apamin. Thus, the major part of an increase in the currents by isoproterenol is iberiotoxin sensitive.

We examined the effects of isoproterenol on the currents with patch pipette solution containing 0.05 mmol/l EGTA in the presence of both iberiotoxin and apamin. In the presence of 100 nmol/l iberiotoxin and 1 μmol/l apamin, the outward current at +80 mV decreased from 13.34 ± 3.70 to 5.66 ± 0.78 pA/pF (n = 4), whereas the 1 μmol/l isoproterenol-induced increase in the current was negated (5.64 ± 1.31 pA/pF, n = 4). We also examined the influences of both blockers on the potentiation by isoproterenol. The outward currents at +80 mV in the absence and presence of isoproterenol (1 μmol/l), and the addition of both iberiotoxin (100 nmol/l) and apamin (1 μmol/l) on isoproterenol were 7.10 ± 1.79, 11.15 ± 2.81, and 4.03 ± 0.29 pA/pF (n = 4), respectively.

Therefore, the outward currents potentiated by isoproterenol were iberiotoxin- and apamin-sensitive currents, that is the activation of BK_{Ca} and SK_{Ca} channels via β-adrenoceptors.

Effects of High Concentration of EGTA on the Membrane Currents

With 5 mmol/l EGTA pipette solution only small outward currents were elicited at +80 mV (fig. 3A). With 0.05 and 5 mmol/l EGTA pipette solutions, the maximal outward membrane current densities at +80 mV were 9.68 ± 1.25 pA/pF (n = 10) and 4.70 ± 0.86 pA/pF (n = 6, p = 0.014), respectively (fig. 3B). With 5 mmol/l EGTA pipette solution, 1 μmol/l isoproterenol did not affect the membrane currents (fig. 3C). In the absence and presence of 1 nmol/l isoproterenol, the maximal membrane current densities at +80 mV were 4.70 ± 1.05 and 4.80 ± 1.15 pA/pF (n = 5), respectively.

These results suggest that the outward currents of smooth muscle cells would be dependent on the increase in the intracellular Ca^{2+} concentration, which would be

