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Negative modulation of L-type Ca^{2+} channels via β-adrenoceptor stimulation in guinea-pig detrusor smooth muscle cells

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Abstract

β-Adrenergic stimulation enhances the activity of L-type Ca^{2+} channels through mechanisms mediated by adenosine 3′5′-cyclic monophosphate (cAMP) and protein kinase A in cardiac myocytes. However, in smooth muscle cells, the effect of β-adrenoceptor stimulation on the L-type Ca^{2+} channel activity has been controversial, and the exact mechanism is still unclear. The present study was aimed at elucidating the effect of β-adrenergic stimulation upon the activity of L-type Ca^{2+} channels in guinea-pig detrusor smooth muscle cells. Isoproterenol (0.1–1 μM) inhibited Ba^{2+} currents through L-type Ca^{2+} channels (I_{Ba}). Isoproterenol (0.1 μM) shifted the steady-state inactivation curve to negative voltages by 11 mV without affecting activation curves. The stimulation of cAMP-mediated signal transduction pathway by forskolin, 8-bromoadenosine 3′5′-cyclic monophosphate (8-Br-cAMP), or the intracellular application of cAMP also mimicked the effects of isoproterenol on I_{Ba}, which was blocked by the inhibition of protein kinase A. These results indicate that, in detrusor smooth muscles, the stimulation of β-adrenoceptors exerts negative modulation of L-type Ca^{2+} channels via cAMP/protein kinase A-dependent mechanism.

Keywords: β-Adrenoceptor; cAMP; Protein kinase A; Urinary bladder; Smooth muscle; Ca^{2+} channel L-type

1. Introduction

Ca^{2+} influx through the L-Type Ca^{2+} channels is the crucial step in muscle contraction. In many myocytes, the activity of L-type Ca^{2+} channels is modulated via β-adrenoceptors. A number of studies have shown that the activity of cardiac L-type Ca^{2+} channels is enhanced by β-adrenoceptor stimulation via adenosine 3′5′-cyclic monophosphate (cAMP)/protein kinase A pathway (Kamp and Hell, 2000; Keef et al., 2001; McDonald et al., 1994; Xiong and Sperelakis, 1995 for a review). In cardiomyocytes, β-adrenoceptor agonists show the positive inotropic and chronotropic effects through the enhancement of Ca^{2+}-dependent responses mediated by the L-type Ca^{2+} channel (Mukherjee and Spinale, 1998). By contrast, in smooth muscle cells, effects of β-adrenergic stimulation and the activation of cAMP/protein kinase A pathway are highly variable among tissues. In rat portal vein, the stimulation of β-adrenoceptor facilitated Ca^{2+} entry through L-type Ca^{2+} channels by protein kinase A activation (Viard et al., 2000). However, in rabbit ear artery, isoproterenol, a β-adrenoceptor agonist, inhibited voltage-activated Ca^{2+} channel currents (Droogmans et al., 1987). In rat tail artery, elevation of intracellular cAMP concentration, induced by parathyroid hormone (Wang et al., 1991) and pituitary adenylate cyclase activating polypeptide (Chik et al., 1996), reduced the amplitude of L-type Ca^{2+} channel currents. On the other hand, L-type Ca^{2+} channels in guinea-pig gastric smooth muscle did not respond to β-adrenergic stimulation (Mitra and Morad, 1985).

In detrusor smooth muscle cells, β-adrenoceptors play an important role in regulating the contractility. In the bladder-filling phase, the detrusor muscle is relaxed by the activation of β-adrenoceptors (Levin et al., 1980). Isoproterenol attenuates the spontaneous contraction by reducing the magnitude of Ca^{2+} transients in guinea-pig detrusor smooth muscle cells

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(Nakahira et al., 2001). However, recent studies reported that β-adrenoceptor agonists did not affect L-type Ca^{2+} channel current in guinea-pig detrusor smooth muscle cells (Klöckner and Isenberg., 1985; Smith et al., 1999; Kobayashi et al., 2000). The aim of the present study was to elucidate the difference in the modulatory effects of β-adrenoceptor stimulation on the L-type Ca^{2+} channels between cardiac myocytes and smooth muscle cells, and the underlying signal transduction pathway using freshly isolated guinea-pig detrusor smooth muscle cells. The removal of Ca^{2+}-dependent inactivation/facilitation mechanisms, by use of Ba^{2+} as a charge carrier, revealed negative modulation of L-type Ca^{2+} channels by β-adrenergic stimulation through the activation of cAMP/protein kinase A pathway in detrusor smooth muscle cells, which was in contrast to positive modulation of cardiac L-type Ca^{2+} channels.

