxide on the CCh-induced contraction. (**d**) Relationships between the relative value of K_{ATP} channel opener-induced relaxation and the concentration of K_{ATP} channel opener. The peak amplitude of the 10 μM pinacidil-induced relaxation was normalized as 1.0. The following values were used for the curve fitting: pinacidil, $K_i=0.8$ μM, $h=1.2$ ($n=4$); ZD0947, $K_i=3.5$ μM, $h=1.2$ ($n=4$); diazoxide, $K_i=51.3$ μM, $h=1.2$ ($n=4$). Each symbol indicates mean with s.d. shown by vertical lines. Some of s.d. bars are less than the size of the symbols.

of gliclazide inhibited the diazoxide-induced current in a concentration-dependent manner, demonstrating two inhibitory sites: a high-affinity site ($K_{i1}=42.4$ nM) and a low-affinity site ($K_{i2}=84.5$ μM) in Figure 6c.

Immunohistochemical localization of SUR1 and SUR2B proteins in human detrusor

To localize the K_{ATP} channels characterized above, immunohistochemistry was performed to detect the expression of the SUR1 and the SUR2B proteins (Figure 7). As shown in Figure 7, both SUR1 and SUR2B immunoreactivity is clearly visible in the smooth muscle bundles of human detrusor (Figure 7a and c).

Discussion

In the present experiments, we have demonstrated that ZD0947 induces a glibenclamide-sensitive K^+ current, which was inhibited by endogenous ATP in human detrusor myocytes.

Target K^+ channels for ZD0947 in human urinary bladder

A glibenclamide-sensitive, cromakalim-induced, hyperpolarization has been recorded in human urinary bladder myocytes by use of the current-clamp technique (Wammack

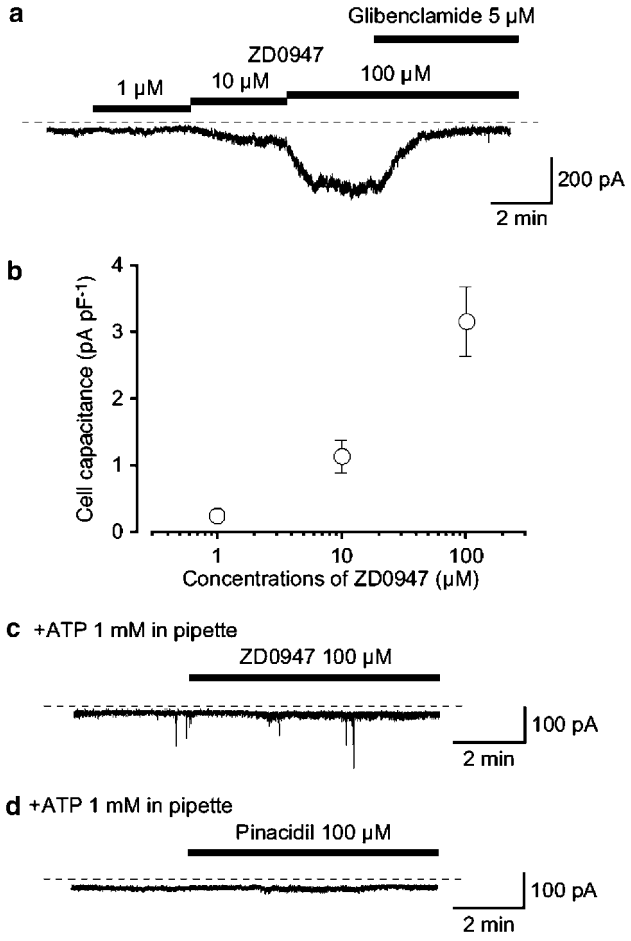

Figure 4 Concentration–response relationships of the ZD0947-induced inward current. (**a**) A cumulative application of ZD0947 caused a concentration-dependent inward current. The dashed line indicates the zero current level. (**b**) Relationship between the current density of inward currents (pA pF^{-1}) and concentrations of ZD0947 (μM). The amplitudes of the ZD0947-induced currents were measured from the current level in the presence of 5 μM glibenclamide. Each symbol indicates mean and $\pm$s.d. of four observations. (**c**, **d**) When 1 mM ATP was included in the pipette solution, neither ZD0947 (**c**) nor pinacidil (**d**) caused a significant inward current. The dashed line indicates the zero current level.

et al., 1994). They reported that neither rat nor guinea-pig is a suitable model to investigate the pharmacological and clinical potential of K_{ATP} channel openers since significant species differences for the effects of K_{ATP} channel openers are present in the urinary bladder. It is clear that human detrusor is the most suitable tissue to study the effects of K_{ATP} channel openers on the activity of K_{ATP} channels in terms of eventual use in urology (Wammack *et al*., 1994). However, for the last decade, little attention has been paid to species differences when the effects of K_{ATP} channel openers were investigated.

