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REVIEW

Signal transduction underlying the control of urinary bladder smooth muscle tone by muscarinic receptors and β-adrenoceptors

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Abstract The normal physiological contraction of the urinary bladder, which is required for voiding, is predominantly mediated by muscarinic receptors, primarily the M_3 subtype, with the M_2 subtype providing a secondary backup role. Bladder relaxation, which is required for urine storage, is mediated by β-adrenoceptors, in most species involving a strong β_3-component. An excessive stimulation of contraction or a reduced relaxation of the detrusor smooth muscle during the storage phase of the micturition cycle may contribute to bladder dysfunction known as the overactive bladder. Therefore, interference with the signal transduction of these receptors may be a viable approach to develop drugs for the treatment of overactive bladder. The prototypical signaling pathway of M_3 receptors is activation of phospholipase C (PLC), and this pathway is also activated in the bladder. Nevertheless, PLC apparently contributes only in a very minor way to bladder contraction. Rather, muscarinic-receptor-mediated bladder contraction involves voltage-operated Ca^{2+} channels and Rho kinase. The prototypical signaling pathway of β-adrenoceptors is an activation of adenylyl cyclase with the subsequent formation of cAMP. Nevertheless, cAMP apparently contributes in a minor way only to β-adrenoceptor-mediated bladder relaxation. BK_{Ca} channels may play a greater role in β-adrenoceptor-mediated bladder relaxation. We conclude that apart from muscarinic receptor antagonists and β-adrenoceptor agonists, inhibitors of Rho kinase and activators of BK_{Ca} channels may have potential to treat an overactive bladder.

Keywords Bladder · Muscarinic receptor · β-adrenoceptor · Phospholipase C · cAMP · Rho kinase · BK_{Ca} channel · L-type Ca^{2+} channel

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Introduction

The urinary bladder stores urine for most of the time and expels it several times a day. The number of voiding events is highly species-dependent, i.e., fairly low (less than eight times per day) in healthy humans, but much higher in some animals species such as rats and particularly in those which use voiding as territorial marking behavior such as dogs. This requires a complex regulation of bladder function, which is under the control of the nervous system (Andersson and Arner 2004). Storage of urine requires the bladder body to distend which is mediated by smooth muscle relaxation of the detrusor. A key physiological mechanism to induce detrusor relaxation is β-adrenoceptor (β-AR) stimulation, which, in most species, involves a strong β_3-component, and in humans, predominantly if not exclusively, occurs via this receptor (Michel and Vrydag 2006). Concomitantly, sympathetic stimulation will also stimulate α_1-adrenoceptors in the bladder neck and urethra to provide bladder outlet resistance and prevent involuntary leakage of urine (Michel and Vrydag 2006). When a certain level of filling of the urinary bladder has been achieved and the central nervous system has decided that it is a socially acceptable time to void, efferent signaling switches from sympathetic

to parasympathetic stimulation. The released acetylcholine then activates muscarinic receptors in the detrusor to stimulate smooth muscle contraction and hence voiding (Abrams et al. 2006; Hegde 2006). This manuscript will review the signal transduction mechanisms involved in detrusor contraction by muscarinic receptors and detrusor relaxation by β-ARs. Other receptors systems, which can contribute to the regulation of bladder smooth muscle tone (Andersson 2006; Chetty et al. 2007; Chopra et al. 2005; Moffatt 2007; Rapp et al. 2005; Ukai et al. 2006), will not be covered. Moreover, the signal transduction underlying smooth muscle tone of the bladder neck or urethra will not be discussed here.

Muscarinic receptor signaling pathways

Although the mammalian bladder expresses more M_2 than M_3 receptors, contraction of the normal detrusor appears to occur largely via M_3 muscarinic receptors (Abrams et al. 2006; Hegde 2006). Muscarinic M_3 receptors in cell types other than detrusor smooth muscle cells can couple to a range of signaling pathways. Among them, stimulation of phospholipase C (PLC) with the subsequent formation of inositol trisphosphate (IP_3) and diacylglycerol (DAG) to release Ca^{2+} from intracellular stores and to activate a protein kinase C (PKC), respectively, are considered as prototypical signaling pathways (Caulfield and Birdsall 1998). However, various other signaling pathways have been demonstrated to be activated after stimulation of M_3 receptors. These include other phospholipases, such as phospholipase D (PLD; Felder 1995; Mamoon et al. 1999; Schmidt et al. 1995) and phospholipase A_2 (PLA_2)/cyclooxygenase (Felder 1995), protein kinases such as phosphatidylinositol-3-kinase (PI-3-kinase; Guizzetti and Costa 2001), tyrosine kinases (Inoue et al. 1994), mitogen-activated protein kinases (MAPK; Larocca and Almazan 1997), particularly those of the extracellular signal-regulated kinase (ERK) family (Wang et al. 1997) and Rho kinases (Schmidt et al. 1999), and also a range of ion channels. The latter group includes ion channels in intracellular organelles such as ryanodine receptors (Caulfield 1993; Simpson et al. 1996) and those in the plasma membrane including various types of K^+ channels, voltage-operated Ca^{2+} channels, and store-operated Ca^{2+} channels (Caulfield 1993). Most of these mechanisms have also been investigated for detrusor smooth muscle.

Phospholipase C

Activation of PLC is the prototypical signaling pathway of M_3 receptors (Caulfield and Birdsall 1998). It is believed to primarily occur via the α-subunits of $G_{q/11}$ G-proteins. Apart from the phosphatidylinositol-PLC (PI-PLC), which generates IP_3, this can also involve phosphatidylcholine-PLC (PC-PLC), both of which will lead to DAG formation (Caulfield and Birdsall 1998; Ehlert et al. 1997). Data in gastrointestinal smooth muscle indicate that PI-PLC may be involved in regulation of the phasic contraction, whereas PC-PLC may contribute to sustaining smooth muscle tone (Makhlouf and Murthy 1997).