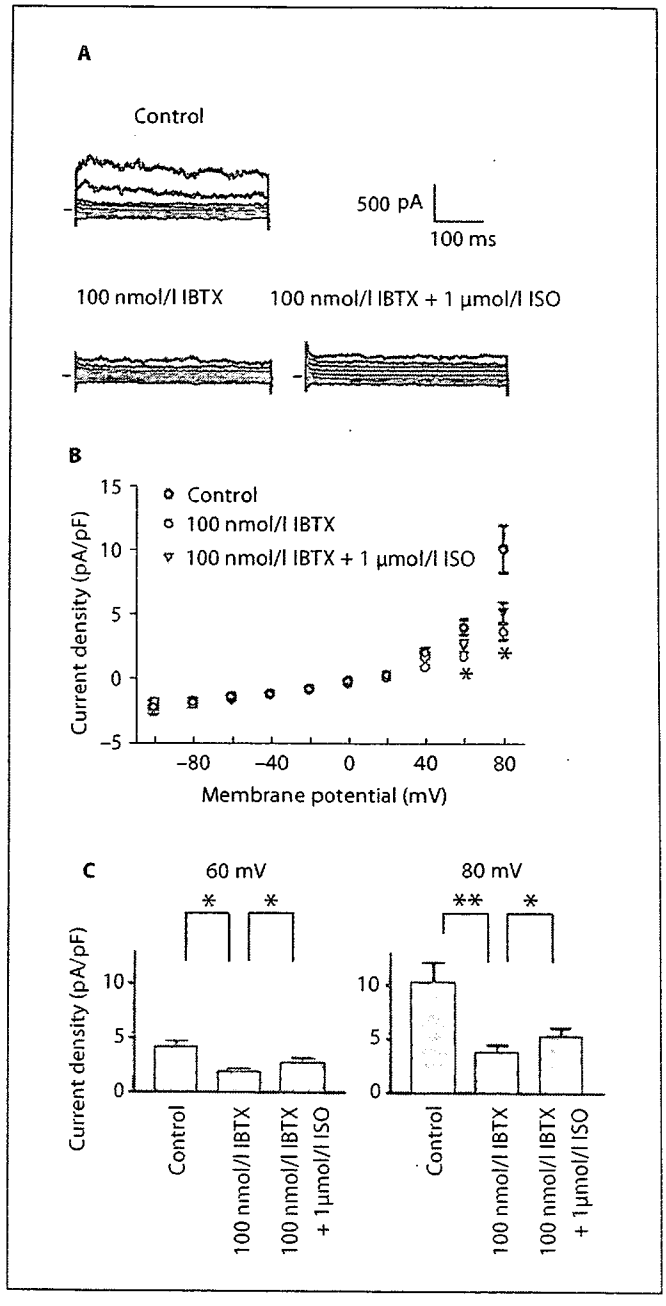

Fig. 2. Influences of iberiotoxin (IBTX) on the membrane currents and the potentiation by isoproterenol (ISO). **A** Representative current traces before (upper) and during (lower left) application of 100 nmol/l iberiotoxin and subsequent addition of 1 μmol/l isoproterenol (lower right) in smooth muscle cells from human urinary bladder. Membrane currents were elicited by 400-ms test pulses from a holding potential of −80 mV to potentials from −100 mV to +80 mV in 20-mV steps. Horizontal bars before the current traces indicate zero current level. **B** Current-voltage relationships of membrane currents in the absence (○) and presence of iberiotoxin (●) and subsequent addition of isoproterenol (▼). Values indicate means ± SEM of 6 experiments. * $p < 0.05$ between 3 states ($p = 0.010$ at +60 mV; $p = 0.005$ at +80 mV). **C** Current densities in the absence and presence of iberiotoxin and subsequent addition of isoproterenol. Membrane currents were elicited by a 400-ms depolarizing test pulse to +60 mV or +80 mV from a holding potential of −80 mV. * $p < 0.05$ between 2 states and ** $p < 0.01$ between 2 states ($n = 6$; $p = 0.013$ between control and iberiotoxin, $p = 0.033$ between iberiotoxin and subsequent addition of isoproterenol at +60 mV; $p = 0.005$ between control and iberiotoxin, $p = 0.034$ between iberiotoxin and subsequent addition of isoproterenol at +80 mV).

negated by the chelating action of intracellular EGTA at 5 mmol/l. With 5 mmol/l EGTA pipette solution, the membrane currents were not affected by β-adrenoceptor stimulation, so it is suggested that the increase in the outward current by β-adrenoceptor stimulation would be from an increase in K_{Ca} currents. These results indicate that the increase in outward membrane currents by the stimulation of β-adrenoceptors is dependent on the increase in the intracellular Ca^{2+} concentration. Taken together, the major part of the outward membrane currents of smooth muscle cells of human urinary bladder consists of K_{Ca} currents, and the stimulation of β-adrenoceptors increases these currents.

Expression of β-Adrenoceptor mRNA in the Cultured Smooth Muscle Cells from Human Urinary Bladder

To examine the expression of β-adrenoceptors in the cells used in this study, we performed the quantification of the expression levels of each β-adrenoceptor subtype by real-time PCR analysis using a cDNA synthesized from the mRNA used as a template. The quantitative data of expression levels of each β-adrenoceptor subtype, summarized in table 1, revealed that the β_2-adrenoceptor subtype was predominantly expressed in the smooth muscle cells from human urinary bladder compared to those of both β_1- and β_3-adrenoceptor subtypes.

Discussion

In the present study we demonstrated that stimulation of β_2-adrenoceptors in the smooth muscle cells of human urinary bladder increases the outward current which is iberiotoxin and apamin sensitive and blocked by intracellular high EGTA. This suggests that K_{Ca} currents are increased by β_2-adrenoceptor stimulation, resulting in the hyperpolarization and relaxation of the cells.

