Levcromakalim and MgGDP Activate Small Conductance ATP-Sensitive K⁺ Channels of K⁺ Channel Pore 6.1/Sulfonylurea Receptor 2A in Pig Detrusor Smooth Muscle Cells: Uncoupling of cAMP Signal Pathways

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ABSTRACT
Pharmacological studies have suggested the existence of ATP-sensitive K⁺ (KATP) channel as a therapeutic target in urinary bladders; however, electrical properties have not yet been shown. Patch-clamp techniques were applied to investigate the properties of KATP channels in pig detrusor cells. In whole-cell configuration, levcromakalim, a KATP channel opener, induced a long-lasting outward current in a concentration-dependent manner. The current-voltage curve of the levcromakalim-induced membrane current intersected at approximately 80 mV. This current was abolished by glibenclamide. Intracellular application of 0.1 mM GDP significantly enhanced the levcromakalim-induced membrane current, whereas cAMP did not. Furthermore, neurotransmitters related to cAMP signaling, such as calcitonin gene-related peptide, vasointestinal peptide, adenosine, and somatostatin, had little effect on the membrane current. In cell-attached configuration, levcromakalim activated K⁺ channels with a unitary conductance of ~12 pS. When the patch configuration was changed to inside-out mode, the K⁺ channel activity ran down. Subsequent application of 1 mM GDP reactivated the channels. The openings of the ~12 pS K⁺ channels in the presence of 1 mM GDP was suppressed by ATP and glibenclamide. In reverse transcription-polymerase chain reaction, K⁺ channel pore 6.1 and sulfonylurea receptor (SUR)2A were predominant in pig detrusor cells. The 12 pS K⁺ channel activated by levcromakalim in pig detrusor smooth muscle cells is a KATP channel. The predominant expression of SUR2A can account for the lack of effect of neurotransmitters related to cAMP.

K⁺ Channels inhibited by intracellular ATP [ATP-sensitive K⁺ (KATP) channels] are widely distributed in cells including muscles, neurons, and endocrine cells, allowing the intracellular energy level to be reflected in the membrane potential (Noma, 1983; Cook and Hales, 1984; Spruce et al., 1985; Ashford et al.), 1988). In the smooth muscle, the existence of KATP channels has been shown in single-channel recordings and by use of pharmacological tools, for example, cromakalim and nicorandil (KATP channel openers) or glibenclamide and tolbutamide (KATP channel blockers) (Quayle et al., 1997).

The functions of the lower urinary tract, such as voiding urine and refilling, are produced by coordinated actions of urinary bladder and urethral smooth muscles. Disharmony of this co-operation is thought to be one of the causes of bladder incontinence (Mostwin, 2002; Brading, 2006). Because pharmacological studies had suggested the existence of KATP channels in the lower urinary tract smooth muscle,
possible interventions with drugs effecting $K_{ATP}$ channels have been explored.

In accordance with previous studies, it has been shown that $K_{ATP}$ channels in pig urethra are activated by cromakalim and intracellular NDPs and inhibited by glibenclamide (Teramoto et al., 1997). On the other hand, $K_{ATP}$ channel properties in pig detrusor smooth muscle cells are poorly understood. Therefore, in the present study, we performed patch-clamp experiments at both whole-cell and single-channel levels accompanied by molecular studies.

**Materials and Methods**

**Animals.** Urinary bladders of white pigs were obtained from a local abattoir. Fat and connective tissues were removed, and smooth muscle bundles were dissected under a binocular dissection microscope.

**Patch Clamp.** Muscle bundles were cut into small pieces (approximately 0.5 × 1 mm²), which were subsequently incubated in a nominally Ca²⁺-free solution for 15 min at 37°C before digestion in an enzyme-containing (0.1% collagenase; type 3; Worthington Biochemicals, Freehold, NJ) Ca²⁺-free solution for 15 min. After rinsing with enzyme- and Ca²⁺-free solution, cells were isolated by mechanical agitation using a fine-bore pipette. The cells used were stored at 4°C in physiological saline solution (PSS; containing 0.5 mM Ca²⁺). After recovery in enzyme-free PSS for 15 min at 37°C before digestion in an enzyme-containing (0.1% collagenase, type 3; Worthington Biochemicals, Freehold, NJ) Ca²⁺-free solution for 15 min. After rinsing with enzyme- and Ca²⁺-free solution, cells were isolated by mechanical agitation using a fine-bore pipette. The cells used were stored at 4°C in physiological saline solution (PSS; containing 0.5 mM Ca²⁺).