Previous studies demonstrated that muscarinic receptor stimulation by carbachol in rat or guinea pig bladder enhances the formation of IP (Kories et al. 2003; Marsh et al. 1996; Nelson et al. 2004; Schneider et al. 2004b). Using the subtype-selective antagonist, darifenacin, the carbachol-stimulated IP formation in the urinary bladder was demonstrated to indeed occur predominantly if not exclusively via M_3 receptors (Kories et al. 2003; Nelson et al. 2004).

An involvement of PLC in bladder tone has been assessed using several inhibitors: U 73,122 and ET-18-OCH_3 were used as PI-PLC inhibitors, D609 as a PC-PLC inhibitor, and neomycin as a non-specific PLC inhibitor. Alternatively, IP_3 receptor inhibitors such as 2-aminoethoxyphenyl borate (2-APB) or heparin have also been used. The effects of 2-APB on bladder function are not easy to understand because this compound was reported to increase the frequency of spontaneous action potentials but nevertheless to reduce contraction amplitudes in the absence of autonomic stimulation in guinea pig detrusor smooth muscle cells (Imai et al. 2002). While studies in guinea pig bladder did not detect inhibitory effects of 2-APB, similar experiments in rat bladder reported this compound to inhibit carbachol-induced calcium sensitization (Durlu-Kandilci and Brading 2006). Similarly, intracellular heparin administration was reported to inhibit acetylcholine-induced contraction of feline detrusor cells (An et al. 2002). The latter study in cats has also suggested that PLC activation may be necessary for muscarinic agonist-induced bladder contraction using the PLC inhibitor neomycin (An et al. 2002). Studies in rats using ET-18-OCH_3 have also proposed an involvement of PI-PLC in rat bladder contraction, although this was not confirmed within the same study when neomycin was used as the inhibitor (Braverman et al. 2006b). In contrast, other groups of investigators using different experimental protocols and the PLC inhibitor U 73,122 have reached somewhat different conclusions, as this compound did not affect carbachol-induced contraction in rat (Schneider et al. 2004b), mouse (Wegener et al. 2004), or human bladder (Schneider et al. 2004a) in concentrations where it fully suppressed IP formation in rat bladder slices (Schneider et al. 2004b). As the contradictory conclusions in rats were based upon different inhibitors (ET-18-OCH_3 and U 73,122) and on different experimental protocols (single vs multiple concentration–response curves per muscle strip), these inves-

tigators have recently collaborated in a crossover study in which each lab used its own protocol to test the inhibitors previously used by the other laboratory (Frazier et al. 2007). In that study, neither of the PI-PLC inhibitors tested [U 73,122 (Fig. 1) and ET-18-OCH_3] exerted a significant inhibition of the carbachol-induced bladder contraction. In confirmation of the earlier study (Braverman et al. 2006b), the PC-PLD inhibitor D609 was quite effective in high concentrations, but at this concentration, it also attenuated contraction in response to the receptor-independent stimulus KCl, indicating that the D609 response was not specific to the muscarinic receptor activation. These crossover experiments indicated that M_3-receptor-mediated PLC activation in the rat bladder is not a major contributor to detrusor contraction.

Phospholipase D

PLD is located in the plasma membrane and can catalyze the hydrolysis of phophatidylcholine to form phosphatidic acid (PA). Hydrolysis of PA by the enzyme PA phosphohydrolase forms DAG. DAG can be converted back into PA by DAG kinase. Although DAG and PA are interchangeable, they do not share the same signaling mechanisms to exert their biological effects. Because DAG can also be generated upon stimulation of PI-PLC and PC-PLC (see above), it is difficult to identify the enzyme responsible for DAG formation. A potential role of DAG will be discussed further below in the context of a role for PKC.

An involvement of PLD in biological responses can be assessed using the inhibitor butan-1-ol and its isomer butan-2-ol, which does not inhibit PLD (Banno et al. 2001). Another PLD inhibitor, which has been used in bladder studies, is para-chloromercuribenzoic acid (An et al. 2002; Yang et al. 2000). In rat bladder slices, carbachol caused only a little, if any, PLD activation compared to the positive control phorbol myristate acetate (Schneider et al. 2004b).

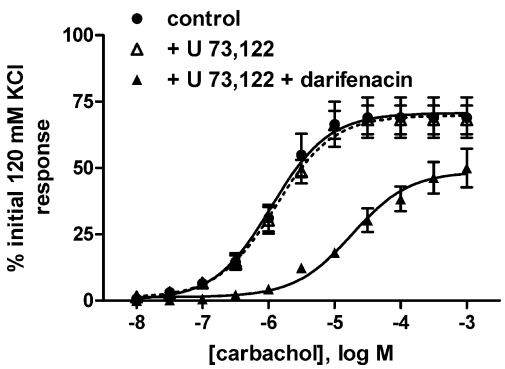

Fig. 1 Effect of U 73,122 (10 μM) alone and in combination with darifenacin (30 nM) on carbachol-induced rat bladder contraction. Taken from (Frazier et al. 2007)

Accordingly, in rat and human urinary bladder, butan-1-ol, as compared to its inactive control, caused only little inhibition, which was limited to its highest concentration (Schneider et al. 2004a). Similarly, para-chloromercuribenzoic acid did not inhibit contractile response to acetylcholine in feline detrusor (An et al. 2002; Yang et al. 2000). Taken together, these data suggest that PLD only plays a minor, if any, role in muscarinic receptor agonist-induced contraction of the urinary bladder.

Phospholipase A_2/cyclooxygenase

PLA_2 specifically catalyzes the hydrolysis of the sn-2 fatty acyl bond of phospholipids to generate free fatty acid and lysophospholipids, comprising arachidonic acid (AA) which can be further metabolized to prostaglandins by cyclooxygenase (Felder 1995). There are two main families of PLA_2 containing several members with common enzymatic activity. One family of PLA_2 is the small molecular weight secretory PLA_2 which consists of mainly calcium-dependent enzymes. The other family consists of both calcium-dependent and calcium-independent cytosolic PLA_2 ($cPLA_2$) isoforms and can be inhibited by 1,1,1-trifluoromethyl-6,9,12,15-eicosatetraen-2-one. Studies into a role for PLA_2 in muscarinic-receptor-induced bladder contraction have mainly focussed on $cPLA_2$. Moreover, several cyclooxygenase products can also generate detrusor smooth muscle contraction (Andersson 2000). At least in rat bladder, protease-activated receptor-2-induced contraction is partly mediated by activation of cyclooxygenase (Nakahara et al. 2004).