In the present experiments, we have demonstrated that ZD0947-induced concentration-dependent human detrusor relaxation is reversibly suppressed by glibenclamide, and that ZD0947-induced concentration-dependent K^+ current was inhibited by glibenclamide and also intracellular ATP in human detrusor myocytes. Given this, the ZD0947-induced detrusor relaxation is likely to be evoked through the activation of the glibenclamide-sensitive K_{ATP} channels in human detrusor myocytes.

Possible existence of SURs in human urinary smooth muscle

In functional expression experiments, pharmacological and electrophysiological studies have indicated that Kir6.2/SUR1 represents the pancreatic β-cell K_{ATP} channel, that Kir6.2/SUR2A is thought to represent the cardiac K_{ATP} channel, whereas Kir6.1/SUR2B represents the smooth muscle-type K_{ATP} channel (Babenko *et al*., 1998). It is generally considered that SURs are responsible not only for sulphonylurea compound-sensitivity but also for interaction with K_{ATP} channel openers (Schwanstecher *et al*., 1998).

In detrusor smooth muscle, the molecular composition of SURs assessed by reverse transcriptase–polymerase chain reaction (RT–PCR) analysis has revealed the presence of mRNA transcripts for SUR1 and SUR2B (guinea-pig, Gopalakrishnan *et al*., 1999; pig and human, Buckner *et al*., 2000). However, Gopalakrishnan *et al*. (1999) concluded that SUR2B but not SUR1 was solely expressed as a SUR protein for regulating the activity of K_{ATP} channels in guinea-pig urinary bladder, since there was much less sensitivity to diazoxide in comparison to that of pancreatic β-cells and no high-affinity binding site for [^{3}H]glyburide (Gopalakrishnan *et al*., 1999; Buckner *et al*., 2000). However, no functional study has yet been undertaken of human urinary bladder smooth muscle K_{ATP} channels to establish the possible involvement of SUR1 subunits.

Both SUR1 and SUR2B appear to share responsiveness to diazoxide, whereas SUR2A shows no response to this compound (Babenko *et al*., 1998). On the other hand, pinacidil activates K_{ATP} channels through SUR2 (SUR2A and SUR2B) but not through SUR1 (Babenko *et al*., 1998). So, in the present experiments, we utilized diazoxide as an agonist for SUR1 and pinacidil as a selective agonist for SUR2, but not for SUR1. We have demonstrated that both diazoxide and pinacidil caused a concentration-dependent relaxation in human detrusor, suggesting the presence of SUR1 and SUR2, although the potency of the pinacidil-induced relaxation was much effective than that of diazoxide. The same order of the potency was observed in other smooth muscles (rat portal vein and urinary bladder, Edwards *et al*., 1991).

At concentrations of less than 1 μM, gliclazide is generally believed to exhibit selective inhibitory effects on SUR1 but not SUR2A or SUR2B in recombinant K_{ATP} channels. Only much higher concentrations of gliclazide ($\geqslant 3\,\mu$M) directly block the channel pore region in K_{ATP} channels (Gribble and Ashcroft, 2000). In the present experiments, we have used gliclazide ($<1\,\mu$M) as an antagonist for SUR1 but not for SUR2 (SUR2A and SUR2B). The diazoxide-induced K_{ATP} currents were significantly inhibited by gliclazide ($\geqslant 1\,\mu$M) although the gliclazide (1 μM)-sensitive component was less than 20% of the diazoxide-induced K_{ATP} currents.

Further, we have directly demonstrated that not only SUR1 but also SUR2B immunoreactivity is clearly visible in human detrusor smooth muscle bundles. These results suggest that