**Patch-clamp methods** were performed at room temperature (21–23°C) as described previously (Kajikawa et al., 2002). In brief, patch electrodes (3–5 MΩ) were made from borosilicate capillary (G-1.5; Narishige, Tokyo, Japan). All input voltage steps were generated by a HEKA system (ITC16 and Macintosh Iic) InstrucTech Corporation, Port Washington, NY, and Apple Computer UK Limited, Uxbridge, UK, respectively, applied to the clamped cell through an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, Germany), and all data were stored using a video tape recorder (Panasonic AG-6200; Osaka, Japan).

**Reverse-Transcription-Polymerase Chain Reaction.** The procedures are essentially the same as previously used for detection of canonical transient receptor potential channels (Asano et al., 1999; Kajikawa et al., 2005). Total RNA was isolated from pig detrusor using the RNeasy purification kit (QIAGEN, Tokyo, Japan). After treatment with RQ1 DNase (Promega, Tokyo, Japan), the total RNA was subjected to reverse-transcription (RT) reaction. Each RT (10-µl reaction volume) was performed using 1 µg of RNA, 12.5 pmoles of random hexamer, and 100 U of Moloney murine leukemia virus reverse transcriptase, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Then, the RT sample (1 µl) was used as a template for polymerase chain reaction (PCR) reaction (20 µl), whereas in the analyses of myosin heavy polypeptide (MYH7) and myosin light polypeptide (MYL6) the reaction volume was 25 µl, containing 0.5 µl of 10-fold-diluted RT sample as the template. To evaluate contribution of genomic DNA, samples without RT were used as controls. Because cDNA sequences for pig SUR2A, SUR2B, and Kir6.2 have not yet been published, the PCR primers were designed in sequences conserved between human and mouse. The sequence of the primers is indicated in Table 1.

The reaction condition was as follows: 1) in Kir6.1, after 95°C for 3 min, 35 cycles of 95°C for 35 s, 58°C for 35 s, and 72°C for 40 s; 2) in Kir6.2, after 95°C for 3 min, 35 cycles of 95°C for 35 s, 61°C for 35 s, and 72°C for 40 s; 3) in SUR2A, after 95°C for 3 min, 3 cycles of 51°C for 40 s and 72°C for 40 s, and subsequently 33 cycles of 95°C for 35 s, 56°C for 35 s, and 72°C for 40 s; 4) in SUR2B, after 95°C for 3 min, 3 cycles of 49°C for 40 s and 72°C for 40 s, and subsequently 33 cycles of 95°C for 35 s, 54°C for 35 s, and 72°C for 40 s; 5) in MYH7 and MYL6, after 95°C for 3 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The amplicons (10 µl) were run on 2.5% agarose gel and stained with ethidium bromide. SUR1 was undetectable under any PCR conditions.

**Solutions.** The ionic compositions of PSS and of high-K⁺ solution for whole-cell recordings were as follows. PSS: 138 mM NaCl, 6 mM KCl, 2.4 mM CaCl₂, 12 mM glucose, and 5 mM HEPES. High-K⁺ solution: 140 mM KCl, 1.0 mM MgCl₂, 0.3 mM EGTA, 0.1 mM ATP, and 5 mM HEPES. For single-channel recordings, the pipette and bath solutions were Ca²⁺-free PSS: 140.4 mM NaCl, 6 mM KCl, 12 mM glucose, 5 mM HEPES with levcromakalim 100 µM, and high-K⁺ solution: 140 mM KCl, 1.0 mM MgCl₂, 0.3 mM EGTA, and

### Table 1

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<th>Clones</th>
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* In particular, primers for SUR1 and Kir6.1 were designed using fragments of SUR1 and Kir6.1 cDNA in pig, respectively.
5 mM HEPES, respectively. The pH of the solutions were adjusted to 7.3 (25°C) with Tris base.

Drugs. Levocromakalim [(±)-trans-6-cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2H-1-benzopyran-3-ol] was a kind gift from Prof. Arthur Weston (The University of Manchester, Manchester, UK). Glibenclamide (5-chloro-N-[4-(cyclohexylureido)sulfonyl]phenethyl]-2-methoxy-benzamide), adenosine, vasointestinal peptide (VIP), calcitonin gene-related peptide (CGRP), somatostatin, and all nucleotides were purchased from Sigma Chemical (Poole, Dorset, UK).