It has been shown that isoproterenol hyperpolarizes the smooth muscle of the detrusor in guinea pigs via stimulation of β-adrenoceptors [9]. In that study, it was

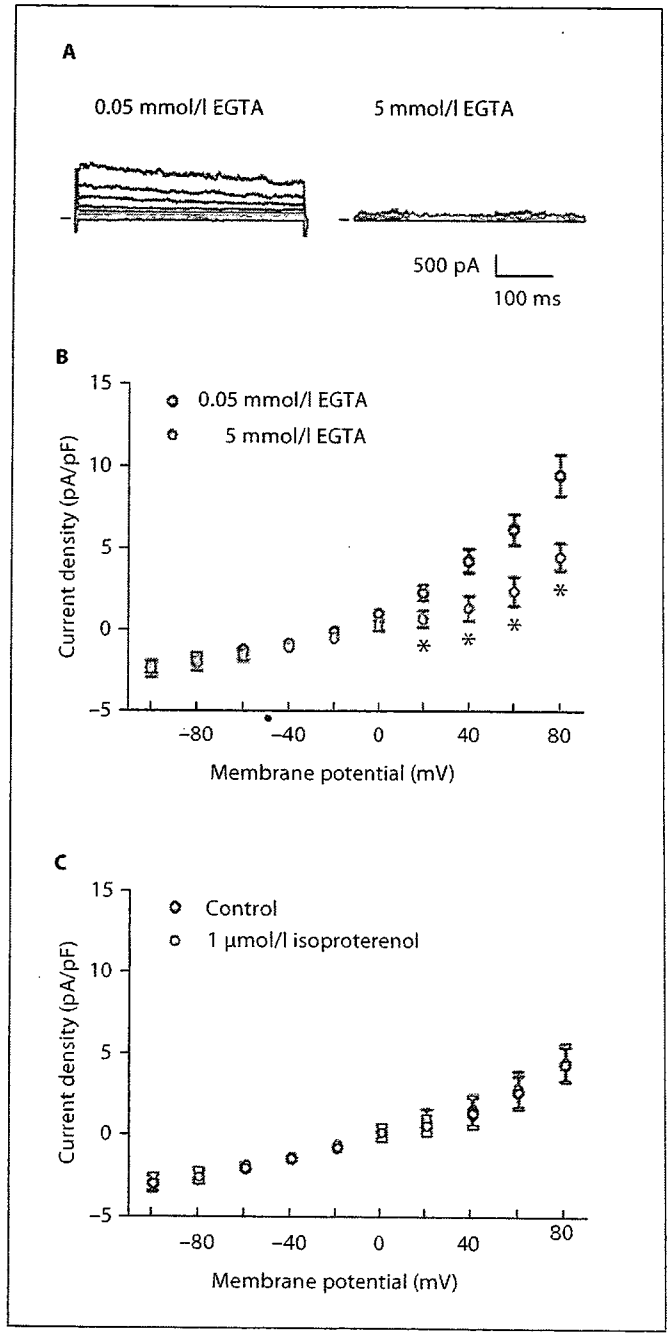

Fig. 3. Influence of 5 mmol/l EGTA pipette solution on the membrane currents of smooth muscle cells from human urinary bladder. **A** Representative current traces with 0.05 mmol/l (left) or 5 mmol/l (right) EGTA pipette solution. Membrane currents were elicited by 400-ms pulses from a holding potential of −80 mV to test potentials from −100 to +80 in 20-mV steps. Horizontal bars before the current traces indicate zero current level. **B** Current-voltage relationships of membrane currents with 0.05 mmol/l (○) and 5 mmol/l (●) EGTA pipette solution. Values indicate means ± SEM of 10 experiments with 0.05 mmol/l and 6 experiments with 5 mmol/l EGTA pipette solution. * $p < 0.05$ versus EGTA 0.05 mmol/l pipette solution ($p = 0.041$ at +20 mV; $p = 0.021$ at +40 mV; $p = 0.016$ at +60 mV; $p = 0.014$ at +80 mV). **C** Effects of isoproterenol on the currents recorded with patch pipette solution containing 5 mmol/l EGTA. Current-voltage relationships of membrane currents in the absence (○) and presence (●) of 1 μmol/l isoproterenol. Values indicate means ± SEM of 5 experiments.

