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Ca^{2+} channel properties in smooth muscle cells of the urinary bladder from pig and human

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Abstract

Ca^{2+} channel properties of pig and human bladder smooth muscle were investigated utilizing standard whole-cell patch clamp techniques. Both the amplitude obtained and the current density of Ca^{2+} channel current evoked by step depolarization were larger in human than in pig myocytes. The inward currents were sensitive to an L-type Ca^{2+} channel antagonist, nifedipine, the effects of which were not significantly different between species. In both species, prior application of ATP (0.1 mM) had no effect on activation of this voltage-sensitive channel current, while a muscarinic receptor agonist, carbachol (0.1 mM), significantly attenuated the amplitude of this current. Furthermore, inclusion of GDP-β-S or Heparin in the pipette abolished or had no effect on the suppression of Ca^{2+} current by carbachol, respectively. These results forward the pig as a good model for the human in detrusor Ca^{2+} channel properties, especially with regard to neural modulation, although voltage-sensitive Ca^{2+} channels seem to make greater contribution in human bladder physiology.

Keywords: Ca^{2+} channel, voltage-sensitive; Detrusor smooth muscle; G-protein; Muscarinic receptor; Purinergic receptor; Dihydropyridine

1. Introduction

As in other types of smooth muscle, there is no doubt that an increase in the intracellular Ca^{2+} concentration is a key process required for the activation of contraction in bladder smooth muscle. However, debate remains as to whether this increase is due to influx from the extracellular space and/or release from intracellular stores and, as to the importance of each mechanism in different species and with respect to the transmitter studied. In smaller mammals, it is well known that neuronal stimulation releases both acetylcholine and ATP (Ambache and Zar, 1970; Burnstock et al., 1972; Kasakov and Burnstock, 1983; Moss and Burnstock, 1985). However, in normal human detrusor, neurally mediated contractions are thought to occur through the actions of acetylcholine (Tagliani et al., 1997), although some studies have suggested a minor purinergic component (e.g. Luheshi and Zar, 1990). Studies of calcium utilisation in the guinea-pig have highlighted the importance of extracellular Ca^{2+} in response to ATP (Iacovou et al., 1990; Kura et al., 1992a; Nakayama, 1993; Hashitani and Suzuki, 1995) while acetylcholine has been shown to cause inositol trisphosphate (IP_3) induced release from intracellular stores (Noronha-Blob et al., 1989; Iacovou et al., 1990), although other investigators have also suggested a role for extracellular Ca^{2+} in the response to acetylcholine (Hashitani et al., 2000). In the rabbit, ATP and acetylcholine have been proposed to cause either/both extracellular Ca^{2+} influx and release from stores (Batra et al., 1987; Fovaeus et al., 1987; Kishi et al., 1992). Similar results have been found in rat detrusor (Munro and Wendt, 1994; Yu et al., 1996; Mimata et al., 1997). In human bladder smooth muscle, the contribution of intracellular store release and extracellular influx to cholinergic responses often differs between studies (Harriss et al., 1995; King et al., 1998; Masters et al., 1999; Wu et al., 1999; Visser and Van Mastrigt, 2000). As such several studies have demonstrated an importance of extracellular Ca^{2+} entry in the contraction induced by muscarinic and/or purinergic stimulation, the most common entry pathway of which is through dihydropyridine-sensitive Ca^{2+} channels.

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Considering that in the lower urinary tract, muscarinic receptor activation plays a major physiological role, as do purinergic mechanisms (for review see Brading, 1992, it is important to clarify not only the general properties of Ca^{2+} channels within detrusor smooth muscle but also the potential modulation mediated by muscarinic or purinerigic agonists. Ca^{2+} channels in other smooth muscles have been reported to be modulated by many neurotransmitters, including muscarinic and purinerigic stimulation (Beech, 1993). Additionally, the pig lower urinary tract is heavily utilised as, and thought to be a good model of, the human. Humans and pigs share similar anatomical features of the detrusor system and similar neuronal control in voiding. However, physiological and/or pharmacological comparison between these species at the microscopic level is still unknown. There are a few reports concerning ion channels of human urinary bladder but none investigating porcine detrusor (Montgomery and Fry, 1992; Gallegos and Fry, 1994; Sui et al., 2001a,b).

The purpose of the present study was to reveal Ca^{2+} channel properties in pig urinary bladder in direct comparison with those of the human and, secondly to investigate whether purinergic and muscarinic stimulation modulates Ca^{2+} channel activity in both tissues and to clarify the underlying mechanism.

2. Methods

2.1. Preparation of cells

Porcine urinary bladders were obtained from a local abattoir and human tissue obtained from cadaveric organ donors with ethical consent. Tissue was transported to the laboratory in cold (4 °C) physiological saline solution and, subsequently, after removing the urothelium, bladder smooth muscle samples were dissected into small pieces (about 1–2 mm cube) with the aid of a dissecting microscope. Samples were then incubated in a nominally Ca^{2+}-free solution for 20 min (at 36 °C). Subsequently, the pieces were pretreated with 0.05% papain for 8 min, then incubated with 0.15% collagenase and 0.05% trypsin inhibitor for 15 min in a Ca^{2+}-free, Mg^{2+}-free solution containing 0.1% bovine serum albumin (BSA). Finally, after washing with Ca^{2+}-free solution, the digested pieces were placed in a test tube and single cells were yielded through gentle agitation. Single cells were kept in a low Ca^{2+} (0.5 mM) solution with 0.5% BSA and stored at 5 °C. All experiments were performed within 3 h of di-gestion.

2.2. Membrane current recording

Whole-cell membrane currents were recorded using a standard patch clamp technique, as described previously (Smith et al., 1999). A patch clamp amplifier (EPC-7, List, Germany) was operated through a Macintosh computer equipped with an AD/DA converter (ITC-16, INSTRUTECH., USA). The resistance of the patch pipette was 3–4 MΩ, when a Cs^{+}-rich pipette solution was used. After rupture of the cell membrane, the series resistance was normally less than 10 MΩ. Series resistance compensation was not applied, however, since the largest inward current recorded was ~300 pA, the clamp potential in the cell membrane would have been altered by at most 3 mV. Pig smooth muscle cells used in this investigation had a mean membrane capacitance of 62 ± 12 pF ($n = 25$), while that in human was 32 ± 5 pF ($n = 14$). The capacitive surge was electrically compensated and a cut-off frequency of 10 kHz was applied to reduce noise. Unless otherwise described, the cell membrane was clamped at −60 mV (the holding potential). All experiments were carried out at room temperature (15–22 °C).

