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Takemoto J[1], Masumiya H[2], Ikeda Y[1], Nakagawa H[1], Yanagisawa T[3], Arai Y[1]
1. Tohoku University Graduate School of Medicine, Department of Urology , 2. Hyogo Medical School, Department of Physiology, 3. Tohoku University Graduate School of Medicine, Department of Molecular Pharmacology

THE OUTWARD POTASSIUM CURRENT IS INCREASED BY BETA AGONISTS IN CULTURED HUMAN URINARY BLADDER SMOOTH MUSCLE CELLS

Hypothesis / aims of study
The beta adrenergic receptors contribute to the relaxation of urinary bladder in various species. It has been demonstrated that human urinary bladder smooth muscle cells have predominantly $beta_3$ adrenoceptor and that $beta_3$ agonists have relaxant effect on human urinary bladder muscle.This relaxant effect of $beta_3$ agonist is supposed to be beneficial to treat overactive bladder. It is well-known that there are species differences in the distribution of beta adrenoceptor subtypes. There have been several difficulties to investigate the electrical activities in isolated human urinary bladder smooth muscle, e.g. the rare availability and low successfulness of isolation to single cells. Thus, we chose the cultured human urinary bladder cells.

The aims of this study are to examine and compare the effects of isoproterenol, a non-selective beta agonist and BRL37344, a selective $beta_3$ agonist, on the whole cell current, and further to elucidate the mechanism of action of beta agonists.

Study design, materials and methods
Human urinary bladder smooth muscle cells were obtained from Cambrex Bio Science, and used at passage 6 to 8. The composition of the extracellular solution was as follows (in mM) 135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5 HEPES, 11.1 Glucose, pH 7.35 with NaOH, and that of intracellular pipette solution was 110 potassium aspartate, 30 KCl, 10 NaCl, 1.0 $MgCl_2$, 10 HEPES, either 0.05 or 5 EGTA, pH 7.25 with KOH. 5mM EGTA pipette solution is thought to abolish calcium-activated potassium current. Cells were hold at -80mV and given 400ms step pulses from -100mV to +80mV. When the whole cell current stabilized, test drug solutions began to be perfused. The effects of drugs were evaluated by the changes in current densities.

Results
Outward-rectifier voltage-dependent current was the main component of the whole cell current. Isopreterenol (1μM) increased the current significantly by 74% (n=5) at +80mV. BRL37344 (1μM) increased the current significantly (n=6) by 57% at +60mV and by 48% at +80mV. 5mM EGTA pipette solution (n=6) attenuated the current significantly, comparing with 0.05mM EGTA pipette solution (n=10), by 67% at +20mV, by 67% at +40mV, by 60% at +60mV, and by 52% at +80mV. 5mM EGTA pipette solution abolished isoproterenol-induced current. Iberiotoxin (100nM), a large conductance calcium-activated potassium channel blocker, also attenuated the current significantly (n=4) by 64% at +80mV. There remained small fraction of isopreterenol-induced current in the presence of iberiotoxin.

Interpretation of results
Iberiotoxin-sensitive large conductance calcium-activated potassium current composed major component of the whole cell current of human urinary bladder smooth muscle. Isopreterenol increased 5mM EGTA-sensitive calcium-activated potassium current (mainly via large conductance calcium-activated potassium channel). BRL37344 also increased the whole cell current, however the efficacy seemed to be smaller than that of isoproterenol, since BRL37344 is a partial agonist of $beta_3$ adrenoceptor.

Increased outward current via $beta_3$ stimulation makes cell hyperpolarize, resulting in inhibition of the excitability and relaxation of bladder muscle.

Concluding message
$Beta_3$ selective agonists like BRL37344 will increase the whole cell current of cultured human urinary bladder smooth muscle, via calcium-activated potassium channel opening. This action of $beta_3$ agonist will be beneficial and these agents are promising stabilizers and relaxants of urinary bladder.

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