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(–ADRENOCEPTOR-MEDIATED ACTIVATION OF BKCA CHANNELS IN URINARY BLADDER SMOOTH MUSCLE CELLS

Hypothesis / aims of study

Urinary bladder smooth muscle relaxation is the main goal during therapy of overactive bladder (OAB). Muscarinic receptor antagonists are still the gold standard in treatment of OAB, but because of high incidence of side effects and low patient compliance alternative drug targets have been discussed during the recent years. Detrusor relaxation can also be induced by activation of either β–adrenoceptors[1,2] elevating cyclic AMP levels, or by promoting calcium-activated potassium channels with large conductance (BK_{Ca} channels) that hyperpolarizes the cells[3].

Here we have studied the interaction between β–adrenoceptor stimulation and BK_{Ca} channel activity in freshly isolated detrusor smooth muscle cells (DSMCs). We have focused on the involved β–adrenoceptor subtypes since this is of interest for estimation of tissue specificity.

Study design, materials and methods

Potassium currents through BK_{Ca} channels (I_{BKCa}) were measured with standard voltage-clamp technique in freshly isolated murine vascular smooth muscle cells (VSMCs) and DSMCs from mouse, pig and man. I_{BKCa} was measured at room temperature (22°C). A system for rapid solution changes allowed addition of test compounds in the close vicinity of the cells. β–Adrenoceptors were stimulated with the non-selective agonist (-)-isoprenaline and the effects on BK_{Ca} channels were measured as changes of I_{BKCa} current amplitude. The following subtype selective antagonists were used as tools: β_1-adrenoceptor antagonist CGP 20712A (300 nM) and β_2-adrenoceptor antagonist ICI 118,551 (50 nM).

Results

BK_{Ca} currents were identified in all smooth muscle cells. Stimulation of β–adrenoceptors with (-)-isoprenaline activated BK_{Ca} channels in a concentration-dependent manner ($-\log EC_{50}$[M]: murine VSMCs 7.0 ± 0.2 (n=3), DSMCs 7.3 ± 0.3 (n=10) in mouse, 7.5 ± 0.2 (n=7) in pig, 6.7 ± 0.4 (n=4) in man). CGP 20712A did not significantly affect the (-)-isoprenaline-evoked effect on BK_{Ca} current. In contrast, ICI 118,551 completely abolished the (-)-isoprenaline-mediated increase of BK_{Ca} current in smooth muscle cells from mouse and pig, but not in human urinary bladder cells. These results suggest that β_2-adrenoceptors are involved in murine and porcine, but not in human smooth muscle cells.

Table: Effects of β–adrenoceptor-agonist and -antagonists on BK_{Ca} current amplitude

	VSMC		DSMC					
	Mouse		Mouse		Pig		Man	
	n	E_{max} (%)	n	E_{max} (%)	n	E_{max} (%)	n	E_{max} (%)
Control	14	100	28	100	12	100	27	100
1 µM (-)-Isoprenaline	5	161±24*	20	128±18**	6	194±49*	12	114±12***
1 µM (-)-Isoprenaline + 300 nM CGP20712A	4	158±29*	4	131±21**	3	185±51*	3	130±28**
1 µM (-)-Isoprenaline + 50 nM ICI 118,551	5	99±15	4	98±8	3	97±30	12	125±13***

* p<0.05, **p<0.01, ***p<0.001 substance versus control, ratio paired *t*-test

Interpretation of the results

Activation of BK_{Ca} channels is mediated via β_2-adrenoceptors in smooth muscle cells from mouse and pig, but not from human urinary bladder. Opening of human detrusor BK_{Ca} channels probably requires β_3-adrenoceptors.

Concluding message

In summary (-)-isoprenaline-evoked BK_{Ca} channel activation is mediated through β_2-adrenoceptors in murine and porcine smooth muscles, whereas in human detrusor only β_3-adrenoceptors are probably involved. Therefore, when targeting β-adrenoceptors for therapy of OAB the following aspects are of importance: (i) β-Adrenoceptor-mediated BK_{Ca} channel activation should be characterized in various smooth muscle tissues, i.e. detrusor and blood vessels and (ii) organ selectivity of BK_{Ca} channel activation could be transfered by different β-adrenoceptor subtypes.

References

1. JPET (2009) 328(1):213-222
2. Br J Pharmacol (2006) 147(2):88-119
3. Pharmacol Ther (2006) 110(1):103-116

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What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**All patients had given informed written consent in accordance with the regulations of the local hospital ethical committee (permission no. EK 194092004).** **All animal experiments were performed in accordance to the regulations of the local legislation committee (permission 24-9168.24-1-2002-8 of the Dresden Regierungspräsidium).**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**