2.3. Solutions and chemicals

The 'normal' bathing solution had the following composition (mM): NaCl, 140; KCl, 6; $CaCl_2$, 2.4; glucose, 12 and HEPES, 5; pH was adjusted to 7.4 with Tris base. The ionic composition of the bathing solution was modified iso-osmotically. For example, when voltage-sensitive Ca^{2+} channel current was recorded, instead of 2.4 mM $CaCl_2$, 10 mM $BaCl_2$ was added to the bathing solution, and the composition of NaCl was reduced isosmotically. In order to block K^{+} currents, we used a Cs^{+}-rich pipette solution having the following composition (mM): CsCl, 144; $MgCl_2$, 2; EGTA (ethyleneglycol-bis-(fl-aminoethylether) *N*,*N*,*N*′,*N*′-tetraacetic acid), 0.05; ATP, 2; HEPES/Tris, 10 (pH 7.2). In some experiments, 1 mM GDP-β-S (guanosine 5′-*O*-(2-thiodiphosphate)) or 5 mg/ml heparin was added to the pipette solution, in order to suppress G-protein actions or antagonise IP_3 receptors, respectively.

The following chemicals, drugs and enzymes were used in the present study: ATP (disodium salt), nifedipine, GDP-β-S (trilithium salt), collagenase (type H), papain, Trypsin inhibitors, carbamyl choline chloride (carbachol) heparin, and bovine serum albumin from Sigma (St. Louis, USA).

2.4. Statistics and analyses

Numerical data are expressed as mean ± standard deviation. When differences between means were evaluated by paired *t*-tests, a *P*-value of less than 0.05 was taken as a statistically significant difference.

Activation parameters were obtained by the same procedures described in Nakayama and Brading (1995). Step pulses of various potentials (from −100 to +50 mV) were applied to isolated detrusor cells. The peak amplitude of the Ca^{2+} channel current (I_{peak}) measured was plotted against the clamp potential (V) of the amplifier. The resultant

current–voltage (I–V) relationship (the data from -50 to $+50$ mV) was fitted to the following two equations:

$$I_{peak}(V) = d_{\infty}(V)G_{max}(V - V_{rev}), \quad (1a)$$

$$d_{\infty}(V) = 1/[1 + \exp\{(V_{50a} - V)/S_a\}], \quad (1b)$$

where G_{max} and V_{rev} are the maximum conductance and reversal potential of the Ca^{2+} channel current. $d_{\infty}(V)$ indicates voltage dependence of the degree of activation based on the Boltzmann distribution, where V_{50a} is the half-maximal activation potential, and S_a is the slope factor for activation.

Inactivation curve was obtained by applying conditioning steps of various potentials (from -100 to $+100$ mV, 1 s) before a test step (0 mV, 100–200 ms). The degree of inactivation was estimated as $I_{peak}(V)/I_{peakmax}$ (the peak amplitude of the test pulse current evoked after a conditioning step of -100 mV), and plotted against the conditioning potential. The inactivation curve obtained was clearly U-shaped: The degree of inactivation consistently increased from $+20$ mV. The data points from -100 to 0 mV was thus fitted to a Boltzmann's equation (Nakayama and Brading, 1993b):

$$f_{\infty}(V) = 1/[1 + \exp\{(V_{50i} - V)/S_i\}], \quad (2)$$

where V_{50i} is the half-inactivation potential and S_i is the slope factor for inactivation. A modified simplex program was used