Table 1. Comparison of the expression levels of each β-adrenoceptor subtype in the smooth muscle cells from human urinary bladder by real-time PCR

β-Adrenoceptor subtype	mRNA level normalized by GAPDH mRNA level, n × 10^{-6}
β_1	ND
β_2	2.92 ± 1.10
β_3	ND

Data are expressed as the mean ± SEM (n = 5). ND = Not detected.

suggested that the activity of the sodium pump was facilitated through the cAMP/PKA pathway. It has also been shown by other studies that hyperpolarization of the membrane of the detrusor smooth muscle could be induced through activation of various K^+ channels. Voltage-dependent K^+ channels [21], ATP-sensitive K^+ channels [22], K_{Ca} channels [23] and TREK-1 ($K_{2P}2.1$)channels [24] have been identified in the human detrusor muscle. Using knockout mice, it was shown that the BK_{Ca} channel plays an important role in slowing the excitability and contractility of the detrusor smooth muscle [25]. It was also shown that NS-1619, a BK_{Ca} channel opener, has a relaxant effect on guinea pig bladder smooth muscle [26]. Among the K^+ channels identified in the smooth muscle, the ATP-sensitive K^+ channel is known to be modulated by stimulation of β-adrenoceptors [27]. Kobayashi et al. [10] also showed that isoproterenol and forskolin facilitated iberiotoxin-sensitive BK_{Ca} channels in the smooth muscle cells from the urinary bladder of guinea pigs. The membrane currents potentiated by β-adrenoceptor agonists observed in the present study were voltage- and intracellular Ca^{2+}-dependent outward currents and also iberiotoxin and apamin sensitive. Therefore, it is likely that K_{Ca} currents are potentiated by β-adrenoceptor stimulation in the smooth muscle cells of human urinary bladder. In guinea-pigs' urinary bladder muscles, it was shown that stimulation of β-adrenoceptors activates BK_{Ca} channels by elevating Ca^{2+} influx through voltage-dependent Ca^{2+} channels and by increasing Ca^{2+} sparks [11]. In rat urinary bladder smooth muscle, β_2-adrenoceptors can simultaneously interact with both BK_{Ca} and L-type Ca^{2+} channels in vivo, which enables the assembly of a unique, highly localized signal transduction complex that mediates the Ca^{2+}- and phosphorylation-dependent modulation of the BK_{Ca} current [28]. In human urinary bladder smooth muscle, the β_2-adrenoceptor in the signaling complex seems to work like it does in rats. The BK_{Ca} channel interactions with its surrounding signaling partners and its targeting to cell-specific microdomains have been briefly reviewed [29]. To our knowledge, we have shown for the first time that β-adrenoceptor stimulation increases the BK_{Ca} and SK_{Ca} currents of the smooth muscle cells of human urinary bladder. An increase in the BK_{Ca} and SK_{Ca} currents of the human bladder smooth muscle cells by β-adrenoceptor agonists could hyperpolarize the muscle cells, leading to the inhibition of excitation, and the relaxation of the urinary detrusor muscle.

It is well known that isoproterenol relaxes human detrusor muscle [2]. There have been many functional studies on the β-adrenoceptor subtypes mediating relaxation of the urinary bladder. In rabbit bladder it was demonstrated that relaxation of the detrusor muscle is predominantly mediated by β_2-adrenoceptors. It was also proposed that β_2- and β_3-adrenoceptors are involved in the relaxation of rat urinary bladder [30]. A limited number of studies have also suggested the predominant involvement of β_3-adrenoceptors in the relaxation of the urinary bladder in dogs [30, 31] and monkeys [32]. The functional studies have demonstrated that the β_3-adrenoceptor is an important subtype for relaxation in the human bladder [13–15, 33–36] followed by the β_2-adrenoceptor [37]. Studies using PCR, Northern blots and in situ hybridization have detected mRNAs for the β_1-, β_2- and β_3-adrenoceptor subtypes in the human bladder [13, 14, 38]. Although it appears that more than 95% of all β-adrenoceptor mRNAs are the β_3-subtype in the human bladder, based upon quantitative PCR experiments [15], in the cultured smooth muscle cells of human urinary bladder, β_2-adrenoceptor mRNAs were detected as a predominant subtype in this study. Therefore, it is probable that isoproterenol and BRL 37344 increase the outward current via stimulation of β_2-adrenoceptors in the cultured smooth muscle cells.

Using cultured cells of human detrusor muscle with patch clamp methods, we have shown that stimulation of β_2-adrenoceptors increases the outward currents involved in BK_{Ca} and SK_{Ca} channels, which are important for the stabilization and relaxation of the muscle.

Acknowledgment

This study was supported by the Grants-in-Aid for Scientific Research from the Uehara Memorial Foundation and the Ministry of Education, Science, Sport and Culture, Japan (18613001).