2. Materials and methods

2.1. Cell isolation

The experimental protocol complies with the guidelines for animal experiments approved by the University of Tokyo. Guinea-pig detrusor smooth muscle cells were prepared as previously described (Kobayashi et al., 2000).

In brief, urinary bladder was dissected from male Hartley guinea-pigs (5–8 weeks old), and connective tissue was removed from detrusor muscle in Ca^{2+}-free Krebs solution (in mM: NaCl 137, KCl 4.7, KH_2PO_4 1.2, $MgCl_2$ 1.2, HEPES 10, glucose 14, pH 7.4 with NaOH). Then, the tissue was digested in Ca^{2+}-free Krebs solution containing 0.3% collagenase, 0.3% trypsin inhibitor and 0.3% bovine serum albumin for 30 min. Digested tissues were agitated with a pipette in order to dissociate single cells. The dissociated cells were stored in Ca^{2+}-free Krebs solution and used for experiments within 8 h after isolation.

2.2. Current recording

Currents were measured in the whole-cell configuration of the patch-clamp technique using Axopatch 200B amplifier (Axon instruments, Foster City, CA). The resistance of the borosilicate glass electrodes ranged between 2 and 4.5 MΩ when filled with the internal solution containing: (in mM) NaCl 5, CsCl 105, tetraethyl-ammonium chloride 30, HEPES 10, Mg-ATP 5, 1,2-bis(*o*-aminophenoxy)etene-*N*,*N*,*N*′,*N*′,-tetraacetic acid (BAPTA) 2, pH 7.2 with CsOH. Cells were perfused with Krebs solution containing 5 mM $BaCl_2$. Whole-cell capacitance was 49.7 ± 12.8 pF (mean ± S.D., $n = 89$). Measurements were excluded from the data when series resistance exceeded 15 MΩ.

Generation of voltage-clamp protocols and acquisition of data were carried out by use of pCLAMP6 software (version 6.0.4, Axon Instruments) as has been previously described (Kobayashi et al., 2000). The series resistance was electronically compensated through the amplifier. Current signals were filtered at 5 kHz before digitization. The linear passive leak currents were digitally subtracted using the P/4 protocol of pCLAMP6 in which passive current components were recorded by applying four sub-episodes of 1/4 of the test pulse amplitude. Measurements were started 4–10 min after the establishment of whole-cell recording. When the intracellular application of compounds was made via patch pipettes, recordings were carried out 8–10 min after rupture of the membrane. Drugs were dissolved in the external solution and applied by the concentration-clamp technique (Adachi-Akahane et al., 1996). All the experiments were performed at room temperature (22–26 °C).

2.3. Drugs

Collagenase was purchased from Yakult (Tokyo, Japan), DL-Isoproterenol hydrochloride, forskolin, bovine serum albumin, trypsin inhibitor, 8-bromoadenosine 3′5′-cyclic monophosphate (8-Br-cAMP), and 8-bromoguanosine 3′5′-cyclic monophosphate (8-Br-cGMP) from Sigma (St. Louis, MO), (9S,10R,12R)-2,3,9,10,11,12-hexahydro-10-methoxy-2,9-dimethyl-1-oxo-9,12-epoxy-1*H*-diindolo-[1,2,3-fg:3,2,1-kl]pyrrolo[3,4-i][1,6]benzodiazocine-10-carboxylic acid methyl ester (KT5823) from Calbiochem (San Diego, CA) and (9*R*,10*S*,12*S*)-2,3,9,10,11,12-hexahydro-10-hydroxyl-9-methyl-1-oxo-9,12-epoxy-1*H*-diindolo-[1,2,3-fg:3-2-1-kl]pyrrolo[3,4-i][1,6]benzodiazocine-10-carboxylic acid hexyl ester (KT5720) from Wako (Osaka, Japan). KT5823 and KT5720 were dissolved in dimethyl sulfoxide (DMSO) at 1 mM as stock solutions, and diluted by Krebs solution so that the final concentration of DMSO was below 0.1%. DMSO had no effect on Ca^{2+} channel currents at least up to 0.1%.

2.4. Statistics and data analysis

All the data were expressed as the mean ± S.E.M. Student's unpaired *t*-test was used for comparison between two groups. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for multiple comparisons. Differences at $P < 0.05$ (two-tailed) were considered to be significant. Steady-state inactivation curves were fitted to a Boltzmann distribution expressed as the following equation:

$$I_{Peak}/I_{Peak.max} = 1/(1 + \exp((V - V_{half.inact})/k)),$$

where $V_{half.inact}$ denotes the half-inactivation potential and k is the slope factor for the inactivation curve. Half-activation potentials for the current–voltage relationships were determined by fitting to the following equation:

$$I_{Peak}/I_{Peak.max} = (V - V_{half.act})/(1 + \exp((V - V_{half.act})/k)),$$

where $V_{half.act}$ represents the half-activation potential and k is the slope factor for the activation curve.