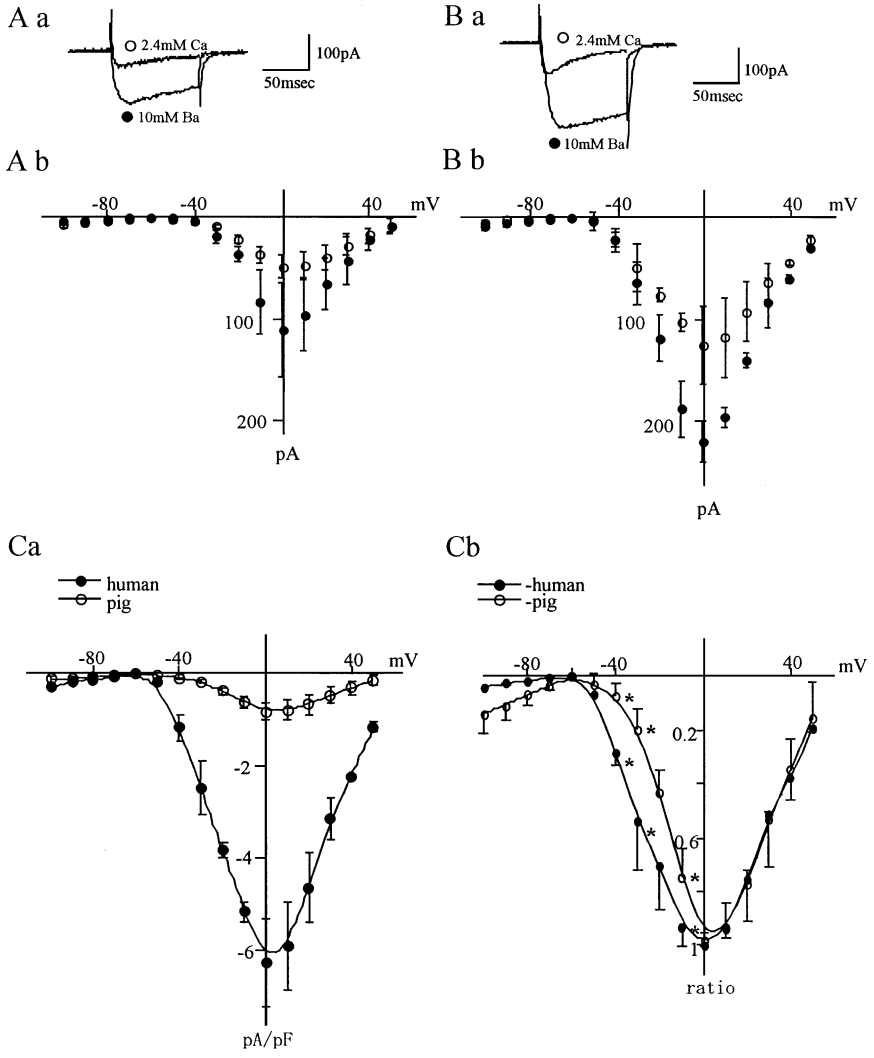

Fig. 1. Features of depolarisation induced inward current and its current–voltage relationships in pig and human detrusor cells. Inward currents were evoked by step depolarization of various potentials (from -100 to $+50$ mV). Representative traces of inward currents evoked by step depolarization to 0 mV (100 ms) are shown in Aa and Ab. The inward currents were obtained from pig (Aa) and human (Ba), respectively. Irrespective of species, the amplitude of the inward current was significantly increased by replacing the charge carrier from 2.4 mM Ca^{2+} (○) to 10 mM Ba^{2+} (●). In Ab (pig) and Bb (human), the peak amplitude (mean ± standard deviation: S.D.) of evoked inward current was plotted against the potential of step depolarization. Open circles (○) and closed circles (●) represent data obtained in the presence of 2.4 mM Ca^{2+} and 10 mM Ba^{2+}, respectively. Each point was obtained from 4 to 6 cells. In Ca, the current density–voltage relationship for the peak I_{Ca} of pig (○) and human (●) is shown. In Cb, the peak amplitude of the inward current (density) was normalized with that upon depolarization to 0 mV. The asterisks indicate significant differences evaluated with Student's t-test ($P < 0.05$).

Table 1
Current density and inactivation time constant of Ca^{2+} channel current at 0 mV in pig (A) and human (B)

	2.4 mM Ca^{2+}	10 mM Ba^{2+}
(A) Pig (n = 6–8)		
Current density (pA/pF)	0.80 ± 0.18	2.2 ± 0.28
Inactivation time constant (ms)	83.9 ± 37.0	173.1 ± 33.9
(B) Human (n = 4–6)		
Current density (pA/pF)	6.3 ± 1.0	8.3 ± 1.1
Inactivation time constant (ms)	39.6 ± 10.5	118.6 ± 17.1

Current density was expressed as the ratio of the peak amplitude (pA) to the membrane capacitance (pF) of the cell used. The inactivation time constant (ms) was obtained by fitting the inward current at 0 mV with a single exponential term.

in the computer fittings for both activation and inactivation parameters.

3. Results

3.1. Voltage-sensitive Ca^{2+} channel currents in pig and human detrusor cells

Detrusor cell inward currents were studied with a Cs^{+} filled pipette using conventional whole cell configuration. A holding potential of −60 mV was normally applied. The membrane capacitances of pig and human detrusor smooth muscle cells were 62 ± 12 pF (n = 25) and 32 ± 5 pF (n = 14), respectively.

Rectangular pulses of various potential (from −100 to +50 mV, in 10 mV steps) were applied to assess current–voltage (I–V) relationship. Typical current traces obtained from pig and human detrusor smooth muscle cells (at 0 mV) are shown in Fig. 1Aa and Ba, respectively. As shown in these current traces, in both spices the voltage-sensitive Ca^{2+} channel current was significantly increased by replacing the charge carrier from 2.4 mM Ca^{2+} (○) to 10 mM Ba^{2+} (●), and the decay of the inward current during depolarization was slower in the presence of Ba^{2+}. The current density and decay time constant are summarized in Table 1.

In Fig. 1Ab (pig) and Bb (human), the peak amplitude of the evoked inward current was plotted against the potential of the rectangular pulse applied. Despite of smaller membrane capacitance, the average amplitude of inward Ca^{2+} current (I_{Ca}: 2.4 mM Ca^{2+} in the bath) at each potential was larger in human (○) than that in pig (●). This tendency was unchanged even when Ba^{2+} was used as a charge carrier (I_{Ba}): 10 mM Ba^{2+} in the bath Fig. 1Ca shows I–V relationships of Ca^{2+} currents normalized with the mem-