A study in rat bladder using 1,1,1-trifluoromethyl-6,9,12,15-eicosatetraen-2-one indicated only a very minor, if any, role for PLA_2 in carbachol-induced contraction (Schneider et al. 2004b). Moreover, in the same study, inhibition of cyclooxygenase by indomethacin at a concentration inhibiting protease-activated receptor-2-mediated contraction (Nakahara et al. 2004) did not inhibit the carbachol response. Similar findings were obtained in feline bladder (An et al. 2002). Hence, the available data suggest that neither of PLA_2 nor cyclooxygenase plays a major role in the muscarinic-receptor-mediated bladder contraction, although this may be different for other contractile stimuli such as protease-activated receptor-2-induced contraction, which is partly mediated by activation of cyclooxygenase (Nakahara et al. 2004) or bradykinin (Kubota et al. 2003).

Protein kinase C

PKC is physiologically activated by DAG, and this can occur subsequent to stimulation of PI-PLC, PC-PLC or PLD (see above) and, in some types of smooth muscle, may contribute to the sustained phase of contraction (Aburto et

al. 1995; Dessy et al. 1998). This involvement can be assessed using PKC inhibitors such as chelerythrine, calphostin C, Gö 6850, and GF 109203X, but most of these inhibitors can have ancillary properties which complicate interpretation of the data. A role for PKC can also be studied using activators such as β-phorbol-12,13-dibutyrate. While the later activates PKC upon short-term exposure, longer exposure can reduce cellular PKC activity. Finally, it should be considered that some muscarinic receptor antagonists such as propiverine and its metabolites also can have PKC inhibitory effects in the bladder (Moritz et al. 2005); however, apart from its muscarinic receptor antagonism, propiverine and some of its metabolites also have calcium channel blocking activity, which further complicates interpretation of data obtained with this compound (Michel and Hegde 2006).

The PKC activator β-phorbol-12,13-dibutyrate augments bladder contractility by electrical field stimulation in mouse and rat (Liu and Lin-Shiau 2000; Weng et al. 2005). While a PKC inhibitor may attenuate muscarinic-receptor-mediated calcium sensitization in the rat or guinea pig bladder (Durlu-Kandilci and Brading 2006), such drugs did not inhibit peak contractile responses to muscarinic agonists in rat (Durlu-Kandilci and Brading 2006; Fleichman et al. 2004), feline (An et al. 2002), or human bladder (Schneider et al. 2004a). Thus, the overall role for PKC in muscarinic-receptor-mediated bladder contraction appears small, which is in line with the negative data regarding an involvement of PI-PLC, PC-PLC, and PLD.

Tyrosine kinases

Tyrosine kinase signaling can be involved in Ca^{2+} sensitization in smooth muscle, and this may involve the activation of RhoA and Rho kinase (ROCK; Somlyo and Somlyo 2003). The role of tyrosine kinases in modulating vascular, visceral, and airway smooth muscle contraction has been documented (Chopra et al. 1997; Dessy et al. 1998; Di Salvo et al. 1994; Grasa et al. 2006; Jin et al. 1996; Jinsi et al. 1996; Steusloff et al. 1995; Tolloczko et al. 2000), specifically for the src family of tyrosine kinases (Roberts 2001; Tolloczko et al. 2002). Tyrosine kinases have also been reported to control Ca^{2+} entry and intracellular Ca^{2+} concentration in vascular smooth muscle (Carter and Kanagy 2002; Lagaud et al. 1999; Toma et al. 1995; Wijetunge et al. 2000).

Tools to asses an involvement of tyrosine kinase include genistein and its negative control daidzein, the src-specific inhibitor PP1, PP2, and its negative control PP3, and the c-kit tyrosine kinase inhibitor (Glivec®). In the guinea pig detrusor smooth muscle, which expressed c-kit positive cells, the c-kit tyrosine kinase inhibitor concentration-dependently suppressed the spontaneous contraction whereas carbachol-induced contraction was only attenuated by a high concentration of the c-kit inhibitor (Kubota et al. 2004). Similarly, neither genistein nor PP2 inhibited the carbachol-induced contraction of rat bladder (Fleichman et al. 2004). Thus, tyrosine kinases apparently do not contribute to muscarinic receptor agonist-induced bladder contraction in a major way.

Mitogen-activated kinase

Studies on a role of the ERK family of MAPK in vascular smooth muscle contraction have yielded conflicting results with positive (Dessy et al. 1998; Fetscher et al. 2001; Roberts 2001; Xiao and Zhang 2002) and negative (Altmann et al. 2003; Janssen et al. 2001; Watts et al. 1998) data having been reported. PD 98,059 and U 126 are inhibitors of ERK activation, and U 124 is a negative control for the latter. ERK signaling has been suggested to be involved in the rat urinary bladder contraction during inflammation induced by uropathogenic *Escherichia coli*, but not in healthy bladder contraction (Weng et al. 2006). Accordingly, inhibition of ERK activation by PD 98,059 or U 126 did not attenuate the carbachol response in the normal rat bladder (Fleichman et al. 2004), indicating that ERK activation is not required for the muscarinic-receptor-induced bladder contraction under physiological condition.

Phosphatidylinositide 3-kinase

PI-3-Ks are enzymes that specifically catalyze phosphorylation of the 3-position in the inositol ring of the phosphoinositides (Rameh and Cantley 1999). They are ubiquitously expressed, and multiple isoforms exist (Northcott et al. 2004; Rameh and Cantley 1999). Activation of PI-3-kinases has been associated with smooth muscle contraction in colonic (Ibitayo et al. 1998) and some preparations of vascular smooth muscle cells via Rho kinase-dependent inhibition of MLCP (Wang et al. 2006; Yoshioka et al. 2007), but they may not be involved in smooth muscle tone of other preparations (Altmann et al. 2003). Wortmannin and LY294002 are commonly used PI-3-kinase inhibitors, and an inactive analogue of LY294002 exists. A single study in rat bladder using wortmannin, LY294002, and the inactive analogue of the latter did not support a role for PI-3-kinase in carbachol-induced contraction (Fleichman et al. 2004).