3. Results

First, in order to investigate the impact of β-adrenoceptor stimulation upon the L-type Ca^{2+} channel in detrusor smooth muscle cells, we studied the effect of isoproterenol on Ca^{2+} channel currents. Ca^{2+} channel currents were measured with 5 mM Ba^{2+} as a charge carrier (I_{Ba}). I_{Ba} was activated by applying rectangular pulses to 0 mV from a holding potential of −60 mV. The $I–V$ relationships of I_{Ba} exhibited typical voltage-dependence of the activation of L-type Ca^{2+} channel currents as shown in Fig. 2. Application of isoproterenol (0.1 μM) via concentration-clamp apparatus caused a reduction of the peak amplitude of I_{Ba} within 1 min. Fig. 1 shows representative time course of isoproterenol-induced inhibition of I_{Ba}. Isoproterenol significantly ($P<0.05$) decreased I_{Ba} density by 37% (from 5.54 ± 0.24 to 3.48 ± 0.51 pA/pF at 0 mV test potential, $n=6$, Fig. 2B). However, when the $I–V$ relationships were normalized to the maximum value of peak I_{Ba} (Fig. 2C), neither depolarizing nor hyperpolarizing shift of the $I–V$ relationships was observed. Accordingly, the activation kinetics was unchanged by isoproterenol. The half-activation potentials were −21.4 ± 1.25 and −21.1 ± 0.99 mV in the absence and presence of isoproterenol, respectively ($n=4$, Fig. 3).

The steady-state inactivation curve was shifted toward hyperpolarized direction (Fig. 3), suggesting that the voltage-dependent inactivation of I_{Ba} was augmented by the treatment with isoproterenol. The shift of the half-inactivation potentials was statistically significant ($P<0.05$, −36.3 ± 3.36 and −47.5 ± 1.27 mV in the absence and presence of isoproterenol, $n=4$).

The decay component of I_{Ba} was fitted well to a single exponential decay curve. The decay rate of I_{Ba} (τ) was 128.9 ± 16.18 and 100.6 ± 14.01 ms in the absence and presence of isoproterenol, respectively.

Since the stimulation of β-adrenoceptor enhances the cAMP production via Gs protein-coupled mechanism, we next examined the role of the intracellular cAMP on the modulation of L-type Ca^{2+} channel function. Application of 200 μM cAMP into cells through patch pipette showed an inhibitory effect similar to that of isoproterenol. In the presence of cAMP, peak I_{Ba} amplitude was, again, smaller

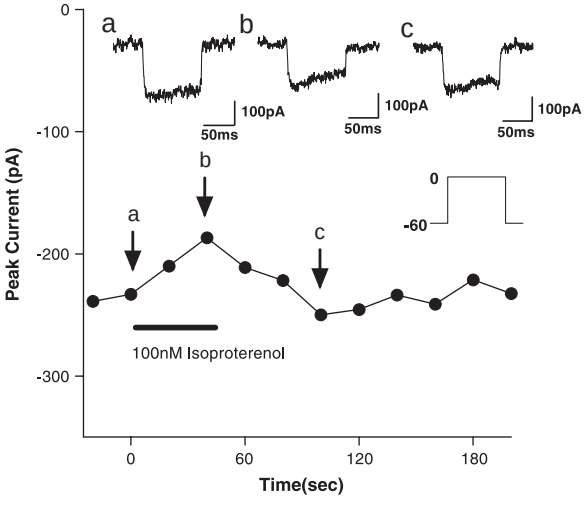

Fig. 1. Representative time-course and current traces of Ba^{2+} currents through L-type Ca^{2+} channels elicited by test pulses from −60 to 0 mV in guinea-pig detrusor smooth muscle cells. Each current trace was recorded just prior to the addition of 0.1 μM of isoproterenol (a), after 40 s (b) and after washout period (c).