References

1 Edvardsen P, Setekleiv J: Distribution of adrenergic receptors in the urinary bladder of cats, rabbits and guinea pigs. Acta Pharmacol Toxicol 1968;26:437–445.

2 Nergardh A, Boreus LO: Autonomic receptor function in the lower urinary tract of man and cat. Scand J Urol Nephrol 1972;6:32–36.

3 Elmer M: Inhibitory β-adrenoceptors in the urinary bladder of rat. Life Sci 1974;15:273–280.

4 Tanaka Y, Horinouchi T, Koike K: New insight into β-adrenoceptors in smooth muscle: distribution of receptor subtypes and molecular mechanisms triggering muscle relaxation. Clin Exp Pharmacol Physiol 2005;32:503–514.

5 Andersson KE, Arner A: Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 2004;84:935–986.

6 Yamagishi T, Yanagisawa T, Satoh K, Taira N: Relaxant mechanisms of cyclic AMP-increasing agents in porcine coronary artery. Eur J Pharmacol 1994;251:253–262.

7 Frazier EP, Mathy MJ, Peters SLM, Michel MC: Does cyclic AMP mediate rat bladder relaxation by isoproterenol? J Pharmacol Exp Ther 2005;313:260–267.

8 Yanagisawa T, Yamagishi T, Okada Y: Hyperpolarization induced by the K^+ channel openers inhibits Ca^{2+} influx and Ca^{2+} release in coronary artery. Cardiovasc Drugs Ther 1993;7:565–574.

9 Nakahira Y, Hashitani H, Fukuta H, Sasaki S, Kohri K, Suzuki H: Effects of isoproterenol on spontaneous excitation in detrusor smooth muscle cells of the guinea pig. J Urol 2001;166:335–340.

10 Kobayashi H, Adachi-Akahane S, Nagao T: Involvement of BK_{Ca} channels in the relaxation of detrusor muscle via β-adrenoceptors. Eur J Pharmacol 2000;404:231–238.

11 Petkov GV, Nelson MT: Differential regulation of Ca^{2+}-activated K^+ channels by β-adrenoceptors in guinea pig urinary bladder smooth muscle. Am J Physiol Cell Physiol 2005;288:C1255–C1263.

12 Nergardh A, Boreus LO, Naglo A-S: Characterization of the adrenergic β-receptor in the urinary bladder of man and cat. Acta Pharmacol Toxicol 1977;40:14–21.

13 Igawa Y, Yamazaki Y, Takeda H, Hayakawa K, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O, Andersson KE: Functional and molecular biological evidence for a possible β_3-adrenoceptor in the human detrusor muscle. Br J Pharmacol 1999;126:819–825.

14 Takeda M, Obara K, Mizusawa T, Tomita Y, Arai K, Tsutsui T, Hatano A, Takahashi K, Nomura S: Evidence for β_3-adrenoceptor subtypes in relaxation of the human urinary bladder detrusor: analysis by molecular biological and pharmacological methods. J Pharmacol Exp Ther 1999;288:1367–1373.

15 Nomiya M, Yamaguchi O: A quantitative analysis of mRNA expression of α_1 and β-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. J Urol 2003;170:649–653.

16 Arch JSK, Kaumann AJ: β_3 and atypical β-adrenoceptors. Med Res Rev 1993;13:663–729.

17 Yanagisawa T, Sato T, Yamada H, Sukegawa J, Nunoki K: Selectivity and potency of agonists for the three subtypes of cloned human β-adrenoceptors expressed in Chinese hamster ovary cells. Tohoku J Exp Med 2000;192:181–193.

18 Masumiya H, Tsujikawa H, Hino N, Ochi R: Modulation of manganese currents by 1,4-dihydropyridines, isoproterenol and forskolin in rabbit ventricular cells. Pflugers Arch 2003;446:695–701.

19 MacDonell KL, Severson DL, Giles WR: Depression of excitability by sphingosine 1-phosphate in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 1998;275:H2291–H2299.

20 Neher E: Correctin for liquid junction potentials in patch clamp experiments. Methods Enzymol 1992;297:123–131.

21 Davies AM, Batchelor TJP, Eardley I, Beech DJ: Potassium channel $K_v\alpha1$ subunit expression and function in human detrusor muscle. J Urol 2002;167:1881–1886.