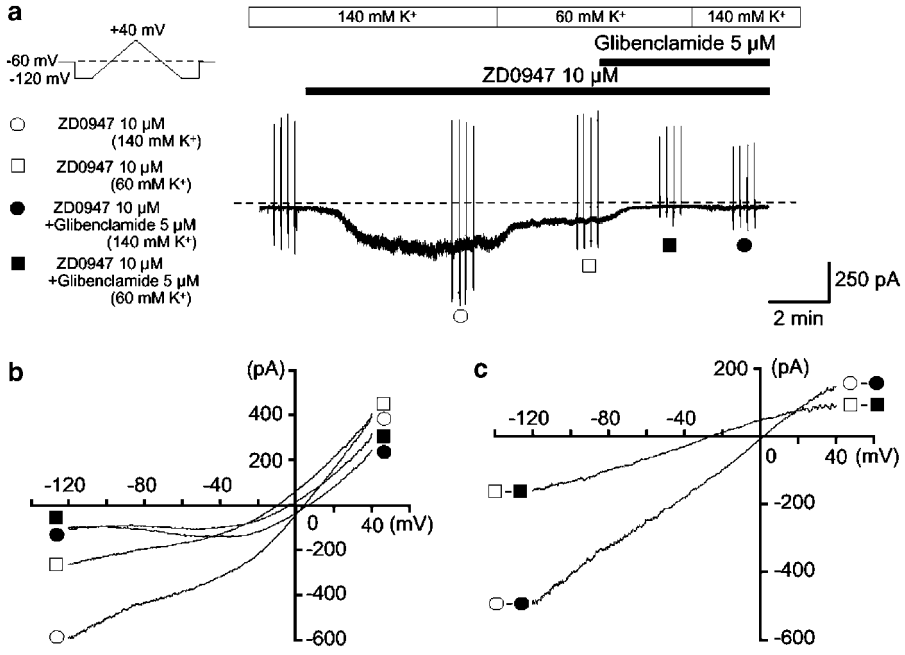

Figure 5 Measurement of the ZD0947-induced membrane current. (**a**) Ramp currents induced by the four ramp potential pulses (see inset) applied every 15 s before and during application of 10 μM ZD0947. In the presence of ZD0947, the ramp membrane currents were obtained in either the 140 mM K^+ solution or the 60 mM K^+ solution. Glibenclamide suppressed the ZD0947-induced membrane current. The vertical deflections indicate ramp currents. The dashed line indicates the zero current level. (**b**) The mean ramp membrane currents (the falling phase of the ramp pulse) on an expanded time scale in several conditions. Each symbol is the same as in (**a**). (**c**) Net membrane current was obtained by subtraction of the two ramp membrane currents (shown in (**b**)) recorded before and during application of ZD0947 in each $[K^+]_o$ condition.

SUR1 may be expressed as well as SUR2B in human urinary bladder.

In the present experiments, gliclazide inhibits the diazoxide- but not the ZD0947-induced currents at low concentrations (between 10 and 100 nM) although, at higher concentrations ($\geqslant$10 μM), this agent did suppress the ZD0947-induced currents. Moreover, in mouse pancreatic β-cells, the activity of pancreatic K_{ATP} channels, which consist of a combination of Kir6.2 and SUR1 proteins (Inagaki *et al*., 1995) was evoked by diazoxide but not by ZD0947 using the cell-attached mode (Teramoto, unpublished observation). These results suggest that ZD0947 may activate K_{ATP} channels mainly through binding to SUR2B in human detrusor myocytes. Further biological studies (such as expression studies) will cast light upon the effects of ZD0947 on the activity of K_{ATP} channels.

Pharmacological implications of K_{ATP} channels in human urinary bladder

Since the synthesis of K_{ATP} channel openers and their introduction to the urological field, their potential use for the treatment of urgency and frequency of micturition has been investigated (Andersson, 1993). Increase in membrane stability is one of the most useful targets for the treatment of OAB since involuntary detrusor activity results from increased sensitivity to depolarizing stimuli. It is generally thought that K_{ATP} channel openers will result in hyperpolarization of detrusor smooth muscle, which leads to a reduced Ca^{2+} influx through the closing of voltage-dependent Ca^{2+} influx pathways (such as voltage-dependent Ca^{2+} channels, nonselective cation channels, etc.). Modulation of K_{ATP} channels allows the contribution of native K_{ATP} channels to be finely tuned, so regulating the contractility of smooth muscle (Teramoto, 2006).

In the urological field, several K_{ATP} channel openers have been developed with the aim of reducing the frequency of micturition with no cardiovascular side effects (Howe *et al*., 1995) and inhibit urinary bladder hyperactivity without changing either blood pressure or heart rate (Wojdan *et al*., 1999). For this, it is essential that the actions of K_{ATP} channel openers on detrusor smooth muscle are more selective than those on other smooth muscles (such as vascular and urethral smooth muscles).

Abdel-Karim *et al*. (2002) reported that ZD0947 shows greater apparent selectivity for urinary bladder mediated effects than for cardiovascular mediated actions in rat. As the therapeutic utility of K_{ATP} channel openers in the treatment of OAB may be limited by hypotension as a result of insufficient selectivity *in vivo* for urinary bladder in comparison with vascular smooth muscle, it is necessary that the effects of K_{ATP} channel openers on human K_{ATP} channels are beneficial *in vivo*. Further studies will be required to investigate the selectivity of ZD0947 for the urinary bladder, in comparison to its effects on human vascular smooth muscle.