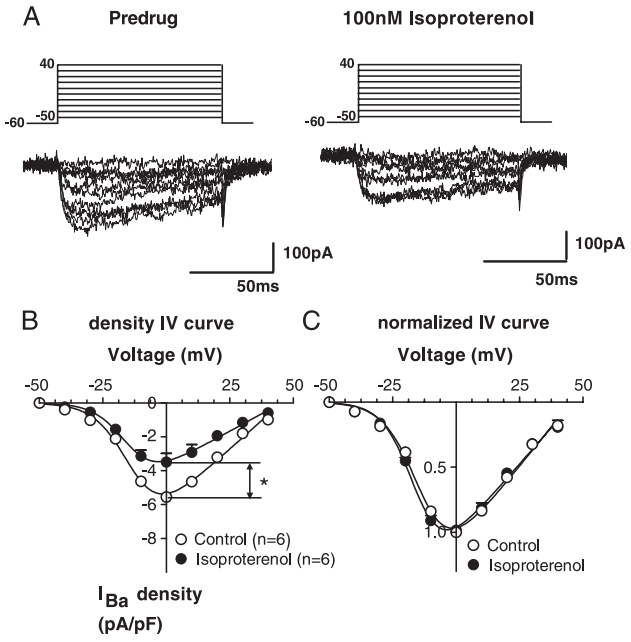

Fig. 2. Current–Voltage relationships of Ba^{2+} currents through L-type Ca^{2+} channels recorded in guinea-pig detrusor smooth muscle cells. Representative current traces are shown in A, in the absence (left panel) and presence (right panel) of isoproterenol (0.1 μM), respectively. $I–V$ relationships in the absence and presence of Isoproterenol are superimposed in B and C. In B, the ordinate represents current density (pA/pF). Isoproterenol significantly decreased I_{Ba} density. In C, currents are normalized by peak current. Each plot represents the mean ± S.E.M., $*P<0.05$, $n=6$.

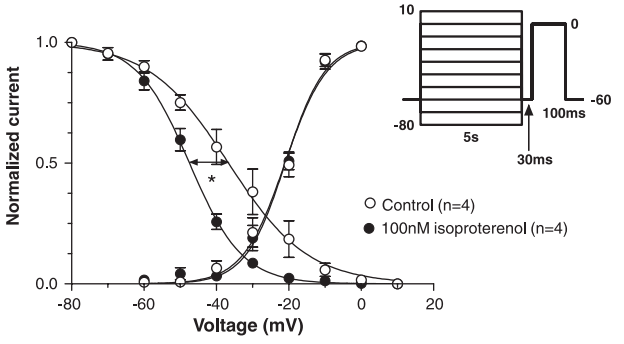

Fig. 3. Steady-state inactivation curves of Ba^{2+} currents through L-type Ca^{2+} channels recorded in guinea-pig detrusor smooth muscle cells in the absence or presence of isoproterenol (0.1 μM). Conditioning pulses (5 s) to various voltages were applied before the test pulse (100 ms). The steady-state inactivation curve was significantly shifted toward hyperpolarized direction by isoproterenol. Each plot represents the mean ± S.E.M., $*P<0.05$, $n=4$.

than control by 30% (from 5.94 ± 1.01 to 4.17 ± 0.87 pA/pF, $n=6$, Fig. 4B) without any shift in $I-V$ relationships (Fig. 4C). The steady-state inactivation curve was significantly ($P<0.05$) shifted toward hyperpolarized direction (−31.3 ± 2.74 and −44.5 ± 4.12 mV in the absence and presence of cAMP, $n=4-5$, Fig. 5). However, the half activation potentials were unchanged (−18.9 ± 1.62 and −22.5 ± 1.31 mV in the absence and presence of cAMP, $n=4-5$, Fig. 5).

Since isoproterenol and cAMP turned out to exhibit the equivalent inhibitory effect on I_{Ba} in detrusor smooth muscle cells, we further examined whether the effect of isoproterenol is mediated by the activation of the cAMP/protein kinase A pathway through β-adrenoceptor activation as shown in Fig. 6. One hundred nanomolar of propranolol significantly ($P<0.05$) antagonized the inhibitory effect of isoproterenol, indicating that isoproterenol reduces I_{Ba} via stimulation of β-adrenoceptors. We also examined the involvement of protein kinase A, which is the major downstream pathway of intracellular cAMP elevation, in the negative modulation of I_{Ba}. KT5720, a highly specific protein kinase A inhibitor, significantly ($P<0.01$) impaired the effect of isoproterenol (Fig. 6A). In contrast, KT5823, a protein kinase G inhibitor, was without effect. Protein kinase A inhibitory peptide (PKI: TTYADFIASGRTGRRNAIHD), at 20 μM, intracellularly applied through patch pipettes, also inhibited the effect of isoproterenol on I_{Ba} (control 28.6 ± 6.84% ($n=7$) vs. +protein kinase A inhibitory peptide 20.8 ± 9.97% ($n=7$), data not shown), although the antagonizing effect was not statistically significant because of the large variation of data. We also examined the effect of H-89, protein kinase A specific inhibitor. However, the treatment of H-89 by itself strongly depressed I_{Ba} at 3 μM, and was not useful for the further evaluation (data not shown). The change in I_{Ba} was 90 ± 2.9%, 121 ± 6.2% and 117 ± 9.9% of predrug value by treatment with propranolol, KT5720 and KT5823, respectively. Interestingly, forskolin (3 μM), in the presence of calyculin A, a protein phosphatase inhibitor, produced marked reduction of I_{Ba}, while either forskolin alone or calyculin A alone hardly affected I_{Ba} (Fig. 6B).