Data Analysis and Statistics. The whole-cell current data low-passed at 1 kHz (−3 dB) by a 3-pole Beessel filter, sampled at 10-ms (continuous traces) or 1-ms intervals (ramp currents), and analyzed on a computer (Macintosh Iici) using the commercial software MacLab 3.5.6 (ADInstruments Pty Ltd., Castle Hill, Australia). For single-channel recording, the stored data were low-passed at 1 kHz and sampled at 1-ms intervals using the same software. Single-channel events were easily detected by eye and were inspected manually. Current traces in the figures were drawn by applying a digital low-pass filter at 400 Hz.

Comparison of means was carried out by Student’s t test; significance, where quoted, refers to p < 0.05. Numerical data are expressed as mean ± S.D. The averaged amplitude of the whole-cell current was obtained from 4 to 6 cells. The averaged frequency and amplitude of the single outward current was calculated from 4 to 6 patches.

Results

The Membrane Current Induced by (Lev)crosmakalim in Pig Detrusor. Whole-cell voltage-clamp experiments were performed in pig detrusor cells to examine the effect of levocromakalim, an established K\(_{ATP}\) channel opener. The membrane capacitance was 60 ± 9 pF (n = 12) when the pipette and bath solutions were Ca\(^{2+}\)-free PSS and high-K\(^+\) solution, respectively. As shown in Fig. 1Ba, bath applications of levocromakalim (30 and 100 µM) induced the outward currents at the holding potential of −40 mV. As long as levocromakalim was present in the bath solution, the outward current was time-independent and long-lasting, indicating that no run-down phenomena were observed. Levocromakalim (> or = 1 µM) induced a sustained outward current at the holding potential of −40 mV in a concentration-dependent manner (EC\(_{50}\), 18 µM; Fig. 1Ab). This outward current was inhibited by bath application of glibenclamide (1 µM), a selective K\(_{ATP}\) channel blocker (Fig. 1Ba). However, glibenclamide, by itself, had little effect on the membrane current in the absence of levocromakalim (n = 4; data not shown).

Instantaneous current-voltage (I-V) relationships were obtained using a ramp-pulse protocol at the times indicated in Fig. 1Ba. The I-V relationships with and without levocromakalim (100 µM) intersected at approximately −80 mV. Levocromakalim (100 µM) induced outward currents above −80 mV and inward currents below −80 mV. This current was clearly inhibited by glibenclamide (1 µM). The same result was obtained from the step-pulse protocol at the holding potential of −60 mV (data not shown). The ramp-pulse protocol is often applied to estimate the charge carrier of the current. The levocromakalim-induced currents intersected at

\[ EC_{50}: 18 \mu M \]

\[ n_H: 1.46 \]

![Fig. 1](image-url) The effect of levocromakalim and glibenclamide on the membrane current of pig detrusor. The whole-cell configuration was established at a holding potential of −40 mV. AA, outward currents were evoked by 30 and 100 µM levocromakalim. Ab, the concentration-response relationship was fitted with a Michaelis-Menten-type equation. Estimated EC\(_{50}\) and Hill’s coefficient were 18 µM and 1.46, respectively. Each point represents an averaged value of the current density (pA/pF), and error bars indicate the S.D. obtained from 4 cells. Ba, a representative trace showing the inhibitory effect of glibenclamide (1 µM) on the levocromakalim (100 µM)-induced outward current. Bb, ramp pulses (from −100 mV to +40 mV for 1 s) were applied before (black circle) and during application of 100 µM levocromakalim (black triangle) and during additional application of glibenclamide (1 µM) (black square). Bb, instantaneous I-V relationships obtained with ramp pulses for each indicated condition in Ba. The lines were averaged from three trials.
-81.7 mV. This value was nearly the same as the theoretical equilibrium potential of K⁺: -81.4 mV (Fig. 1Bb).

**Effect of Intracellular MgGDP and cAMP on the Levromakalim-Induced Outward Current.** In a wide variety of preparations, intracellular NDPs such as GDP, UDP, IDP, and ADP have been reported to augment K<sub>ATP</sub> channel activity (Kajioka et al., 1991; Beech et al., 1993; Kamouchi and Kitamura, 1994; Teramoto et al., 1997). In addition, protein kinase (PKA and/or PKC) have been reported to activate a levromakalim-sensitive K⁺ channel, presumably K<sub>ATP</sub> (Standen et al., 1998). Therefore, the effect of NDP or protein kinase activation is not only of intrinsic interest, but it can also provide good supporting evidence that the target channel of levromakalim is a K<sub>ATP</sub> channel.