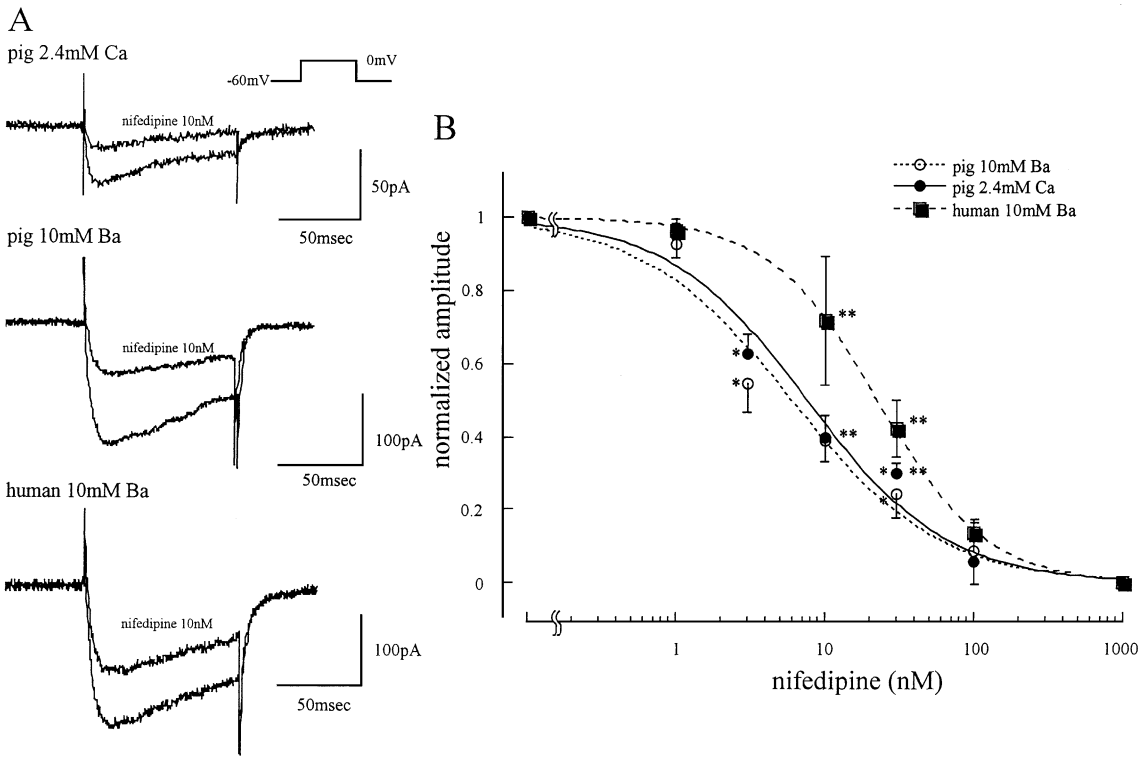

Fig. 2. Effect of nifedipine on ICa^{2+} channel current in pig and human detrusor cells. (A) Superimposed current traces upon depolarization to 0 mV before and after application of 10 nM nifedipine with 2.4 mM Ca^{2+} or 10 mM Ba^{2+} as a charge carrier. (B) The dose–response relationship for nifedipine inhibition of ICa^{2+} channel current. The peak amplitude of the inward current was normalized by taking that before application of nifedipine as 1.0. The dose–response relationship was fitted with a Michaelis–Menten type equation. Each point represents an averaged value and error bars indicate standard deviation obtained from four cells. The asterisks and double asterisks indicate significant difference by Student's t-test ($P < 0.05$) between the amplitude of I_{Ca} and I_{Ba} in pigs or I_{Ba} between pig and human, at each concentration, respectively.

brane capacitance of each cell. This graph clearly indicates that human detrusor cells have not only obviously larger current but also higher Ca^{2+} channel density. In Fig. 1Cb, the $I-V$ relationship shown in Fig. 1Ca was further analysed by normalizing the peak amplitude of the inward current with that upon depolarization to 0 mV. At negative voltage steps, especially at -40, -30 and -10 mV, significant differences between species was observed: The half-maximal activation potential (V_{50a}) was -10.7 mV in pig, while -16.2 mV in human.

In pig detrusor cells, conditioning pulses of 1 s duration were applied to obtain a steady-state inactivation curve in the presence of 10 mM Ba^{2+} (Nakayama and Brading, 1995; Nakayama et al., 2000). The voltage of the conditioning step was changed from -100 to $+100$ mV with 20 mV increment. Above $+20$ mV, the degree of inactivation almost linearly and consistently increased (to $\sim 80\%$ at $+100$ mV) presumably due to conversion of Ca^{2+} channel conformation to the second open state (Nakayama and Brading, 1993a,b). The data points from -100 to 0 mV in the inactivation curve were thus fitted to a Boltzmann equation. The half-maximal inactivation potential (V_{50i}) was -23.2 mV.

3.2. Effect of nifedipine on ICa^{2+} channel current

In order to compare the blocking action of a Ca^{2+} channel antagonist against I_{Ca} and I_{Ba} from pig detrusor cells with that from human, we examined the effect of the dihydropyridine, nifedipine on inward currents in the presence of 2.4 mM extracellular Ca^{2+} or 10 mM extracellular Ba^{2+} for pig detrusor cells and in 10 mM extracellular Ba^{2+} for human detrusor cells. Nifedipine (10 nM) caused dramatic decreases in I_{Ca} and I_{Ba} evoked by step depolarizations of various potentials (between -50 and $+50$ mV). The current–voltage relationships after application of 10 nM nifedipine were, however, not shifted from the control, indicating a lack of voltage-dependency (data not shown). Therefore, we examined the effect of nifedipine on the maximum amplitude of I_{Ca} and I_{Ba} induced by a step depolarization to 0 mV. Fig. 2A shows the representative pig and human currents before and after application of 10 nM nifedipine under the aforementioned conditions. Fig. 2B shows concentration–inhibition curves for maximum inward currents obtained during standard voltage steps to 0 mV from 6 to 8 cells. The ID_{50} values of nifedipine were 5.94 and 7.55 nM for pig cells in 10 mM Ba^{2+} and 2.4 mM Ca^{2+} bathing solution, respectively, and 22.4 nM for human cells in 10 mM Ba^{2+} solution.

3.3. Effect of ATP and carbachol on I_{Ca}

The effect of ATP on voltage-sensitive Ca^{2+} channel current in pig detrusor cells was studied in 2.4 mM extracellular Ca^{2+} solution. Just after application of 100 μM ATP, a transient inward current, which was estimated