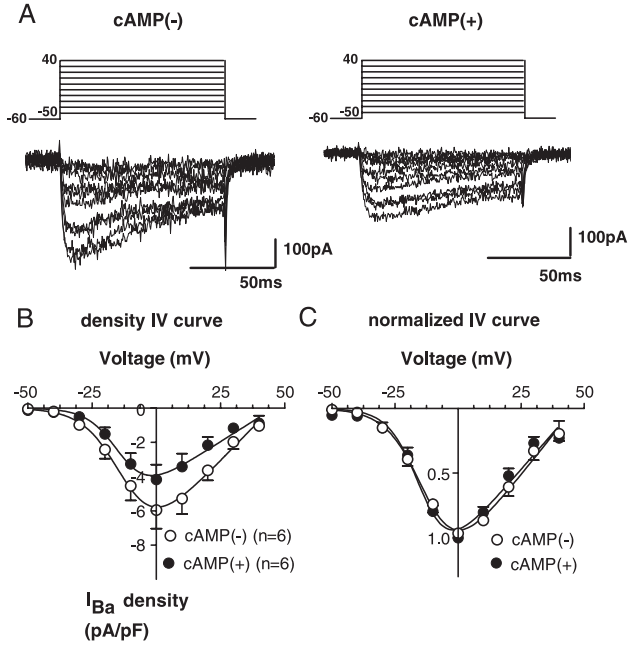

Fig. 4. Current–Voltage relationships of Ba^{2+} currents through L-type Ca^{2+} channels recorded in guinea-pig detrusor smooth muscle cells. Representative current traces are shown in A, in the absence (left panel) and presence (right panel) of cAMP (200 μM), respectively. $I-V$ relationships in the absence and presence of cAMP are superimposed in B and C. In B, the ordinate represents current density (pA/pF). In C, currents are normalized by peak current. Each plot represents the mean ± S.E.M., $n=6$.

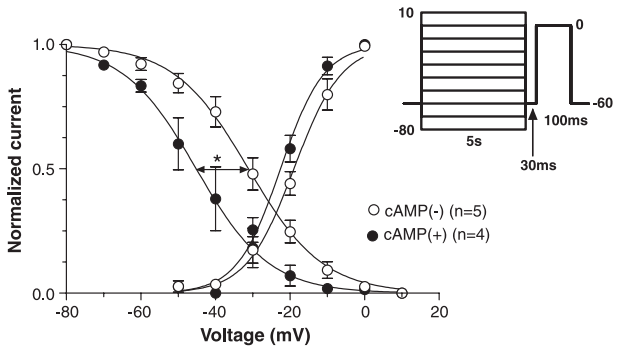

Fig. 5. Steady-state inactivation curves of Ba^{2+} currents through L-type Ca^{2+} channels recorded in guinea-pig detrusor smooth muscle cells in the presence or absence of cAMP (200 μM). Conditioning pulses (5 s) to various voltages were applied before the test pulse (100 ms). The steady-state inactivation curve was significantly shifted toward hyperpolarized direction by cAMP. Each plot represents the mean ± S.E.M., $*P<0.05$, $n=4-5$.