Inclusion of neither GDP (0.1 mM) nor cAMP in the pipette (1 mM MgCl₂ was already contained in the pipette solution; see Material and Methods) had little effect on the membrane current just after the whole-cell configuration was performed. In addition, bath application of glibenclamide (10 μM) hardly suppressed the membrane current under the same condition (data not shown). However, the inclusion of GDP, but not that of cAMP, in the patch pipette significantly facilitated the outward current induced by bath application of levromakalim (10 μM) (Fig. 2A).

The graph in Fig. 2B summarizes the effects of GDP and cAMP on the levromakalim-induced outward current at a holding potential of -40 mV (n = 6 for each). Levromakalim (10 μM) induced outward current of 0.77 ± 0.04 pA/pF in the normal pipette solution containing ATP (0.1 mM). Addition of cAMP (0.1 mM) in the patch pipette had little effect on levromakalim-induced current (0.81 ± 0.03 pA/pF). By contrast, addition of GDP (0.1 mM) in the pipette significantly enhanced levromakalim-induced outward current (2.61 ± 0.21 pA/pF). Simultaneous application of cAMP (0.1 mM) and GDP (0.1 mM) did not further increase the amplitude of levromakalim-induced outward current (2.40 ± 0.17 pA/pF).

**The Effect of Neurotransmitters on the Membrane Current.** K<sub>ATP</sub> channels open under pathophysiological conditions, for example cardiac ischemia, but they may also open under physiological conditions. So far, neurotransmitters such as CGRP, VIP, adenosine, and somatostatin have been shown to activate K<sub>ATP</sub> channels (Nelson et al., 1990; see review in Ashcroft and Roper 1993; Dunne et al., 1989; Dart and Standen, 1993). We performed experiments in whole-cell configuration to investigate the effect of the neurotransmitters listed above on the membrane current. The pipette contained GTP (0.1 mM) and GDP (0.1 mM) in addition to 0.1 mM ATP. CGRP (100 nM) failed to evoke any outward current (Fig. 3A), whereas the subsequent application of levromakalim (10 μM) evoked a sustained outward current in 4 cells. Furthermore, adenosine, VIP, and somatostatin failed to evoke outward current in the respective 4 cells (Fig. 3, B, C, and D, respectively).

**Single-Channel Analysis in Pig Detrusor Smooth Muscle Cells.** In cell-attached or inside-out configuration, with PSS in the pipette and high-K⁺ solution in the bath, only one type of K⁺ channel was recorded, and its unitary conductance was 143 ± 2.3 pS (n = 15) (data not shown). The open probability of this single-channel current was increased by raising [Ca²⁺]<sub>i</sub> in the pipette, indicating that this single-channel current was activated by Ca²⁺.

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**Fig. 2.** Effects of GDP and/or cAMP on levromakalim-induced outward currents recorded from pig detrusor. The whole-cell configuration was established at a holding potential of -40 mV. Aa, the effect of 10 μM levromakalim on the membrane current. The pipette solution contained 140 mM KCl, 1 mM MgCl₂, and 0.3 mM EGTA with 0.1 mM ATP, and the bath solution was PSS. Ab, the inclusion of GDP (0.1 mM) in the pipette significantly enhanced levromakalim (10 μM)-induced outward current. B, a graph summarizing the effects of cAMP (0.1 mM) and/or GDP (0.1 mM) on the membrane currents. Pairs of data showing a statistically significant difference (Student’s t test, p < 0.05). Each column represents the mean value of the current density with the S.D. (n = 4–6).
is due to the opening of a Ca\(^{2+}\)-activated K\(^+\) channel, i.e., the maxi-K\(^+\) channel. Because the aim of this study is to reveal and to clarify the properties of K\(_{\text{ATP}}\) in detrusor smooth muscle cells, the patch pipette was filled with a Ca\(^{2+}\)-free solution. Under this condition, maxi-K\(^+\) channels were still open, but the open probability was considerably reduced.

