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The Modulation Of Detrusor Contractility By Agents Influencing Ion Channel Activity

Original research article/Review

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Abstract **Background:** This study specified the role of several significant ion channels regulating the metabolism of calcium ions in contraction and relaxation of human detrusor muscle in order to identify possible target for future drugs that are capable of treating diseases resulting from impaired detrusor activity, e.g. overactive bladder. Although this disease can be successfully treated with muscarinic receptor antagonists or β_3 agonist, many patients may not be suitable for chronic therapy, especially due to the relatively high side effects of the treatment.
Material and Methods: The study used the isolated detrusor tissue samples, which were obtained from the macroscopic healthy tissue of urinary bladder from 19 patients undergoing a total prostatectomy because of localized prostate cancer. Each biological sample was prepared into 8 strips. We used oxybutynin and mirabegron as control drugs and several blockers of specific subtypes calcium and potassium ion channels as tested substances. The contractility of bladder was investigated by an organ tissue bath method in vitro and contraction was induced by carbachol.
Results: The amplitude of contraction was successfully decreased by positive control drugs and, from tested agents, the comparable effect had the substance capable of influencing IP_3 receptors and Orai-STIM channels and combination consisting of drugs possessing an inhibitory effect on IP_3 receptors, L- and T-type voltage-gated calcium channels and Orai-STIM channels.
Conclusion: The present work represents a new finding about handling Ca^{2+} in urinary bladder contraction and pointed to a dominant role of IP_3 receptor-mediated pathway in the regulation of Ca^{2+} metabolism, which may represent a future strategy in pharmacotherapy of impaired detrusor activity.

Keywords *Overactive bladder – calcium channels – smooth muscle reactivity*

INTRODUCTION

Overactive bladder (OAB) is specifically defined by the International Continence Society as an urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence, in the absence of urinary tract infection or other obvious pathology (Gormley et al., 2015). OAB occurs in both men and women and it has a significant impact on the quality of life (Sharaf and Hasim, 2017). The impaired physiological contractile or relaxing function of the detrusor muscle is regarded as the most significant reason for OAB.

The relaxation of detrusor is mediated by a tonic release of norepinephrine, which activates β_3 adrenergic receptors and also by intrinsic urinary bladder (UB) smooth muscle (SM) properties. The contraction is achieved by acetylcholine, which activates UB SM, mainly M3 muscarinic receptors (Andersson, 2015), and can be reversed either by muscarinic receptor antagonists (e.g. oxybutynin, tolterodine, fesoterodine or trospium) or β_3 receptor agonists, e.g. mirabegron (Cernecka et al., 2015).

Several treatment options are available for OAB, including bladder and behavioural training, pharmacologic treatment, and surgical therapies. The antimuscarinics used to treat OAB are recognised to be effective in the improvement of OAB symptoms, but the associated side effects such as dry mouth, constipation, headache, and blurred vision occurred relatively often, leading to the discontinuation of therapy (Maman

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et al., 2014; Rajalabaya et al. 2016). The approval of the β_3-adrenoceptor agonist mirabegron has added a new class of pharmacotherapy for OAB. In 12-week trials, mirabegron (25, 50, and 100 mg) demonstrated significant reductions, compared with placebo, in micturition and incontinence episode frequency, with an incidence of antimuscarinic-associated adverse events similar to placebo (Abrams et al., 2015). However, patients who use mirabegron could suffer from hepatotoxic, cardiovascular and CNS toxic adverse effects (Sharaf and Hasim, 2017).

So there is still a need to find other treatment options. From literature, the importance of calcium ions (Ca^{2+}) is well known for an appropriate contractile function of the detrusor muscle. Increase in intracellular Ca^{2+}, resulting in contraction, occur primarily due to extracellular Ca^{2+} entry through plasmalemmal ion channels or release of Ca^{2+} from the endoplasmic reticulum (ER). In addition to Ca^{2+} homeostasis, administration of both pharmacological groups, generally used for OAB treatment, influence transport of Ca^{2+} through the plasma membrane (mirabegron) or its release from ER (antimuscarinics) (Cernecka et al., 2015). The generation and propagation of UB SM action potential results especially from Ca^{2+} entry through L-type voltage-gated ion channels (VGCC) (Parajuli et al., 2016). Several recent studies documented that M3 receptor-mediated detrusor contractions also require Ca^{2+} influx via L-type VGCC (Hedge, 2006). T-type channel activity is important at membrane potentials near the resting level (Sui et al., 2007). Nonselective cation channels, such as transient receptor potential (TRP) channels, significantly contribute to extracellular Ca^{2+} entry pathways in SM cells.