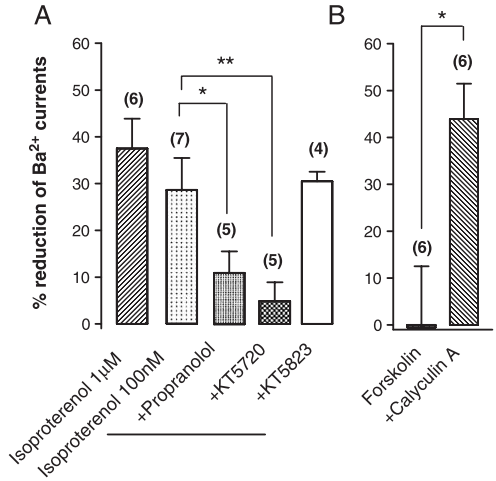

Fig. 6. Effects of isoproterenol (1 μM, 0.1 μM) and forskolin (3 μM) on Ba^{2+} currents through L-type Ca^{2+} channels recorded in guinea-pig detrusor smooth muscle cells. Each column represents the percent reduction of the peak amplitude of Ba^{2+} currents in the presence or absence of propranolol (100 nM), KT5720 (1 μM), KT5823 (1 μM), or calyculin A (1 μM), respectively. (A) Propranolol and KT5720 significantly inhibited the effect of isoproterenol. (B) Calyculin A significantly enhanced the effect of forskolin. Each column represents the mean ± S.E.M., $*P<0.05$, $**P<0.01$, $n=4-7$.

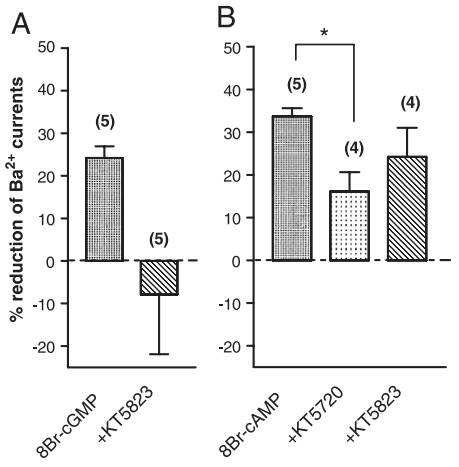

Fig. 7. Effects of 8Br-cGMP (100 μM), 8Br-cAMP (100 μM) on Ba^{2+} currents through L-type Ca^{2+} channels recorded in guinea-pig detrusor smooth muscle cells. Each column represents the reduction of the peak amplitude of Ba^{2+} currents in the presence or absence of KT5720 (1 μM) and KT5823 (1 μM). KT5720 significantly inhibited the effect of 8-Br-cAMP. Each column represents the mean ± S.E.M., $*P<0.05$, $n=4-5$.

We further examined the involvement of protein kinase G in the inhibition of I_{Ba}, since the activation of cAMP production can affect cGMP/Protein kinase G system and vice versa based on the recent report that the action of cAMP and cGMP is mediated partly by cross-activation of opposing protein kinases in rabbit portal vein (Ruiz-Velasco et al., 1998). As shown in Fig. 7, both 8-Br-cAMP and 8-Br-cGMP, membrane permeable analogs of cAMP and cGMP, respectively, inhibited I_{Ba} in the detrusor muscle. The effect of 8-Br-cGMP on I_{Ba} was reversed by KT5823, thus confirming that the inhibitory effect of 8-Br-cGMP on I_{Ba} was mediated by protein kinase G. However, the effect of KT5823 on the 8-Br-cGMP-induced decrease of I_{Ba} was not significant ($P=0.055$) because of large variability of the effect of 8-Br-cGMP in the presence of KT5823, which might be due to the variability of the contribution of cGMP signaling pathway among the detrusor myocytes. In contrast, KT5823 was ineffective on the 8-Br-cAMP-induced inhibition, even though KT5720 significantly attenuated the effect of 8-Br-cAMP. These results indicate that the inhibitory effect of β-adrenoceptor/cAMP on I_{Ba} is not mediated by protein kinase G but by protein kinase A.

4. Discussion

In the present study, we demonstrated the inhibitory modulation of L-type Ca^{2+} channels via β-adrenoceptor stimulation in detrusor smooth muscle cells. L-type Ca^{2+} channels are responsible for voltage-dependent Ca^{2+} influx in guinea-pig detrusor smooth muscle cells (Klöckner and Isenberg, 1985) and capable of generating action potentials (Brading and Mostwin, 1989). Accordingly, the inhibition of L-type Ca^{2+} channels in urinary bladder suppresses the contraction induced by membrane depolarization. Thus, L-type Ca^{2+} channels have been suggested to play important roles in the regulation of detrusor contractility. In the present report, we applied Ba^{2+} as a charge carrier in order to exclude the involvement of the Ca^{2+}-dependent facilitation/inactivation processes in the effect of β-adrenergic stimulation on L-type Ca^{2+} channel currents.

Freshly isolated detrusor smooth muscles are known to express L-type and T-type Ca^{2+} channels in guinea pigs (Sui et al., 2001). Under our experimental condition, 36% of peak I_{Ba} remained even after treatment with 5 μM nitrendipine, an L-type Ca^{2+} channel specific blocker, at a holding potential of −90mV. However, at a holding potential of −60 mV, nitrendipine blocked more than 85% of I_{Ba}. Thus, in our hand, I_{Ba} recorded at a holding potential of −60mV was mainly composed of L-type Ca^{2+} channel currents.