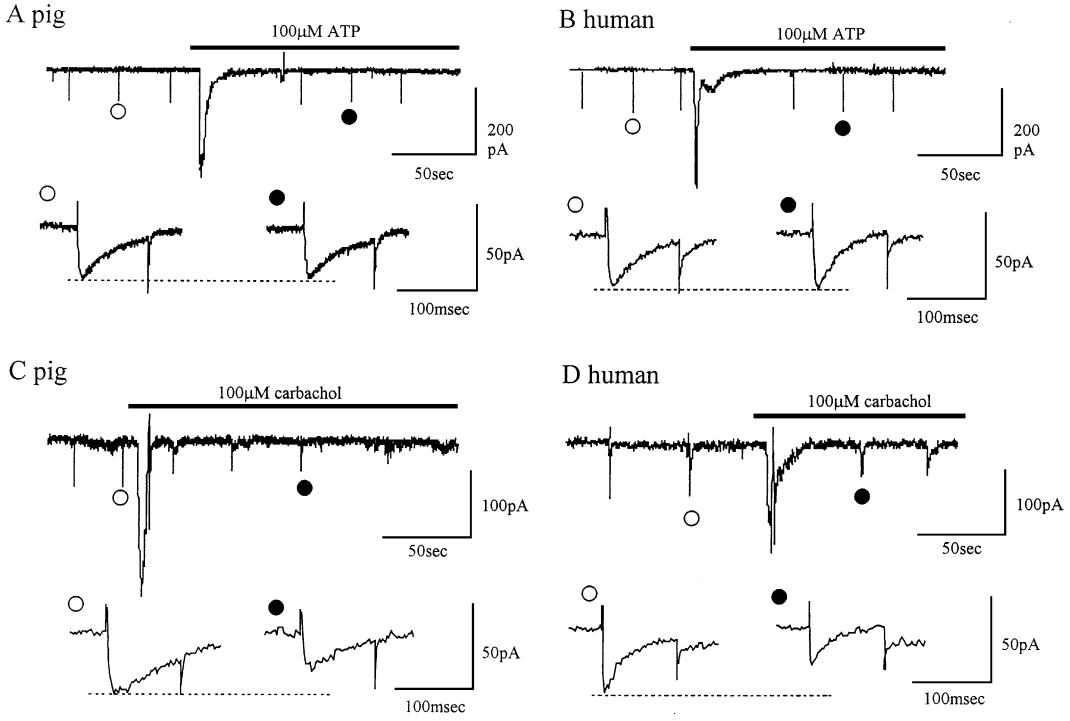

Fig. 3. Effects of ATP (A and B) and carbachol (C and D) on membrane current (at -60 mV, holding potential) and Ca^{2+} channel current evoked by depolarization to 0 mV (100 ms). Applications of 100 μM ATP (A and B) or 100 μM carbachol (C and D) induced transient inward currents. Pig detrusor cells were used in A and C, while human cells were used in B and D. Representative Ca^{2+} channel currents before (○) and after drug application (●) are shown expanded below each current trace recorded continuously.

to be induced by the activation of a non-selective cation channel, was observed, as previously reported by Inoue and Brading (1991) and Nakayama (1993). However, there was no difference in the amplitude (0.97 against control: $n=7$) or decay of the inward current recorded upon depolarization to 0 mV before and after application of ATP (Fig. 3A). In contrast, 100 μM carbachol induced a transient inward current presumably through activation of Ca^{2+}-activated Cl^- channels (unpublished observation), while the amplitude of the depolarization-induced current was decreased by 0.56 ± 0.11 against control current in the absence of carbachol ($n=6$) (Fig. 3C). Essentially the same results were obtained from human detrusor cells upon applications of ATP (1.04 ± 0.06: $n=4$) and carbachol (0.51 ± 0.10: $n=4$) (Fig. 3B and D).

3.4. Inhibitory action of carbachol I_{Ba} of human detrusor cells

Fig. 4A illustrates the current–voltage relationships of I_{Ba} obtained from human detrusor cells with or without 100 μM carbachol. When each value shown in Fig. 4A was normalized to the peak amplitude at 0 mV, the current–voltage relationships were nearly identical to each other (graphs not shown). We thus judged that the inhibitory action of carbachol on voltage-sensitive Ca^{2+} channels is voltage-independent. Presumably, muscarinic receptor agonists decrease number of available channels.

When the pipette solution contained 1 mM GDP-β-S, which would completely inhibit the dissociation of α and βγ complexes of GTP-binding proteins, an application of carbachol little changed the amplitude of I_{Ba}, as indi-