In conclusion, we have demonstrated that ZD0947 causes a detrusor relaxation through activation of

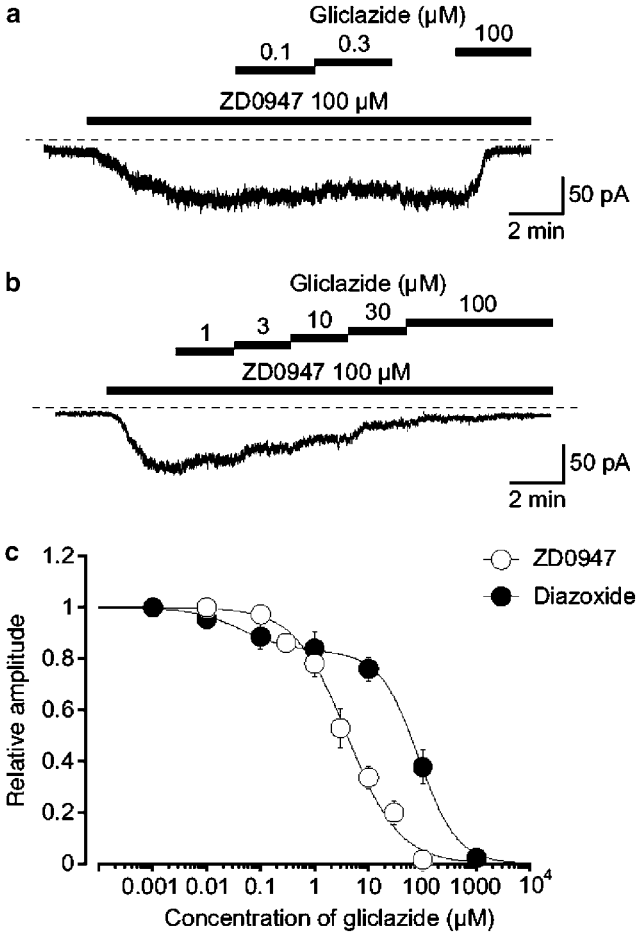

Figure 6 Inhibitory effects of gliclazide on the ZD0947- and the diazoxide-induced inward membrane currents at −60 mV in nystatin-perforated patch configuration. (**a, b**) Cumulative application of gliclazide caused a concentration-dependent inhibition of the ZD0947-induced currents. The dashed line indicates zero current. (**c**) Relationship between relative inhibition of K_{ATP} channel openers-induced current and the concentration of gliclazide. The following values were used for the curve fitting: 100 μM ZD0947, $K_i = 4.0$ μM, $h = 0.9$; 500 μM diazoxide, $K_{i1} = 42.4$ nM, $h1 = 0.8$; $K_{i2} = 84.5$ μM, $h2 = 1.2$. Each symbol indicates the mean of four observations with ±s.d. shown by vertical lines. Some of s.d. bars are less than the size of the symbols.

glibenclamide-sensitive K_{ATP} channels in human urinary bladder.

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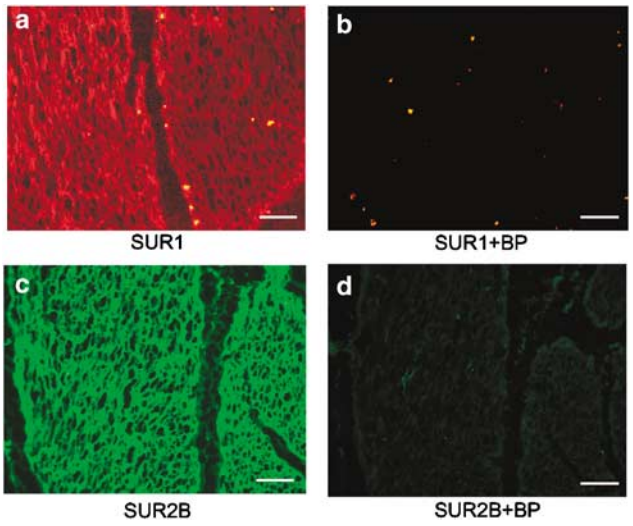

Figure 7 Fluorescent images of immunoreactivity for SUR1 and SUR2B in the human detrusor bundles. (**a, b**) SUR1 immunoreactivity and negative control (in the presence of the blocking peptide (BP)). (**c, d**) SUR2B immunoreactivity and negative control (in the presence of the BP). Note that the scale bar (white line) represents 100 μm in each picture.

Conflict of interest

The authors state no conflict of interest.

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