22 Buckner SA, Milicic I, Daza A, Davies-Taber R, Scott VES, Sullivan JP, Brioni JD: Pharmacological and molecular analysis of ATP-sensitive K^+ channels in the pig and human detrusor. Eur J Pharmacol 2000;400:287–295.

23 Montgomery BSI, Fry CH: The action potential and membrane currents in isolated human detrusor smooth muscle cells. J Urol 1992;147:176–184.

24 Tertyshnikova S, Knox RJ, Plym MJ, Thalody G, Griffin C, Neelands T, Harden DG, Singor L, Weaver D, Myers RA, Lodge NJ: BL-1249 [(5,6,7,8-tetrahydro-naphthalen-1-yl)-(2-[1*H*-tetrazol-5-yl]-phenyl)amine]: a putative potassium channel opener with bladder-relaxant properties. J Pharmacol Exp Ther 2005;313:250–259.

25 Meredith AL, Thorneloe KS, Werner ME, Nelson MT, Aldrich RW: Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+}-activated K^+ channel. J Biol Chem 2004;279:36746–36752.

26 Malysz J, Buckner SA, Daza AV, Milicic I, Perez-Medrano A, Gopalakrishnan M: Functional characterization of large conductance calcium-activated K^+ channel openers in bladder and vascular smooth muscle. Naunyn Schmiedebergs Arch Pharmacol 2004;369:481–489.

27 Hudman D, Elliott RA, Norman RI: K_{ATP} channels mediate the β_2-adrenoceptor agonist-induced relaxation of rat detrusor muscle. Eur J Pharmacol 2000;397:169–176.

28 Liu G, Shi J, Yang L, Cao L, Park SM, Cui J, Marx SO: Assembly of a Ca^{2+}-dependent BK channel signaling complex by binding to β_2 adrenergic receptor. EMBO J 2004;23:2196–2205.

29 Lu R, Alioua A, Kumar Y, Eghbali M, Stefani E, Toro L: MaxiK channel partners: physiological impact. J Physiol 2006;570:65–72.

30 Yamazaki Y, Takeda H, Akahane M, Igawa Y, Nishizawa O, Ajisawa Y: Species differences in the distribution of β-adrenoceptor subtypes in bladder smooth muscle. Br J Pharmacol 1998;124:593–599.

31 Takeda H, Matsuzawa A, Igawa Y, Yamazaki Y, Kaidoh K, Akahane S, Kojima M, Miyata H, Akahane M, Nishizawa O: Functional characterization of β-adrenoceptor subtypes in the canine and rat lower urinary tract. J Urol 2003;170:654–658.

32 Takeda H, Yamazaki Y, Akahane M, Akahane S, Miyata H, Igawa Y, Nishizawa O: Characterization of β-adrenoceptor subtype in bladder smooth muscle of cynomolgus monkey. Jpn J Pharmacol 2002;88:108–113.

33 Igawa Y, Yamazaki Y, Takeda H, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O: Possible β_3-adrenoceptor-mediated relaxation of the human detrusor. Acta Physiol Scand 1998;164:117–118.

34 Igawa Y, Yamazaki Y, Takeda H, Kaidoh K, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O, Andersson KE: Relaxant effects of isoproterenol and selective β_3-adrenoceptor agonists on normal, low compliant and hyperreflexic human bladders. J Urol 2001;165:240–244.

35 Morita T, Iizuka H, Iwata T, Kondo S: Function and distribution of β_3-adrenoceptors in rat, rabbit and human urinary bladder and external urethral sphincter. J Smooth Muscle Res 2000;36:21–32.

36 Badawi JK, Uecelehan H, Hatzinger M, Michel MS, Haferkamp A, Bross S: Relaxant effects of β-adrenergic agonists on porcine and human detrusor muscle. Acta Physiol Scand 2005;185:151–159.

37 Badawi JK, Seja T, Uecelehan H, Honeck P, Kwon S-T, Bross S, Langbein S: Relaxation of human detrusor muscle by selective β_2 and β_3 agonists and endogenous catecholamines. Urology 2007;69:785–790.

38 Fujimura T, Tamura K, Tsutsumi T, Yamamoto T, Nakamura K, Koibuchi Y, Kobayashi M, Yamaguchi O: Expression and possible functional role of the β_3-adrenoceptor in human and rat detrusor muscle. J Urol 1999;161:680–685.