The application of levcromakalim (100 \(\mu\)M) in the patch pipette evoked another single-channel current with a smaller amplitude (outward current of approximately 1 pA) (Fig. 4, A and B). After this observation, the recording condition was altered from cell-attached to inside-out mode in the same patch. The conversion of the patch-clamp configuration suppressed the opening of the small conductance channel. It is interesting to note that the subsequent application of GDP to the intracellular side of the patch reactivated these small conductance channels, whereas there was no change in the open probability and amplitude of maxi-K\(^+\) channels (Fig. 4A). Figure 4C shows the open-time histogram of smaller single-channel currents obtained at inside-out patch configuration with 1 mM GDP in the bath. The mean open time was 2790 ± 890 ms (\(n = 7\)) under the same conditions.

**Unitary Conductance of the Levcromakalim and Mg-GDP-Activated Channel in the Pig Detrusor.** Figure 5A shows single-channel currents recorded at various potentials in the inside-out configuration. For these measurements, the patch pipette was filled with a Ca\(^{2+}\)-free PSS containing levcromakalim (100 \(\mu\)M), and high-K\(^+\) solution containing GDP (1 mM) was superfused in the bath. Figure 5B summarizes the I-V relationship of the GDP-activated K\(^+\) channel. The average unitary conductance was 12.4 ± 2.6 pS (\(n = 5\)).

**The Effect of ATP on 12 pS K\(^+\) Channel.** Figure 6A shows an example of the effect of ATP on the 12 pS K\(^+\) channel in the inside-out configuration. In the presence of levcromakalim (100 \(\mu\)M) in the pipette, the application of 1 mM GDP activated the channel (Fig. 6Aa). The mean open time was 1961 ms in this membrane patch (Fig. 6Ba). Additional application of 0.1 mM ATP increased the number of short closures during the channel openings, and thereby the mean open time was markedly reduced to 170 ± 62 ms (\(n = 5\)) (Fig. 6, Ab and Bb). When the concentration of ATP was increased to 1 mM, the open time was further reduced so that it was too low to measure. The subsequent removal of ATP allowed the 12 pS K\(^+\) channel to reactivate (Fig. 6Ad), indicating the reversibility of the effect of ATP. (In the presence of 1 mM Mg\(^{2+}\), concentrations of free ATP\(^4-\) were estimated to be 23 and 410 \(\mu\)M, when 0.1 and 1 mM ATP were applied, respectively.)

**The Effect of Glibenclamide on 12 pS K\(^+\) Channel.** Figure 7 shows the effect of glibenclamide on the 12 pS K\(^+\) channel. Similar to Fig. 6Aa, GDP (1 mM) induced long opening of the 12 pS K\(^+\) channel in the inside-out patch configuration with levcromakalim (100 \(\mu\)M) in the pipette (Fig. 7Aa). The subsequent application of glibenclamide (1 \(\mu\)M) reduced the openings, decreasing the mean open time from 2028 to 380 ms in this membrane patch (Fig. 7Ab). On average, the open time decreased from 1770 ± 515 to 432 ± 261 ms in the presence of 1 \(\mu\)M glibenclamide (\(n = 5\)) (Fig. 6, Ab and Bb). It was hard to show the reversibility of the 12 pS K\(^+\) channel after application of glibenclamide; however, as shown in this recording (Fig. 7Ac), washing out with a GDP-containing solution for more than 15 min occasionally allowed recovery of the 12 pS K\(^+\) channel activity.

**RT-PCR.** K\(_{\text{ATP}}\) channels are known to consist of an inward rectifier Kir and a SUR. RT-PCR was performed to determine the subunits that form K\(_{\text{ATP}}\) channels in pig detrusor smooth muscle cells. The specific primers for Kir6.1, Kir6.2, SUR1, SUR2A, and SUR2B cDNA were designed to produce amplons of 403, 386, 174, 376, and 285 basepairs, respectively (Table 1). Figure 8 shows results suggesting that Kir6.1 and SUR2A are the predominant components, rather than Kir6.2 and SUR2B, for K\(_{\text{ATP}}\) channels in pig detrusor smooth muscle cells. SUR1 was not detectable under any PCR conditions (data not shown). To verify the muscle type, MYH7 and MYL6 (cardiac and smooth muscle markers, respectively) were also examined, and only MYL6 was detected.