Rho kinase

ROCK are serine/threonine kinases of ~160 kDa and play a role in various cellular functions (Riento and Ridley 2003; Somlyo and Somlyo 2003). Two isoforms designated as ROCK I (also known as ROK_{β} or p160ROCK) and ROCK II (ROK_{α} or Rho kinase) exist. These isoforms and their physiological activator RhoA are expressed in many

tissues, including urinary bladder, at both the mRNA and protein level (Chang et al. 2006; Takahashi et al. 2004; Wibberley et al. 2003). ROCK activation can enhance smooth muscle contraction by multiple mechanisms (Riento and Ridley 2003; Somlyo and Somlyo 2003).

Y-27,632, fasudil, and HA-1077 are ROCK inhibitors which are commonly used to assess the involvement of this kinase in biological responses. In the urinary bladder of humans (Fig. 2) and other mammals, Y-27,632, fasudil, and HA-1077 inhibit both phasic and sustained contraction induced by several stimuli including muscarinic receptor agonists (Braverman et al. 2006a; Fleichman et al. 2004; Jezior et al. 2001; Schneider et al. 2004a,b; Speich et al. 2005; Takahashi et al. 2004; Wibberley et al. 2003). Whereas all available studies indicate that ROCK inhibitors reduced potency of muscarinic receptor agonist carbachol in the bladder, results regarding the carbachol efficacy are conflicting (Braverman et al. 2006b; Fleichman et al. 2004; Schneider et al. 2004a, 2005; Takahashi et al. 2004). Based upon a reduction of the potency of M_3-selective antagonist darifenacin in the presence of Y-27,632, it has been proposed that ROCK may have a specific role in the M_2 receptor contribution to bladder tone (Braverman et al. 2006b). As the overall role of M_2 receptors in bladder tone requires further clarification (Abrams et al. 2006; Hegde 2006), this proposal is awaiting confirmation by other investigators. In contrast to the consistently reported role of ROCK in muscarinic-receptor-mediated bladder contraction in vitro, ROCK inhibitors were reported not to affect physiological bladder contraction in vivo in healthy animals, but only under pathophysiological conditions (Rajasekaran et al. 2005). A possible role of ROCK in bladder dysfunction and its treatment has recently been reviewed (Peters et al. 2006).

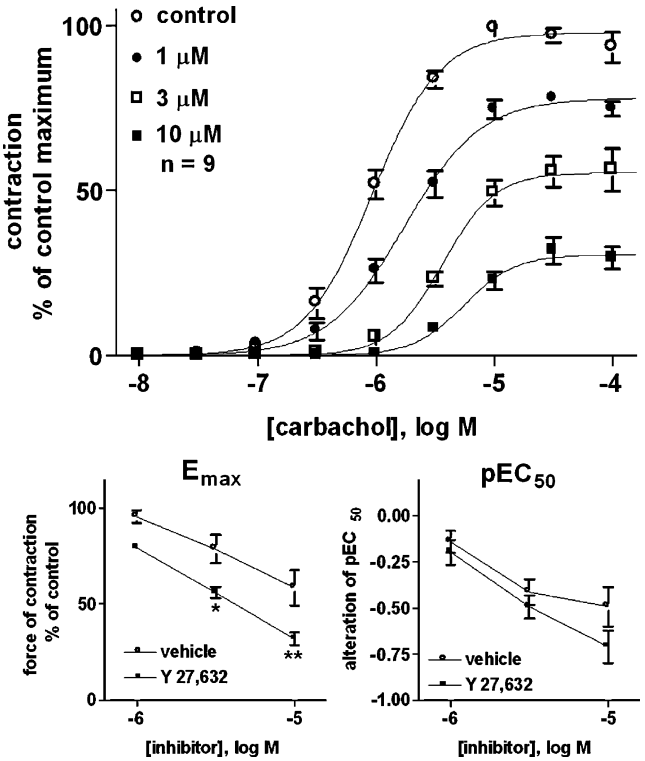

Fig. 2 Effects of the Rho kinase inhibitor Y 27,632 and its vehicle on carbachol-induced contraction. The *upper panel* shows carbachol concentration-response curves in the absence and presence of various Y 27,632 concentrations. The *lower two panels* show alterations of pEC_{50} and E_{max} relative to matched time controls. *$p<0.05$, **$p<0.01$ vs matching time controls in the presence of vehicle in a two-way analysis of variance followed by Dunnet post-tests. Taken from (Schneider et al. 2004a)

Ca^{2+} channels

Similar to all other types of smooth muscle, bladder contraction requires elevation of intracellular Ca^{2+} concentrations. While several studies have demonstrated that removal of extracellular Ca^{2+} will impair muscarinic-receptor-mediated bladder contraction (Jezior et al. 2001; Visser and van Mastrigt 2000), the relative roles of influx from the extracellular space and of mobilization from intracellular stores remain a subject of discussion and possibly are species-dependent (Wuest et al. 2007). At least four types of Ca^{2+} channels can be involved in bladder smooth muscle contraction, including voltage-operated channels, store-operated channels, IP_3-receptors, and ryanodine receptors. Voltage-operated and store-operated Ca^{2+} channels are abundant in the plasma membrane and contribute to the influx of Ca^{2+} from extracellular space, whereas Ca^{2+} release from intracellular stores involves activation of IP_3 and ryanodine receptors in the membranes of the sarcoplasmic reticulum.