In cardiomyocytes and baby-hamster kidney (BHK) cells, isoproterenol is reported to facilitate I_{Ba} through the hyperpolarizing shift of activation potential (Naguro et al., 2001). In contrast, in guinea-pig detrusor smooth muscle cells, isoproterenol inhibited the peak I_{Ba}, but did not change the voltage dependence of the activation of L-type Ca^{2+} channel currents (Fig. 2).

In addition, as shown in Fig. 3, steady-state inactivation curve was shifted leftward upon exposure to isoproterenol. This may indicate that, in the presence of β-adrenoceptor stimulation, L-type Ca^{2+} channels are more susceptible to the voltage-dependent inactivation, and thus the availability of Ca^{2+} channels is reduced even at −60mV. Therefore, the reduction of peak I_{Ba} produced by isoproterenol can be explained by the augmentation of the voltage-dependent inactivation of L-type Ca^{2+} channels. In addition to the shift of half-inactivation potential, the slope of steady state inactivation curve seemed to be different between control and isoproterenol-treated groups. This change could involve the time-dependent change of the inactivation kinetics in the paired measurements taken before and after the treatment with isoproterenol. In order to exclude such possibility, we measured the steady-state inactivation curves with and without the intracellular application of cAMP in the same time protocol (Fig. 5). As a result, the intracellular application of cAMP shifted half-inactivation potential to negative voltages as compared with time-matched control, suggesting that the hyperpolarizing shift of the inactivation curve was mediated via cAMP-dependent mechanism. In isolated rat ventricular myocyte, isoproterenol did not affect the steady-state inactivation curve, while activation curve was significantly shifted to the left (Katsube et al., 1996). Accordingly, the stimulation of cAMP/protein kinase A pathway may activate different regulatory mechanisms between L-type Ca^{2+} channels of detrusor muscles and cardiomyocytes.

The inhibitory effect of β-adrenoceptor agonist on L-type Ca^{2+} channel current has not been demonstrated in guinea-pig detrusor smooth muscle cells (Klöckner and Isenberg., 1985; Smith et al., 1999; Kobayashi et al., 2000). One of the possible reasons for the discrepancy could be the difference

in holding potential. Our result showed that the attenuation of L-type channel currents by β-adrenergic stimulation was due to the shift of the inactivation curves by the activation of protein kinase A pathway. Accordingly, the effect of protein kinase A phosphorylation on the availability of L-type Ca^{2+} channels is dependent on the resting membrane potential, which makes depolarized cells more susceptive to channel inactivation by protein kinase A. Previous studies may have failed to detect the inhibitory effect of β-adrenoceptor stimulation because cells were held at hyperpolarized potential enough to mask the inactivation in the steady state. Another possibility could be the difference in the charge carrier for L-type Ca^{2+} channel currents and the intracellular Ca^{2+} buffering condition. We used Ba^{2+} as a charge carrier through L-type Ca^{2+} channels, and added 2 mM BAPTA in the internal solution. On the other hand, Klöckner and Smith measured Ca^{2+} channel currents (I_{Ca}) with an internal solution containing 1–2 mM EGTA, which binds Ca^{2+} with slower on-rate compared to that of BAPTA. The rise in submembrane Ca^{2+} concentration has been reported to affect L-type Ca^{2+} channel activity if the buffering of intracellular Ca^{2+} is insufficient (Bates and Gurney, 1999). The previously reported differences in the action of β-adrenoceptor agonists on detrusor muscle L-type Ca^{2+} channels may be explained by the presence of the intracellular Ca^{2+}-dependent feedback regulatory mechanisms. Ca^{2+}-dependent facilitation of L-type Ca^{2+} channels is a well-known phenomenon, and reported to involve Ca^{2+}/calmodulin-dependent protein kinase (Dzhura et al., 2000; McCarron et al., 1997), which may have masked the inhibitory effect of β-adrenoceptor agonists in L-type Ca^{2+} channels. Our explanation is also supported by the fact that Ba^{2+} instead of Ca^{2+} was used as a charge carrier in most of the reports that demonstrated the inhibitory effects of cAMP signaling on L-type Ca^{2+} channels (Chik et al., 1996; Droogmans et al., 1987; Wang et al., 1991) in smooth muscle cells.