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**Fig. 3.** Effects of various neurotransmitters on membrane current recorded under the whole-cell configuration of pig detrusor. A, CGRP (100 nM) was applied to the bath solution. After washing out 100 nM CGRP, the application of 10 \(\mu\)M levcromakalim caused long-lasting outward current. The additional application of 10 \(\mu\)M glibenclamide almost totally suppressed the outward current induced by levcromakalim. B, the effect of 1 \(\mu\)M VIP on the membrane current. C, the effect of 10 \(\mu\)M adenosine on the membrane current. D, the effect of 1 \(\mu\)M somatostatin on the membrane current. The holding membrane potential was −40 mV throughout. The pipette solution was a high-K\(^+\) solution with 0.1 mM ATP and 0.1 mM GDP including 0.1 mM GTP, and the bath solution was PSS.
Discussion

K\textsubscript{ATP} Channels in Lower Urinary Tract. Whole-cell recordings in the present study revealed that levocromakalim, a cromakalim enantiomer established as a K\textsubscript{ATP} channel opener, induced a long-lasting K\textsuperscript{+} current in a dose-dependent manner, and that glibenclamide suppressed it. In single-channel recordings using levocromakalim in the patch pipette, the activity of the 12 pS K\textsuperscript{+} channel was enhanced by MgGDP and suppressed by ATP and glibenclamide. Taken together, these results indicate that K\textsubscript{ATP} channels of small conductance exist in pig urinary bladder smooth muscle cells.

In cell-attached patch-clamp experiments, if levocromakalim was used in the pipette, 12 pS K\textsuperscript{+} channels were also activated. This channel activity was completely suppressed by switching the patch-clamp configuration into the inside-out mode. It is noteworthy that the subsequent application of GDP in the intracellular medium restored the channel activity. GDP alone does not activate the K\textsubscript{ATP} channels, but potentiates the action of levocromakalim (Kajioka et al., 1991; Beech et al., 1993; Teramoto et al., 1997). Therefore, the molecular components forming K\textsubscript{ATP} channels in detrusor cells seem to differ between species. Molecular identification, including regulators, may be required to clarify details of K\textsubscript{ATP} channel properties.

Pharmacological characteristics of the K\textsubscript{ATP} channels observed in pig detrusor cells are comparable with those described in pig urethra, i.e., activation by cromakalim and GDP; inhibition by glibenclamide and ATP. However, it is noteworthy that the amplitude of the levocromakalim-induced outward current was approximately three times larger in the detrusor than in the urethra (~180 pA in detrusor versus ~60 pA in urethra during application of 100 \mu M levocromakalim; Fig. 1) (Teramoto et al., 1997). In addition, the channel conductances were slightly different (12 pS in detrusor and 20 pS in urethra recorded under the same ionic conditions: 5 mM K\textsuperscript{+} in extracellular/140 mM K\textsuperscript{+} in intracellular medium). Therefore, the density of K\textsubscript{ATP} channels in

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**Fig. 4.** Single-channel currents were recorded from pig detrusor cells using the cell-attached configuration followed by inside-out patch configuration at a holding potential of 0 mV. Ca\textsuperscript{2+}-free solution containing levocromakalim (100 \mu M) was in the pipette, and high-K\textsuperscript{+} solution was in the bath. A, the image shows disappearance of 12 pS K\textsuperscript{+} channel opening after membrane excision and an ability of GDP to reactivate the channel in the inside-out membrane. B, traces at a faster speed are shown in each indicated position of A. C, the open-time histogram was drawn from a 20-min record. A histogram was fitted with a single exponential curve.
detrusor is estimated to be ~5 times greater than in the urethra.

Molecular Components. SUR, the regulatory subunit of the K<sub>ATP</sub> channels, is thought to be distributed in a cell- and tissue-specific manner, i.e., predominant expression of SUR1 in pancreatic β-cells, SUR2A in cardiac cells, and SUR2B in smooth muscle cells (Isomoto et al., 1996). Interestingly, our RT-PCR suggests that K<sub>ATP</sub> channels in pig detrusor mainly consist of Kir6.1 and SUR2A, instead of SUR2B. Furthermore, immunohistochemistry has recently shown coexpression of SUR1 and SUR2B in human detrusor, although SUR2B plays the major role (Scott et al., 2004; Aishima et al., 2006). In contrast to the generalized rule of distribution, detrusor smooth muscle cells seem to express unique SUR isoforms depending upon species.