In response to a variety of stimuli, Ca^{2+}metabolism-controlling channels in the ER, i.e. ryanodine receptors (RyRs) and inositol-trisphosphate receptors (IP_3 Rs), mediate the efflux of Ca^{2+} from the ER into the cytoplasm of the cell (Hill-Eubanks et al., 2011). The antimuscarinic agents inhibit processes triggered by the activation of M3 receptors, especially emptying of Ca^{2+} from SR through the activation of IP_3 Rs. Large-conductance calcium-activated potassium channels (BK^+_{Ca}) stimulated by β3 agonists indirectly inhibit L-type VGCC (Sutovska et al., 2007). There is also the strong evidence that BK^+_{Ca} channel activity is inhibited upon the activation of M3 receptors and this mechanism contributes to suppressed UBSM contraction on administered antimuscarinics (Parajuli et al., 2016).

Extracellular Ca^{2+} influx in response to the depletion of intracellular Ca^{2+} stores, a process termed store-operated Ca^{2+} entry (SOCE), is known to play an important role in a number of cell types. The ubiquitously expressed STIM proteins serve as ER Ca^{2+} sensors and members of the Orai family (Feske et al., 2016) of transmembrane proteins as the entities responsible for mediating Ca^{2+} entry. Several morphological studies have shown that STIM and Orai family members are expressed in SM, and, under the test conditions, are capable of functionally coupling store depletion to extracellular Ca^{2+} entry (Hill-Eubanks et al., 2011). The Orai-STIM pathway role was examined in the rat model of OAB (Zhao et al., 2014), but not in humans.

MATERIAL AND METHOD

The primary objective of the current study was to evaluate the potential efficacy of different modulators of ion channel activity and their combinations in the suppression of UBSM activity compared with mirabegron and oxybutynin monotherapy.

All processes were approved by the Institutional Ethics Committee of the Jessenius Faculty of Medicine registered in the Institutional Review Board or the Institutional Ethic Board Office (IRB 00005636) in accordance with Slovakian and European legislation (decision No. EK1249/2013, EK1880/2016). Patient recruitment was conducted by using information sheets, and written informed consent was obtained. The samples of urinary bladder were collected in cooperation with the Clinic of Urology. Samples were collected from 19 male patients undergoing total prostatectomy for localized carcinoma (cT2N0M0) without OAB symptoms, systemic chronic disease, and therapy influencing ion channels activity administered systemically, neurogenic bladder and/or inflammation of lower and upper urinary tract before operation. Urinary bladder specimen was immersed into Krebs-Henseleit's buffer of the following composition (nM): NaCl, 112.9; KCl, 4.7; $CaCl_2$, 2.8; $MgSO_4$, 0.5; $NaHCO_3$, 24.9; and glucose, 11.1.

UBSM reactivity was evaluated using well-described organ tissue bath methodology (Franova et al., 2009). Prior to drug administration, urinary bladder samples were incubated for 1 hour in Krebs-Henseleit solution. Carbachol (1μM) was applied directly into the chamber to induce SM contractions. Cumulative doses (1μM, 10μM, 100μM and 1 mM) of the tested substances able to modulate transport of Ca^2+ ions**,** i.e. diltiazem, SKF96365 (1-[β-(3-(4-methoxyphenyl)propoxy)-4-methoxyphenethyl]-1H-imidazole hydrochloride**)**, 2APB (2-aminoethoxydiphenyl borate), potassium channels opener NS1619, their combinations and reference drugs mirabegron and oxybutynin were injected into the chamber 5 min after carbachol application. The amplitude of UBSM contraction was recorded and data were normalized on the weight of each sample.