The group of voltage-operated Ca^{2+} channels contains several types, among which, the L-type appears most relevant and has been most frequently studied with regard to the bladder. L-type Ca^{2+} channels can be inhibited by drugs such as nifedipine or diltiazem. Moreover, knockout mice have been generated, which lack a crucial subunit of L-type Ca^{2+} channels (Wegener et al. 2004). Various investigators have shown that L-type Ca^{2+} channel inhibitors reduce muscarinic-receptor-mediated detrusor contraction in rat (Schneider et al. 2004b), mouse (Wuest et al. 2007), rabbit (Zderic et al. 1994), pig (Buckner et al. 2002; Uchida et al. 1994; Wuest et al. 2007), and human (Fig. 3; Masters et al. 1999; Schneider et al. 2004a; Wuest et al. 2007). Interestingly, inhibition of bladder contraction was observed at inhibitor concentrations which are lower than those typically required to inhibit the contraction of vascular smooth muscle. Accordingly, knockout mice for the Cav1.2 L-type Ca^{2+} channel exhibited markedly reduced detrusor contraction in response to muscarinic

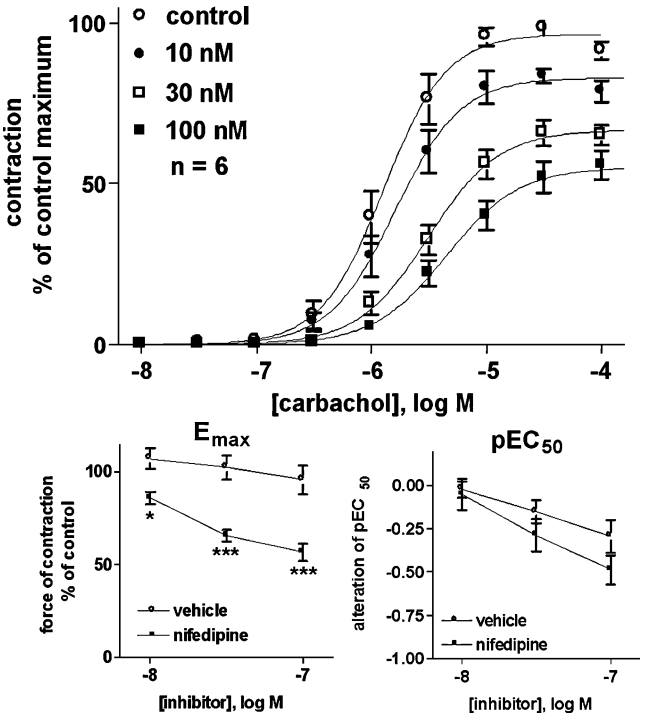

Fig. 3 Effects of the Ca^{2+} channel inhibitor nifedipine and its vehicle on carbachol-induced contraction. The *upper panel* shows carbachol concentration-response curves in the absence and presence of various nifedipine concentrations. The *lower two panels* show alterations of pEC_{50} and E_{max} relative to matched time controls. $*p<0.05$, $***p<0.001$ vs matching time controls in the presence of vehicle in a two-way analysis of variance followed by Dunnet post-tests. Taken from (Schneider et al. 2004a)

agonists in vitro and also had dilated bladders in vivo (Wegener et al. 2004). While it has been proposed that Q-type voltage-operated Ca^{2+} channels may also contribute to bladder tone under some circumstances, N- and T-type Ca^{2+} channels apparently are not involved (Frew and Lundy 1995). Similarly, non-selective cation channels, Ca^{2+}-activated Cl^- channels, and Na^+/Ca^{2+} exchanger do not play major role in Ca^{2+} influx in the bladder (Ganitkevich and Isenberg 1992; Nakayama and Brading 1993).

While the relative role of L-type Ca^{2+} channels in the muscarinic-receptor-mediated detrusor contraction may differ between species (Wuest et al. 2007), the above data clearly demonstrate their overall importance. However, this does not exclude the possibility that their role may be indirectly, e.g., related to the filling of intracellular stores acted upon by other signaling pathways. Moreover, a number of relevant questions remain to be answered. Firstly, the specific mechanism of how muscarinic receptor activation couples to L-type Ca^{2+} channels remains to be elucidated. In this regard, it has been proposed that at least in guinea pigs, the Ca^{2+} needed for contraction enters the cell through voltage-dependent Ca^{2+} channels and is then pumped into an intracellular store from where it is released by muscarinic agonists (Rivera and Brading 2006). Other work in guinea pigs also supports a role for L-type Ca^{2+} channels in maintaining Ca^{2+} entry and refilling intracellular stores in detrusor smooth muscle (Wu et al. 2002). Secondly, some electrophysiological studies across multiple species have reported that muscarinic agonists may inhibit the current through L-type Ca^{2+} channels (Kajioka et al. 2002; Yoshino and Yabu 1995). Those authors argued that this may involve Ca^{2+}-mediated inactivation of Ca^{2+} channels triggered by the release of Ca^{2+} from IP_3 and thapsigargin-sensitive internal stores or by a G-protein-mediated mechanism. The reason for the discrepancy between these electrophysiological studies and the contractility data based upon pharmacological channel inhibitors and knockout mice remains to be elucidated. Thirdly, some clinically used muscarinic receptor antagonists including oxybutynin (Kachur et al. 1988) and propiverine (Wuest et al. 2006) and/or their metabolites (Michel and Hegde 2006) also have direct inhibitory effects on L-type Ca^{2+} channels. How much this may contribute to their clinical effects on the bladder remains to be determined. Finally, inhibitors of L-type Ca^{2+} channels are frequently used in the treatment of cardiovascular disease, but during their use, no major adverse events on bladder function have surfaced. The reasons for a major role of such channels in bladder contractility vs a lack of adverse effects of their inhibitors on the bladder may relate to pharmacokinetic properties of the clinically used drugs, which may not reach sufficiently high concentrations in bladder tissue.

Calcium can also enter the cell from the extracellular space via store-operated Ca^{2+} channels which are sensitive to the inhibitor SK&F 96,365. However, SK&F 96,365 was reported to exert only minor, if any, inhibition of carbachol-induced rat bladder contraction (Schneider et al. 2004b). Therefore, it is difficult to judge other findings reporting that suppression of IP_3 release may impair store-operated Ca^{2+} channel function (Bootman et al. 2002).