In the vascular smooth muscle, neurotransmitters such as CGRP, adenosine, and isoproterenol have been shown to activate K<sub>ATP</sub> channels via G protein-coupled cAMP formation (for review, see Standen and Quayle, 1998). However, in the present study, the intracellular application of cAMP did not potentiate the levcromakalim-induced K<sub>ATP</sub> channel current (Fig. 2). Furthermore, the extracellular applications of CGRP, adenosine, VIP, and somatostatin, all of which require cAMP formation for channel activation, caused no outward current (Fig. 3). It is known that SUR2B possesses PKA and PKC phosphorylation sites in the C terminus, whereas PKC but not PKA modulates cardiac K<sub>ATP</sub> channels, indicating that SUR2A lacks PKA phosphorylation sites (Wang et al., 2007). Furthermore, It has been reported that the vascular isoform (Kir6.1 and SUR2B) of K<sub>ATP</sub> channels is a target of VIP (Yang et al., 2008). The unique expression of SUR2A in pig detrusor cells may account for the lack of the effect of neurotransmitters on the K<sub>ATP</sub> channel activation.

The ATP sensitivity of K<sub>ATP</sub> channels is reportedly affected by both Kir and SUR isoforms. It has been shown that in the presence of Mg<sup>2+</sup>, only high concentrations (>10 mM) of ATP inhibited the K<sub>ATP</sub> channel of SUR2B/Kir6.1 in human embryonic kidney cells, whereas the K<sub>ATP</sub> channel of SUR2B/Kir6.2 has much higher ATP sensitivity with the IC<sub>50</sub> of 70 μM (Quast et al., 2004; Isomoto et al., 1996; Yamada et al., 1997; Satoh et al., 1998). On the other hand, rather low concentrations of ATP (~100 μM) suppress the activity of SUR1/Kir6.1 K<sub>ATP</sub> channels expressed in COSm6 cells (Babenko and Bryan, 2002). Our present study revealed that 100 μM ATP (free ATP<sup>−</sup> = 23 μM in the presence of 1 mM Mg<sup>2+</sup>) markedly reduced the mean open time of SUR2A/Kir6.1 K<sub>ATP</sub> channels in pig detrusor cells. This result agrees well with previously reported IC<sub>50</sub> values (20–100 μM) for K<sub>ATP</sub> channels of SUR2A/Kir6.2 (Ashcroft and Ashcroft, 1990; Babenko et al., 1999). Therefore, the high ATP sensitivity of K<sub>ATP</sub> channels in detrusor cells is attributable to SUR2A.

Role of K<sub>ATP</sub> Channels and Therapeutic Potential. In smooth muscle, the resting membrane potential that is mainly determined by the K<sup>+</sup> channel activity plays an im-

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**Fig. 5.** Relationships between the holding potential and the amplitude of the single-channel current of levcromakalim- and GDP-activated channels. The single-channel currents were recorded from the inside-out patch membranes with the following condition. The pipette solution was Ca<sup>2+</sup>-free PSS with levcromakalim (100 μM), and the bath solution was high-K<sup>+</sup> solution with GDP (1 mM). A, a representative trace at each holding potential (from −20 to +30 mV with every 10 mV increment) obtained from the same patch. The amplitude of the maxi-K<sup>+</sup> current recorded was not full size. B, I-V relationships of the levcromakalim- and GDP-activated channels. Symbols indicate the mean value of levcromakalim- and GDP-activated channels, with a S.D. of six observations. The line was drawn by the least-squares method.
A major role in regulating the contractility through the control of voltage-dependent Ca$^{2+}$ channel activity (for review, see Kuriyama et al., 1998). However, KATP channels are unlikely to make a major contribution to the resting membrane potential in the pig detrusor cells, because KATP channel openings were observed only when the patch pipette contained levcromakalim in the present study (also see review in Fry et al., 1998). Furthermore, KATP channels in pig detrusor cells are not subject to the neural and hormonal controls, unlike in pancreatic β-cells and some vascular smooth muscle cells such as rabbit mesenteric artery, in which KATP channels are activated by adenosine and several other neuropeptides (Dunne et al., 1989; Nelson et al., 1990; Dart and Standen, 1993; Kleppisch and Nelson, 1995). It is presumed that K$^{+}_{ATP}$ channels play a major role under pathophysiological conditions such as ischemia, which reduces intracellular ATP and consequently increase NDPs, including ADP and GDP.