2APB and SKF 96365 were purchased from TOCRIS (USA), and all other drugs from Sigma Aldrich (SR). 2APB was dissolved in 10% DMSO, and the remaining chemicals in water for injection.

Statistical analysis was performed in R (R Core Team 2015), using libraries WRS2 (Mair et al., 2016) and car (Fox and Weisberg, 2011). Normality of the data was assessed by quantile-quantile plots with bootstrap confidence intervals. Since the normalized amplitudes of contraction were not found to be Gaussian, the robust ANOVA was used for evaluation. Post-hoc confidence intervals were adjusted to control the family-wise error rate. Results with *p*-value below 0.05 were considered statistically significant.

RESULTS

From each urinary bladder sample 8 specimens were prepared and we tested the following monotherapies and combination (all in concentration c=1μM – 1mM): diltiazem (DIL), SKF96365 (SKF), 2APB, SKF + DIL, SKF + DIL + 2APB and SKF + DIL + 2APB + NS1619.

According to the obtained data, 2APB had a similar relaxing effect compared to reference drugs oxybutynin (OXY) and mirabegron (MIR) with effective onset seen in the lowest tested concentration of drugs (Fig. 1) and relatively low variability in responses (Fig. 2). DIL and SKF in monotherapies had a similar effect, which was lower than both positive controls, but their combination did not augmented decrease of UBSM contractility. Adding 2APB and both 2APB and NS1619 in combination with DIL + SKF led to statistically significant improvement of the relaxing effect. However, the effect of SKF+DIL+2APB combination was still significantly lower than that of MIR (Figs. 1 and 2).

DISCUSSION

The involvement of Ca^{2+} ion channels, such as VGCC (L-type and T-type), IP_3 Rs and SOCE (TRPC superfamily and Orai-STIM channels) and K^+ channels, especially BK^+_{Ca}, in UB SM contractility was examined using the pharmacological method in the present study. The individual ion channels activity was modulated by DIL, SKF, 2APB, NS1619 and their combinations and detrusor contractility was determined by the organ tissue bath method. This method was previously described in detail by Franova et al. 2009 and is generally used to test the efficacy of drugs that modulate SM tone in, for example, airways SM, uterine SM as well as UB SM.

The reference drugs OXY and MIR, clinically used for OAB treatment, effectively and dose-dependently decreased the contractile curve of isolated UB SM induced by carbachol with only insignificant differences of the effectiveness. As the mechanism of action of OXY and MIR is eminently regulated by Ca^{2+} and K^+ ions, this strongly supported the importance of calcium and potassium ion channels in detrusor physiology.

The results showed a significant role of IP_3 Rs and Orai-STIM channels in the process of contraction and relaxation of UB SM, because the efficacy of the tested drug 2APB was comparable to the generally used OXY and MIR. 2APB is used regularly in the research on the manipulation of intracellular Ca^{2+} release. It was initially found to block IP_3 Rs and was later shown to inhibit SOCE dose-dependently, independent of IP_3 Rs inhibition (DeHaven et al., 2008). Regardless it was expected that either monotherapy by DIL and SKF or by their combination did not cause significant a decrease in the amplitude of contraction induced by carbachol. We presumed especially a synergic effect of SKF+DIL combination because of the influencing T- and L-type VGCC as well as Orai-STIM channels. However, SKF is able to also block BK^+_{Ca} in higher concentrations, which probably explains such an effect (Tanahashi et al., 2016). A combination of SKF+DIL+2APB possessed significantly higher effects than SKF+DIL and, moreover, the isolated UBSM relaxation as similar to that on OXY. However, the effectiveness comparable to MIR alone was achieved by a combination of drugs SKF+DIL+2APB+NS1619.

CONCLUSIONS

Our experimental results confirmed the key role of IP_3 Rs, Orai-STIM and BK^+_{Ca} channels in the detrusor muscle physiology and pathophysiology. Furthermore, drugs able to inhibit IP_3 Rs, such as 2APB or agents influencing Orai-STIM and BK^+_{Ca} channels should be useful in new strategies drawn for the development of OAB treatment. Other possible approach should be the optimization of current treatments aimed at maintaining the balance between K^+ and Ca^{2+} ions, which seems to be essential for the right function of UB SM contractility.

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