With regard to channels mediating calcium mobilization from intracellular stores, a possible role of the IP_3 receptor as a Ca^{2+} channel involved in bladder contraction has been discussed in the PLC section. Another type of channel possibly involved in mobilization of calcium from intracellular stores is the ryanodine receptor, which can be inhibited by ryanodine and is expressed in the human urinary bladder (Chambers et al. 1999). Ryanodine has been reported to inhibit bladder contraction in rabbits (Zderic et al. 1994), guinea pig (Buckner et al. 2002), and human bladder (Visser and van Mastrigt 2000), but other investigators did not confirm such inhibition in mouse, pig, or human bladder (Wuest et al. 2007). Ryanodine receptors have also been implied in the muscarinic stimulation of large transient inward and small oscillating inward currents in porcine bladder (Kajioka et al. 2005), but the relationship

of this involvement with detrusor smooth muscle tone remains to be defined.

β-Adrenoceptor relaxation signaling pathways

Noradrenaline released from the hypogastric nerves activates β-AR and induces detrusor smooth muscle relaxation. All three cloned β-AR subtypes are present in the urinary bladder of rat and humans at the mRNA level, and at least in humans, the β_3-AR is by far the most abundantly expressed subtype (Fujimura et al. 1999; Matsubara et al. 2002; Seguchi et al. 1998). Studies at the protein level are difficult to interpret because none of the available radioligands is suited to detect β_3-AR (Baker 2005; Hoffmann et al. 2004; Niclauss et al. 2006). Functionally, it appears that detrusor relaxation involves a strong β_3-AR component in most species and may be the predominant, if not sole, mediator of bladder relaxation in humans (Michel and Vrydag 2006). Additional contributions by other β-AR subtypes are less well defined and may differ between species (Michel and Vrydag 2006). Most studies on signal transduction processes underlying β-AR-mediated bladder relaxation have not specifically studied receptor subtypes, and hence, it remains possible that some of the effects described below actually represent a mixture of effects mediated by multiple subtypes.

Cyclic AMP formation

The prototypical signaling pathway of β-AR is activation of adenylyl cyclase to elevate intracellular cAMP concentrations (Bylund et al. 1994). Sequentially, cAMP activates protein kinase A (PKA) or alternative effector molecules such as epac (Schmidt et al. 2007) to mediate smooth muscle relaxation. Hence, β-AR agonists such as isoprenaline can elevate cAMP formation in rat bladder smooth muscle cells (Derweesh et al. 2000; Ma et al. 2002; Uchida et al. 2005). The functional role of adenylyl cyclase can be tested by inhibitors such as SQ 22,536 or Rp-cAMPs, whereas that of PKA can be assessed by inhibitors including H7 and H89.

To test the role of cAMP formation and PKA in bladder relaxation, three studies have recently been reported from rat bladder. In one study using the β_2-agonist clenbuterol against field stimulation-induced tone, a PKA inhibitor was reported to block relaxation while not affecting the field stimulation-induced contraction (Hudman et al. 2000). Two other studies investigated used isoprenaline as the agonist (one additionally included a β_3-selective agonist) against the effects of isoprenaline on both passive tension and KCl-induced tone (Fig. 4; Frazier et al. 2005; Uchida et al. 2005). Both studies have used adenylyl cyclase inhibitors such as SQ 22,536 and Rp-cAMPs and also PKA inhibitors such as H7 and H89. While their quantitative findings differ slightly, they both show that cAMP formation does not contribute to relaxation against KCl-induced tone. While some contribution exists for relaxation against passive tension, even under those conditions, it may account for only a minor part of the response. One of the studies additionally demonstrates that a guanylyl cyclase also is not involved to a major extent (Frazier et al. 2005). These findings indicate that the cAMP/PKA pathway may contribute to bladder relaxation by a β_2-AR, but less, if at all, to that induced by a β_3-selective or a non-selective

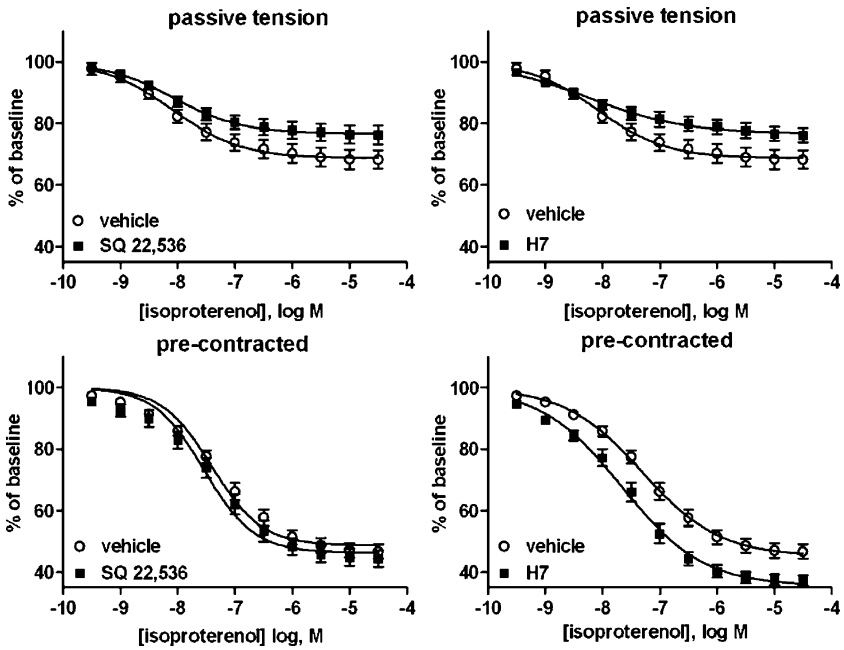

Fig. 4 Effect of the adenylyl cyclase inhibitor SQ 22,536 (1 μM) and the protein kinase A inhibitor H7 (10 μM) on isoproterenol-induced relaxation of rat urinary bladder. Taken from (Frazier et al. 2005)

agonist. The latter findings are in line with recent data from various other types of smooth muscle which have indicated that various types of K^+ channels may be more important in β-AR-mediated relaxation than cAMP (Ferro 2006).