K$^{+}_{ATP}$ channel openers were first used as antihypertensive agents. Such chemicals may also have potential in the treatment of other disorders (Fujii et al., 1990; Edward et al., 1991), for example, to stabilize overactive bladder. The sustained activation of this channel current seen in the whole-cell recordings implies a long-lasting therapeutic effect of K$^{+}_{ATP}$ channel openers on urinary bladder instability. Ideally, the effort should be put into developing bladder-sensitive K$^{+}_{ATP}$ channel openers because micturition is coordinated by simultaneous contraction of detrusor and relaxation of urethral smooth muscle, both of which express K$^{+}_{ATP}$ channels of similar characteristics. However, because the density of K$^{+}_{ATP}$ channels is significantly larger in detrusor cells than in the urethra, it is highly probable that detrusor-specific K$^{+}_{ATP}$ channel openers could be developed depending on the difference in the channel density. In addition, as described earlier, detrusor cells seem to express K$^{+}_{ATP}$ channels containing unique SUR isoforms (SUR1 and 2B in human and SUR2A in pig). It is probable that the ratio of Kir6.X and SUR isoforms affects the response to K$^{+}_{ATP}$ channel openers. Furthermore, pathophysiological conditions may alter these ratios as well as the channel density. Investigation into the molecular components of K$^{+}_{ATP}$ channels may help the development of K$^{+}_{ATP}$ channel openers for tissue-specific and disease-specific treatments. Substantially, a novel K$^{+}_{ATP}$ channel opener like A-251179, which is highly selective to Kir6.2/SUR2B, has potential for the treatment of overactive bladder (Shieh et al., 2007).

In conclusion, this is the first report addressing single-channel activity of K$^{+}_{ATP}$ channels in pig detrusor smooth muscle cells. They express K$^{+}_{ATP}$ channels with unitary conductance of ~12 pS. These K$^{+}_{ATP}$ channels are activated by

**Fig. 6.** Effect of ATP on 12 pS K$^{+}$ channel. Ca$^{2+}$-free PSS containing levcromakalim (100 μM) and high-K$^{+}$ solution containing GDP (1 mM) were in the pipette and bath, respectively. The inside-out patch configuration was established at a holding potential of 0 mV. Aa, long opening of the 12 pS K$^{+}$ channel was observed in the presence of levcromakalim (100 μM) in the pipette and GDP (1 mM) in the bath solution. In the same patch, 0.1 mM ATP (Ab) and 1 mM ATP (Ac) were applied to the bath solution, and subsequently ATP was removed from the bath solution (Ad). B, open-time histograms obtained with (Bb) or without (Ba) 0.1 mM ATP in the presence of 1 mM GDP. All histograms were fitted with a single exponential curve with the time constant noted in each histogram. The open-time histogram was drawn from the records for 10 min (0 mM ATP) or 2 min (0.1 mM ATP).
levcromakalim and GDP, and they are suppressed by glibenclamide and ATP, but neurotransmitters had little excitatory effect on them. These characteristics are consistent with those in pig urethra. However, the density of K<sub>ATP</sub> channels seems to be significantly higher in pig bladder. This difference may help the development of K<sub>ATP</sub> channel openers for treatment of bladder instability, which requires suppression of bladder contraction and maintenance of urethral tone.

RT-PCR examinations suggested predominant expression of Kir6.1/SUR2A. The unique SUR isoform may account for the lack of effect of neurotransmitters on K<sub>ATP</sub> channel activity in pig detrusor cells.

Fig. 7. Effect of glibenclamide on the 12 pS K<sup>+</sup> channel. The inside-out patch configuration was established at a holding potential of 0 mV. Long opening of this channel was observed with levcromakalim (100 µM) in the pipette and GDP (1 mM) in the bath solution (Aa). The addition and subsequent removal of glibenclamide (1 µM), respectively, reduced (Ab) and restored the 12 pS K<sup>+</sup> channel opening (Ac). Open-time histograms obtained in the absence (Ba) or the presence (Bb) of glibenclamide (1 µM) with levcromakalim (100 µM) and GDP (1 mM). Histograms were drawn from the records for 10 min (control) or 5 min (1 µM glibenclamide), and they were fitted with a single exponential term.

Fig. 8. RT-PCR detection of Kir6.X and SUR2X in pig detrusor smooth muscle cells. MYH7 and MYL6, cardiac, and smooth muscle markers, respectively, were also examined.

References
Babenko AP and Bryan J (2002) SUR-dependent modulation of K<sub>ATP</sub> channels by an...