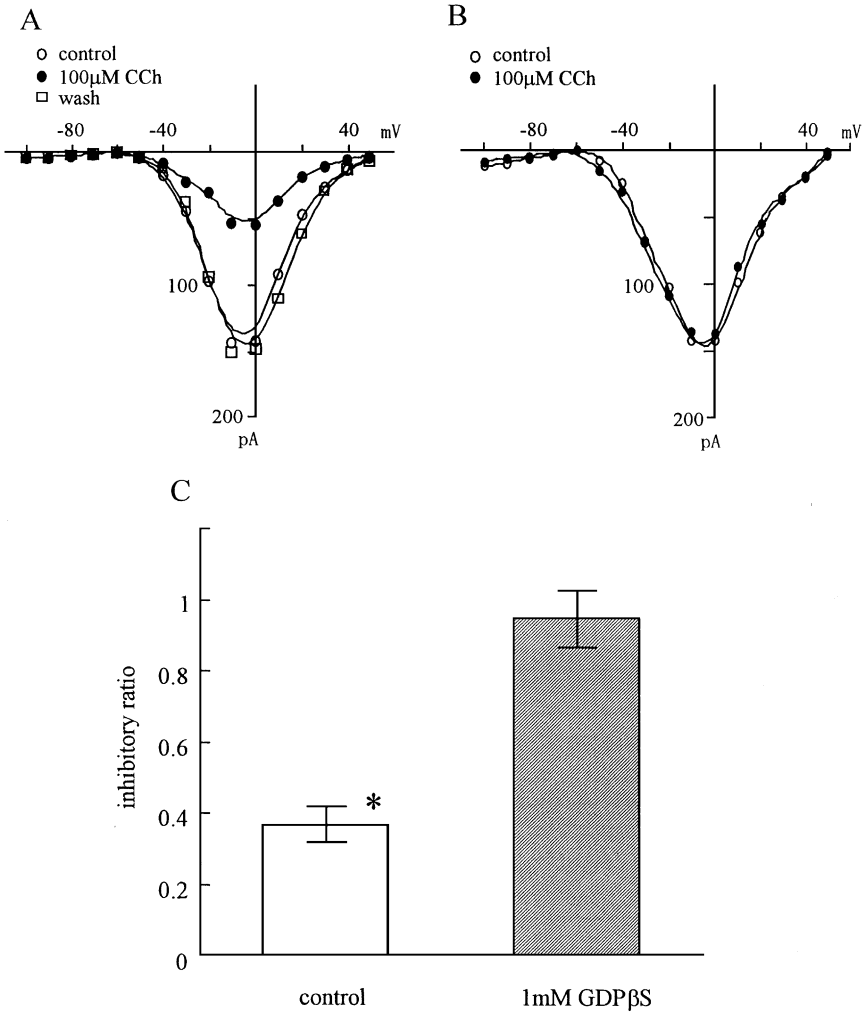

Fig. 4. Effect of carbachol on depolarization-induced inward current in human detrusor cells. In the presence of 10 mM Ba^{2+} as a charge carrier, Ca^{2+} channel (Ba^{2+}) currents (I_{Ba}) were evoked by rectangular pulses of −100 to +50 mV (in 10 mV steps). In A, the current–voltage relationships of I_{Ba} were obtained before (○) and after (●) an application of 100 μM carbachol, and after removal of carbachol (□). The current–voltage relationships of I_{Ba} in B were obtained before (○) and after (●) an application of 100 μM carbachol with 1 mM GDP-β-S in the patch pipette. The averages of carbachol-induced reduction of I_{Ba} with or without 1 mM GDP-β-S in the patch pipette are shown in C. The current amplitude was expressed relative to that obtained before application of carbachol. The asterisk indicates a significant difference evaluated with Student's *t*-test ($P<0.05$).

cated by the current–voltage relationships in Fig. 4B. Fig. 4C summarizes the effect of GDP-β-S on the inhibitory action of 100 μM carbachol on I_{Ba}. The amplitude of each I_{Ba} was normalized to that induced by the step depolarization to 0 mV before application of 100 μM carbachol. Applications of carbachol (100 μM) reduced I_{Ba} to 0.37 ± 0.05 ($n = 5$) relative to control responses, while in the presence of 1 mM GDP-β-S in the pipette, carbachol failed to inhibit I_{Ba} (0.95 ± 0.08, $n = 4$).

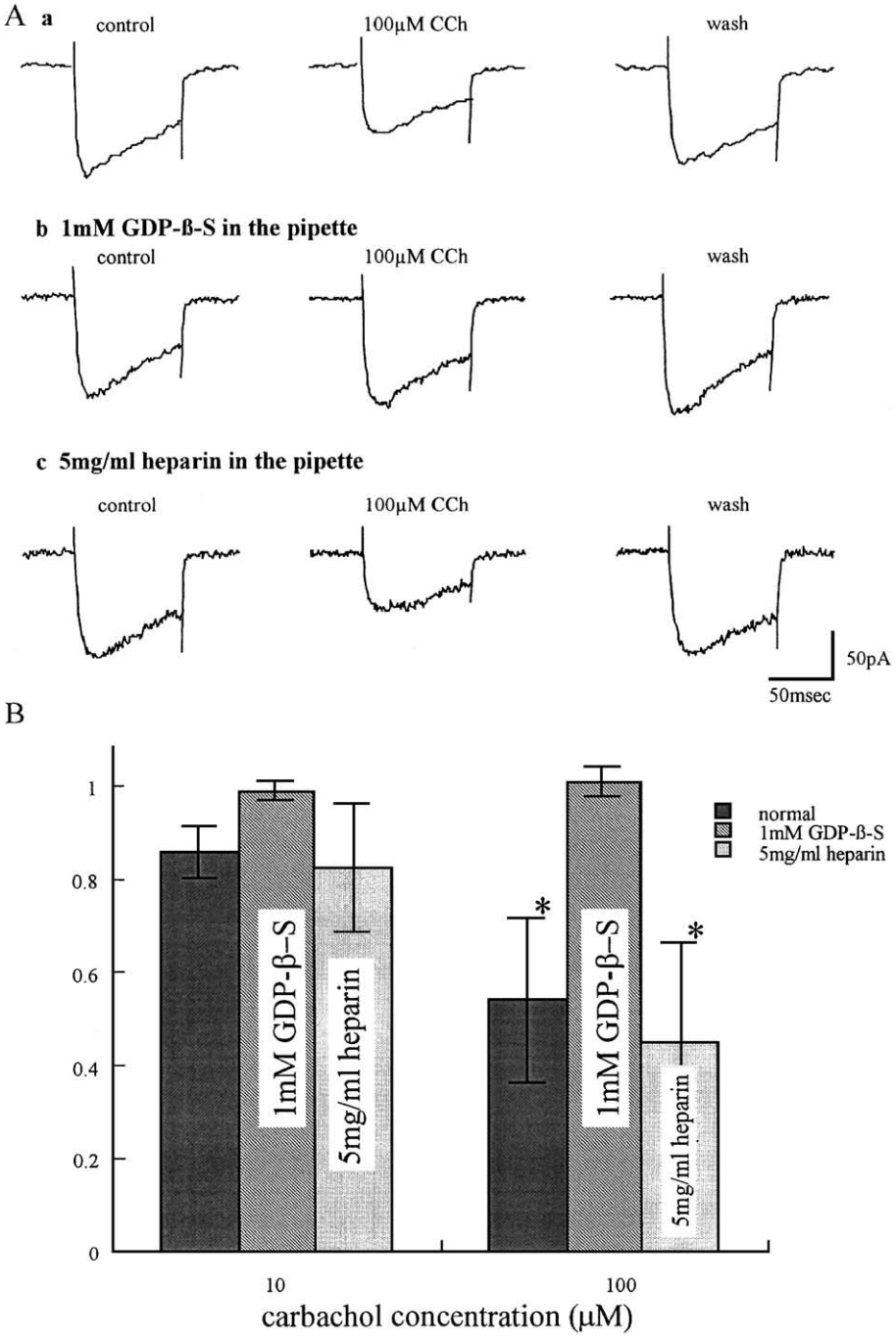

Fig. 5. Effects of GDP-β-S or heparin on the carbachol-induced reduction of voltage-sensitive Ca^{2+} channel current (I_{Ba}) in pig detrusor cells. In A, current traces were obtained in the absence (a), and presence (b) of 1 mM GDP-β-S or 5 mg/ml heparin (c) in the pipette. The inward currents were evoked by step depolarizations to 0 mV (100 ms) in the presence of 10 mM Ba^{2+} as a charge carrier. An application of 100 μM carbachol reduced I_{Ba} (a). On the other hand, in the presence of GDP-β-S, carbachol did not reduce I_{Ba} (b). In the presence of heparin, carbachol still caused the reduction of I_{Ba} (c). The graphs in B show the averages of (10 and 100 μM) carbachol-induced reduction of I_{Ba} with 'normal' pipette solution, and with 1 mM GDP-β-S or 5 mg/ml heparin in the pipette. The current amplitude was expressed relative to that obtained before carbachol application. The asterisks indicate significant differences evaluated with Student's *t*-test ($P < 0.05$).