K^+ channels

Numerous types of K^+ channels exist, which are not only activated under distinct conditions but also mediate different physiological functions (Brading 1992). Channels which have been investigated in the bladder include voltage-gated (K_v) channels (Davies et al. 2002; Thorneloe and Nelson 2003), small conductance (SK) channels (Heppner et al. 2003; Herrera et al. 2000; Herrera and Nelson 2002), ATP-sensitive (K_{ATP}) channels (Bonev and Nelson 1993; Buckner et al. 2000; Gopalakrishnan et al. 1999), and large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) channels (Klockner and Isenberg 1985; Markwardt and Isenberg 1992; Petkov et al. 2001; Suarez-Kurtz et al. 1991; Trivedi et al. 1995; Zografos et al. 1992). Many of these channels can be activated by β-AR stimulation, leading to an efflux of potassium and, hence, a hyperpolarization and reduced tone of the smooth muscle cells (Ferro 2006). The available data indicate that K_{ATP} and BK_{Ca} channels are the two types of K^+ channels which provide the greatest contributions to detrusor smooth muscle tone.

K_v channels are expressed in human urinary bladder (Davies et al. 2002) and have been reported to be functionally important in regulating detrusor tone both in mouse (Thorneloe and Nelson 2003) and human bladder (Chen et al. 2004; Davies et al. 2002). However, no data on their possible involvement in β-AR-mediated bladder relaxation has been reported.

K_{ATP} channels are expressed in rat (Ha et al. 1993), guinea pig (Gopalakrishnan et al. 1999), pig and human detrusor (Buckner et al. 2000). Their functional role can be assessed by activators such as pinacidil, cromakalim, or rimakalim or by inhibitors such as glibenclamide. Their overall role in the regulation of bladder smooth muscle tone is documented by numerous in vitro and in vivo studies demonstrating that K_{ATP} channel openers can relax bladder smooth muscle. Such data were obtained for several representatives of this drug class including pinacidil (Edwards et al. 1991; Malmgren et al. 1990; Vijayakumar et al. 2007), cromakalim (de Moura et al. 1993; Foster et al. 1989a,b; Fujii et al. 1990; Malmgren et al. 1990; Vijayakumar et al. 2007), lemakalim (Bonev and Nelson 1993), rimakalim (Wuest et al. 2005), ZD097 (Aishima et al. 2006), ZD 6169, and WAY-133537 (Gopalakrishnan et al. 2002). Such functional data were reported from various species including rat (Edwards et al. 1991; Hudman et al. 2000), mouse (de Moura et al. 1993), guinea pig (Bonev and Nelson 1993; Foster et al. 1989a; Fujii et al. 1990; Gopalakrishnan et al. 1999), pig (de Moura et al. 1993; Foster et al. 1989b; Uchida et al. 1994), goat (Vijayakumar et al. 2007), and humans (Aishima et al. 2006; de Moura et al. 1993; Foster et al. 1989b; Gopalakrishnan et al. 2002). This broad spectrum of species shows that the role of K_{ATP} channels for bladder smooth muscle tone stretches across mammalian species. Moreover, K_{ATP} channel openers reduce both spontaneous and agonist-induced contraction of the detrusor, independent of the contractile stimulus being used (Buckner et al. 2000; Malmgren et al. 1990). Their effects also are independent of the presence of a functional urothelium, with the possible exception of pigs (de Moura et al. 1993), underscoring their primary direct effects on the smooth muscle cells. However, a key problem with the use of K_{ATP} channel openers is that they also work on vascular smooth muscle cells and, therefore, lower blood pressure in vivo (Foster et al. 1989a,b). Therefore, it is not surprising that a clinical study using a K_{ATP} channel opener in OAB patients at a non-hypotensive dose failed to detect symptom improvement (Chapple et al. 2006)

While the above data clearly demonstrate the importance of K_{ATP} channels in the regulation of bladder tone, they do not allow direct conclusions regarding their role in β-AR-mediated bladder relaxation. This can be assessed by testing the effects of K_{ATP} channel inhibitors such as glibenclamide against β-AR stimulation (Frazier et al. 2005; Hudman et al. 2000). Glibenclamide abolished relaxation of field stimulation-induced contraction by the β_2-AR agonist clenbuterol in rat bladder (Hudman et al. 2000). On the other hand, glibenclamide failed to attenuate the relaxant effect of isoprenaline in rat bladder against passive or KCl-induced tension (Frazier et al. 2005). These data highlight the possibility that different β-AR subtypes in the bladder may utilize distinct signaling pathways to elicit smooth muscle relaxation.

SK channels are expressed in the urinary bladder (Chen et al. 2004), and their role in bladder function has been reviewed previously (Heppner et al. 2003). SK channels are Ca^{2+}-sensitive and can be activated by the influx of Ca^{2+} via voltage-operated Ca^{2+} channels (Herrera and Nelson 2002). They can influence the function of ryanodine receptors in the detrusor (Herrera et al. 2000). In guinea pigs, SK channels play a role as a negative feedback element to reduce the amplitude and duration of detrusor contraction (Herrera et al. 2000). In vivo studies in genetically modified mice either overexpressing the SK_3 isoform in the bladder or having an inducible knockdown of this channel demonstrated a greater bladder capacity in the overexpression as compared to the WT mice and those with reduced SK_3 expression. Moreover, the mice with suppressed SK_3 expression exhibited marked increases of non-voiding contraction, suggesting a role of these channels

in limiting non-voiding contraction (Herrera et al. 2003). However, an involvement of these channels in the β-AR-mediated bladder relaxation has not been reported.