Isoproterenol has been reported to inhibit L-type Ca^{2+} channel currents via cAMP/protein kinase A pathway in several smooth muscle preparations other than urinary bladder. In rat aorta, isoproterenol inhibited Ca^{2+} influx via the activation of cAMP pathway (Orlov et al., 1996). In rabbit portal vein, the effect of isoproterenol on L-type Ca^{2+} channels is reported to be biphasic (Xiong et al., 1994): the initial stimulatory phase is mediated by the direct interaction between L-type Ca^{2+} channel and G protein, while the latter inhibitory phase is mediated by cAMP/protein kinase A pathway. However, the fast facilitation of L-type Ca^{2+} channel current was not observed in the present study. We also demonstrated that the inhibitory effect of isoproterenol on the detrusor L-type Ca^{2+} channel is mediated by the elevation of cAMP concentration, because both the intracellular application of cAMP (Figs. 4 and 5) and the application of 8-Br-cAMP (Fig. 7) mimicked the effect of isoproterenol (Figs. 1, 2 and 6). In addition, KT5720 blocked both the isoproterenol-induced (Fig. 6) and the 8-Br-cAMP-induced (Fig. 7) inhibition of I_{Ba}. Thus, the β-adrenoceptor-mediated blockade of I_{Ba} seems to be mediated by phosphorylation by protein kinase A. As shown in Fig. 6, the experiment with a selective inhibitor of protein kinase G (KT5823) demonstrated that the activation of protein kinase G by 8-Br-cGMP inhibited the activity of L-type Ca^{2+} channels in detrusor muscle. However, KT5823 did not inhibit the effect of isoproterenol or 8-Br-cAMP (Figs. 6 and 7). Ruiz-Velasco et al. (1998) reported that 8-Br-cAMP, at the same concentration as we tested, inhibits L-type Ca^{2+} channels by protein kinase G-dependent mechanisms in rabbit portal vein. However, in detrusor smooth muscle cells, our results indicate that protein kinase G is not involved in the effect of 8-Br-cAMP or isoproterenol. There may be tissue-specific mechanisms for cyclic nucleotide-mediated modulation of L-type Ca^{2+} channels among smooth muscles.

In the present study, forskolin failed to affect I_{Ba} in the absence of calyculin A (Fig. 6). L-type Ca^{2+} channel α_{1C} subunit has been reported to be associated with protein phosphatase 2A (Davare et al., 2000). Thus, the activity of Ca^{2+} channels appears to be regulated by both phosphorylation by protein kinase A and dephosphorylation by protein phosphatase 2A. In detrusor muscle, local signaling from the β-adrenoceptor to the Ca^{2+} channel may not be mimicked by the macroscopic rise of cAMP level by forskolin in the presence of local phosphatase activity.

We also examined the effect of isoproterenol under the present experimental conditions on guinea-pig and rat ventricular myocytes. Both ventricular myocytes showed the enhancement of I_{Ba} in response to isoproterenol (data not shown), consistent with the previously reported feature of ventricular myocyte (Katsube et al., 1996), suggesting that L-type Ca^{2+} channels of detrusor smooth muscle cells and cardiac myocytes are regulated via different mechanisms. As for the role of protein kinase A in cardiac myocytes, the phosphorylation of the serine residue in the COOH-terminal of α_{1C} subunit ($Ca_V1.2$) has been reported to be required for the enhancement of L-type Ca^{2+} channel current (Naguro et al., 2001). However, the critical protein kinase A phosphorylation site responsible for the attenuation of L-type Ca^{2+} channel currents has not been reported within α_{1C} subunit, nor in auxiliary subunits of L-type Ca^{2+} channels. Extensive future studies should be carried out to clarify the site of action of protein kinase A in detrusor smooth muscle cells.

In the previous study, we showed that the stimulation of β-adrenoceptors increases the activity of BK_{Ca} channels in guinea pig detrusor muscle cells (Kobayashi et al., 2000). The relative significance of BK_{Ca} channel facilitation and L-type channel inhibition as the downstream pathways of β-adrenergic stimulation is not clear from the present study. However, BK_{Ca} channel facilitation and L-type channel inhibition may work in synergy for the reduction of muscle tone, because the opening of BK_{Ca} channels is dependent on both the membrane potential and the intracellular Ca^{2+}

concentration, and thus can function as a negative feedback mechanism for the voltage-dependent Ca^{2+} influx.

In conclusion, the present study demonstrates that, in contrast to cardiac myocytes, the stimulation of β-adrenoceptors exerts negative modulation of L-type Ca^{2+} channel function in detrusor smooth muscle cells via cAMP/protein kinase A pathway, which may contribute to the understanding of the difference in the response to β-adrenoceptor stimulation between cardiomyocytes and smooth muscle cells.

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