3.5. Inhibitory action of carbachol on I_{Ba} of pig detrusor cells

The effects of carbachol on I_{Ba} in the presence of GDP-β-S or heparin in the patch pipette were examined in pig detrusor cells. In Fig. 5Aa, after observing the control Ca^{2+} channel current (at 0 mV for 100 ms), 100 μM carbachol was applied to the bathing solution. The application of 100 μM carbachol markedly reduced the amplitude of I_{Ba}, while subsequent washout almost fully restored it. The series of current traces in Fig. 5Ab clearly show that carbachol had little effect on I_{Ba} in the presence of GDP-β-S. However, when heparin (5 mg/ml) was present in the pipette solution, carbachol continued to cause suppression (Fig. 5Ac). Fig. 5B summarizes the degree of carbachol-induced suppression on voltage-sensitive Ca^{2+} channel current when different concentrations (10 and 100 μM) of carbachol were used. The peak amplitude in the presence of 10 μM carbachol (normalized relative to that under carbachol-free conditions) was 0.86 ± 0.06 in 'normal' pipette solution, and 0.98 ± 0.02 and 0.82 ± 0.14 with the addition of 1 mM GDP-β-S and 5 mg/ml heparin, respectively ($n = 4–6$). After 100 μM carbachol was applied, the peak amplitude was 0.54 ± 0.18 in 'normal' pipette solution, and 1.09 ± 0.03 and 0.45 ± 0.21 with 1 mM GDP-β-S and 5 mg/ml heparin in the pipette, respectively ($n = 4–6$).

4. Discussion

4.1. Similarities and dissimilarities of L-type Ca^{2+} channel in human and pig detrusor cells

Our results show that only a single type of voltage-sensitive Ca^{2+} channel (L-type) occurs in both human and pig urinary bladder smooth muscle cells, since $I–V$ relationships did not change when the holding potential was shifted from -60 to -80 mV (data not shown); furthermore, the L-type Ca^{2+} channel antagonist nifedipine completely inhibited the inward current evoked by the depolarizing pulse (Fig. 1) in both tissues. In this study, Ca^{2+} or Ba^{2+} was used as a charge carrier. As expected (Nakayama and Brading, 1993b), Ba^{2+} currents through L-type Ca^{2+} channels decayed more slowly than Ca^{2+} currents in both tissues (Fig. 1). However, the amplitude of voltage-sensitive Ca^{2+} channel current recorded from pig detrusor cells was significantly smaller than those in human and guinea-pig, even if the current density was taken into consideration. When the current density of voltage-sensitive Ca^{2+} channel current was compared between species in our hands, the order was: guinea-pig > human>pig. This result is also consistent with previous papers from other groups (Klöckner and Isenberg, 1985; Montgomery and Fry, 1992; Gallegos and Fry, 1994; Sui et al., 2001a). Interestingly, the degree of activation at the threshold potential (-40 mV) is higher in human than in pig (Fig. 1Cb) and guinea-pig detrusor cells (Fig. 2 in Klöckner and Isenberg, 1985). Further at several negative steps, the amplitude of Ca^{2+} channel current was significantly larger in human than in pig. These results suggest that Ca^{2+} channels of human detrusor cells may play an important role in mictrition. The species differences in the threshold of channel activation might be caused by the modulation of mainly S4 segments of channel protein, which are thought to construct its voltage sensor, as the differences in the amino acid sequence of each segment is considerable between species, however molecular biological investigations would be necessary to elucidate such differences (see review Benzanilla, 2000).

4.2. Sensitivities to nifedipine

It is generally accepted that the contraction induced by various neurotransmitters (for example, ATP or acetylcholine) or Ca^{2+} influx is inhibited by dihydropyridine derivatives such as nifedipine in a dose-dependent manner in urinary bladders. ATP or carbachol could first depolarize the membrane via the opening of non-selective cation channels or activation of Ca^{2+}-activated Cl^- channels, respectively. Subsequently, the dihydropyridine-sensitive Ca^{2+} channel may be opened by the induced membrane depolarization (Fovaeus et al., 1987; Bo and Burnstock, 1990; Kishi et al., 1992; Uchida et al., 1994). Interestingly, the IC_{50} values or the maximum inhibitory concentrations of nifedipine obtained from the above reports were almost identical to the results of our present patch clamp study. It suggests that the degree of Ca^{2+} channel activation reflects the strength of muscle contraction not only directly but also accordingly.

Actually, there were significant differences of inhibitory actions by nifedipine at some concentrations. However, irrespective of the differences of charge carrier or species, 1 μM nifedipine completely inhibited Ca^{2+} channel current (Fig. 2). In a previous patch clamp study, nifedipine was also shown to decrease Ca^{2+} current in guinea-pig urinary bladder smooth muscle cells (Kura et al., 1992b). The IC_{50} value was not calculated in this tissue but 100 nM nifedipine almost completely abolished the Ca^{2+} current. Therefore, the inhibitory action of nifedipine in the guinea-pig can be estimated to be similar to that found presently in human or pig. In addition the IC_{50} or maximum inhibitory concentrations found presently are similar to those measured previously in vascular smooth muscle cells. It has been reported that the K_d value for another dihydropiridine (nicardipine) was 24 nM in rabbit mesenteric artery as measured using patch clamp methodology (Terada et al., 1987a,b) and that maximal inhibition of KCl-induced contraction in various rabbit vessels was achieved with 100 nM nifedipine (Dunn et al., 1991). Therefore, it is likely that in both vascular and non-vascular smooth muscle cells, dihydropyridine receptors have pharmacologically similar features irrespective of species. Clinically, there might be interesting differences between patients suffering from

bladder activity with or without taking Ca^{2+} channel antagonists for hypertension (King et al., 1998).