A role for BK_{Ca} channels can be assessed pharmacologically by inhibitors such as charybdotoxin or iberiotoxin. Functional evidence, based upon pharmacological modulation as well as genetically modified animals, demonstrates a role for BK_{Ca} channels in regulating detrusor tone in rats (Christ et al. 2001; Malysz et al. 2004), mice (Meredith et al. 2004; Petkov et al. 2001), guinea pigs (Grant and Zuzack 1991; Klockner and Isenberg 1985; Markwardt and Isenberg 1992; Suarez-Kurtz et al. 1991; Woods et al. 2001; Zografos et al. 1992), pigs and humans (Trivedi et al. 1995). They have been reported to mediate repolarizing of the membrane potential and maintaining the resting potential (Heppner et al. 1997; Karicheti and Christ 2001). Moreover, at least in guinea pigs, they have been proposed to provide a negative feedback element to reduce the amplitude and duration of detrusor contraction (Herrera et al. 2000). In mice, this role is regulated by the smooth-muscle-specific pore-forming β_1-subunit (Petkov et al. 2001). Similar to SK channels, BK_{Ca} channels are Ca^{2+}-sensitive and can be activated by the influx of Ca^{2+} via voltage-operated Ca^{2+} channels (Herrera and Nelson 2002). In contrast to SK channels, BK_{Ca} channels are also activated by the Ca^{2+}-induced Ca^{2+} spark by ryanodine receptors, and such activation has been reported in the detrusor of guinea pigs (Herrera et al. 2001; Herrera and Nelson 2002; Ohi et al. 2001) and humans (Chambers et al. 1999). Accordingly, it has been suggested that BK_{Ca} channels and ryanodine receptors are co-localized at the sub-plasmalemma and in sarcoplasmic reticulum fragments to generate Ca^{2+} sparks to activate spontaneous transient outward currents (Ohi et al. 2001). The strength of coupling of Ca^{2+} sparks to BK_{Ca} channels is regulated by membrane potential and stimuli which modulates Ca^{2+} sensitivity (Herrera et al. 2001). Thus, BK_{Ca} are important in the regulation of bladder tone, and activators of BK_{Ca} channels have been synthesized as possible drugs for the treatment of overactive bladder (Hewawasam et al. 2002; Sheldon et al. 1997; Turner et al. 2003).

In guinea pig bladder, stimulation of β-AR activates BK_{Ca} channels via Ca^{2+}-sensitive mechanisms by means of increase of Ca^{2+} influx and Ca^{2+} sparks (Petkov and Nelson 2005). Some investigators have demonstrated the involvement of BK_{Ca} channels in β-AR-mediated bladder relaxation using BK_{Ca} channel inhibitors such as iberiotoxin and charybdotoxin in rats (Frazier et al. 2005; Uchida et al. 2005) and guinea pigs (Kobayashi et al. 2000; Petkov and Nelson 2005). Studies in rats have demonstrated that the role of BK_{Ca} channels in β-AR-mediated relaxation may depend on the experimental conditions, i.e., being considerable in the presence of KCl-induced tone but absent in the presence of passive tension only (Fig. 5; Frazier et al. 2005; Uchida et al. 2005). While a study in guinea pigs has reported that β-AR relaxation is mediated by facilitation of BK_{Ca} channels following the activation of cAMP/PKA pathways (Kobayashi et al. 2000), studies in rats do not support the idea of a concomitant involvement of BK_{Ca} channels and the cAMP/PKA pathway (Frazier et al. 2005; Uchida et al. 2005).

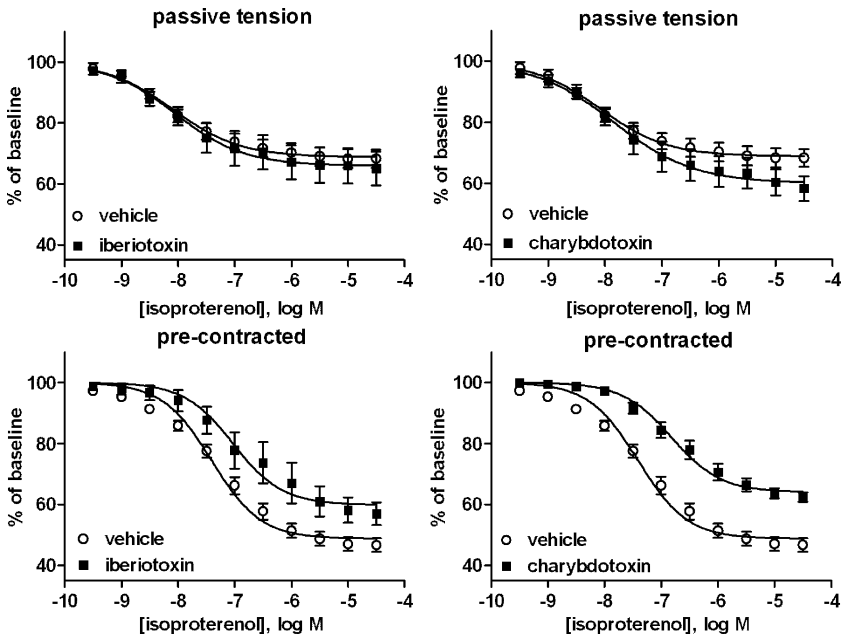

Fig. 5 Effects of the BK_{Ca} channel inhibitors iberiotoxin (30 nM) and charybdotoxin (30 nM) on isoproterenol-induced relaxation of rat urinary bladder. Taken from (Frazier et al. 2005)

Conclusion

Muscarinic receptors, specifically their M_3 subtype, are an important physiological mediator of bladder contraction. Although an activation of PLC is the prototypical signaling pathway of these receptors and this pathway is also activated in the bladder, PLC apparently only contributes to bladder contraction in a very minor way. Rather, muscarinic-receptor-mediated bladder contraction involves voltage-operated Ca^{2+} channels and ROCK. While currently available inhibitors of voltage-operated Ca^{2+} channels have little effect on bladder function in vivo in patients, ROCK inhibitors may have potential as drugs for the treatment of an overactive bladder. β-AR, specifically β_3-AR, are important physiological mediators of bladder relaxation, and selective β_3-AR agonists are currently in clinical development for the treatment of an overactive bladder. Although the prototypical signaling pathway of β-AR is an activation of adenylyl cyclase with the subsequent formation of cAMP, cAMP apparently contributes to β-AR-mediated bladder relaxation only in a minor way. BK_{Ca} channels may play a greater role in β-AR-mediated bladder relaxation, and activators of these channels are also under investigation to treat an overactive bladder.

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