4.3. Purinergic and cholinergic regulation of Ca^{2+} channels

Application of the purinoceptor agonist ATP activated a transient inward current at a negative holding potential of −60 mV (Fig. 3). These properties were very similar to those described by previous reports, although the application methods and ionic composition of the solutions were slightly different (Inoue and Brading, 1991; Nakayama, 1993). Such results suggest that purinoceptor stimulation opens non-selective cation channels. In guinea-pig detrusor cells, L-type Ca^{2+} channels became inactivated as a result of an increase in intracellular Ca^{2+} induced by activation of purinoceptors (Nakayama, 1993). However, presently, in pig and human detrusor cells, L-type Ca^{2+} channel activity was not modified by the activation of a purinoceptor agonist (Fig. 3). This difference could of course be explained by species differences, further, the functional coupling between voltage-sensitive Ca^{2+} channel and ATP-activated non-selective cation channel in human and pig tissues might not be so developed as that observed in guinea-pig tissue since the human and pig urinary bladder receive a excitatory innervation from cholinergic nerves but not from non-adrenergic, non-cholinergic (NANC) nerves. Further experiments would be required to elucidate this hypothesis. On the other hand, the muscarinic receptor agonist, carbachol induced a transient inward current, probably through Ca^{2+}-activated Cl^- channels, activated by increased Ca^{2+} via IP_3 formation, in pig and human detrusor cells as previously reported in guinea-pig detrusor cells (Nakayama, 1993).

Applications of carbachol induced suppression of the L-type Ca^{2+} channel current. There was no voltage dependency in the Ca^{2+} channel inhibitory action induced by carbachol (Fig. 4). A G-protein-coupled mechanism is suggested to be involved in this inhibition in pig and human detrusor cells since there was no suppression induced by carbachol when GDP-β-S was present in the patch pipette (Fig. 5). The effects of muscarinic activation on voltage-dependent Ca^{2+} channels are varied among previous reports. Muscarinic receptor agonists increased the magnitude of Ca^{2+} channel current in stomach smooth muscle cells (Clapp et al., 1987), in single cells from the longitudinal smooth muscle layer of rabbit jejunum (Benham et al., 1985) and in trabecular meshwork (a smooth muscle like tissue) (Steinhausen et al., 2000). Conversely, muscarinic suppression of Ca^{2+} current has been reported in intestinal smooth muscle cells (Russell and Aaronson, 1990; Beech, 1993; Unno et al., 1996), in bladder smooth muscle cells (Nakayama, 1993; Yoshino and Yabu, 1995) and in tracheal smooth muscle cells (Yamakage et al., 1995). Furthermore, other neurotransmitters such as histamine, bradykinin and substance P also induced suppression of Ca^{2+} current in intestinal smooth muscle cells (Beech, 1993). It is generally accepted that there are two possible mechanisms underlying muscarinic suppression of Ca^{2+} channel current in smooth muscle cells: (a) muscarinic stimulation induces the production of IP_3 which releases Ca^{2+} from intracellular stores causing a Ca^{2+}-induced inactivation of Ca^{2+} channels: (b) muscarinic receptor agonists activate a G-protein which causes Ca^{2+} channel inhibition via a mechanism which does not require Ca^{2+} release.

Presently, in pig and human detrusor cells at fast phase, namely, during membrane depolarization, there may be some Ca^{2+}-dependent inactivation of Ca^{2+} channels since Ba^{2+} currents through L-type Ca^{2+} channels decayed more slowly than the Ca^{2+} currents. This finding is similar to that observed in other types of smooth muscle cells (Terada et al., 1987a,b; Katzka and Morad, 1989; Langton et al., 1989). However, it is likely that extracellular Ca^{2+} are responsible for Ca^{2+} channel inactivation, not only because the inward currents through the L-type Ca^{2+} channel decayed more slowly when extracellular Ca^{2+} ions were replaced by Ba^{2+} ions, but also because there was no significant difference in the suppression of Ca^{2+} currents by carbachol when high concentrations of the IP_3 receptor antagonist, heparin, was in the patch pipette (Fig. 5). This finding indicates that muscarinic stimulation suppresses Ca^{2+} current irrespective of IP_3-induced Ca^{2+} release. Hence, G-protein activation via muscarinic receptors may play a dominant role in suppression of the Ca^{2+} current. Furthermore, this was supported not only by our observation that activation of the non-G-protein coupled purinergic receptor failed to inhibit Ca^{2+} current (Fig. 3) but also by the report that G-proteins can be directly coupled to Ca^{2+} channels in rat pituitary GH_3 cells (Kleuss et al., 1991). The physiological role of this muscarinic inhibitory action on L-type voltage-dependent Ca^{2+} channels is still unknown. Presumably, this may work as a negative feedback system to prevent cytosolic Ca^{2+} overload.

4.4. Conclusion

In conclusion, humans and pigs share similar anatomical features of the detrusor system and similar neuronal control in voiding urine. The present study carried out using patch clamp techniques revealed that the actions of carbachol and ATP on voltage-sensitive Ca^{2+} channels in human detrusor cells are similar to those in pigs. In this context, it is suggested that investigation into Ca^{2+} channel properties in pig tissue merit for clinical treatments in lower urinary tracts. Since the current density of voltage-sensitive Ca^{2+} channels is significantly larger in human detrusor cells, voltage-sensitive Ca^{2+} channels would play more important roles in human micturition. Even though we have also detected minor differences in the threshold membrane potential of channel activation between humans and pigs, it is suggested that pig bladder is a good model in the context of neural modulation on Ca^{2+} channel. Such knowledge is vital, not just as a direct comparison between

human and pig, but also because such knowledge in isolated detrusor cells is useful to assess the various established models of urinary incontinence to the human state.

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