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COMPREHENSIVE STUDIES OF ATP-SENSITIVE POTASSIUM CHANNELS IN PIG AND HUMAN DETRUSOR SMOOTH MUSCLE CELLS

Hypothesis / aims of study

ATP-sensitive K channels (K_{ATP} channels) are widely distributed in various tissues, thus reflecting the intracellular energy level into the membrane potential. In addition, in detrusor cells, the existence of K_{ATP} channels has been shown using pharmacological tools, for example, cromakalim, nicorandil (K_{ATP} channel openers), glibenclamide, tolbutamide (K_{ATP} channel blockers). The physiological role of K_{ATP} channels is still unclear but nevertheless it is expected that the K_{ATP} channels in the lower urinary tract may be potential therapeutic targets in such functional diseases as overactive bladder (OAB). We therefore carried out comprehensive study of the K_{ATP} channels in pig detrusor cells in comparison to humans.

Study design, materials and methods

Urinary bladders of white pigs were obtained from a local abattoir. Human bladders were obtained from patients without prostatic enlargement and lower urinary tact symptoms who were undergone total cystectomy due to bladder carcinoma.

(Patch clamp)

Muscle bundles obtained from detrusor were cut into small pieces which were subsequently incubated in a nominally Ca^{2+}-free solution for 15min at 37˚C before digestion in an enzyme-containing (0.1% collagenase, type 3, Worthington) Ca^{2+}-free solution for 15 min. After rinsing with enzyme and Ca^{2+}-free solution, cells were isolated by mechanical agitation using a fine-bore pipette. Cells obtained were stored at 5°C in physiological salt solution containing 0.5mM Ca^{2+}. Patch clamp technique was performed on isolated pig and human detrusor cells at room temperature using borosilicate capillary electrodes (3-5 MΩ: whole cell, 5-10 MΩ: single channel).

(RT-PCR)

RT-PCR was also performed to determine the sulphonylurea receptor (SUR) subunits which formed K_{ATP} channels. Total RNA was isolated from detrusor using RNeasy purification kit (Qiagen, Tokyo, Japan). After treated with RQ1 DNase (Promega), 1 mg of the total RNA was subjected to RT (reverse-transcription) reaction in a 10 μl reaction volume. RT was performed using random hexamer (12.5 pmol) and MMLV reverse transcriptase (100 U), according to manufacture's instructions (Gibco-BRL, Rockville MD, U.S.A.). Then, the RT sample (1 μl) was used as a template for PCR reaction (20 μl). To evaluate contribution of geneomic DNA, samples without RT were used as controls. Since cDNA sequences for pig SUR2A, SUR2B and Kir6.2 have not yet been published, the PCR primers were designed in sequences conserved between human and mouse.

Results

(Pig detrusor)

In the whole-cell configuration of these experiments, levcromakalim, a K_{ATP} channel opener, induced a long-lasting outward current which was then reversed by glibenclamide in a concentration-dependent manner. The intracellular application of 0.1 mM GDP significantly enhanced the levcromakalim-induced membrane current, while cAMP did not. CGRP, VIP, adenosine and somatostatin had little effect on the membrane current. In the cell-attached configuration of these experiments, levcromakalim activated K^+ channels with a unitary conductance of 12 pS. This 12 pS K^+ channel was then re-activated by the intracellular application of 1 mM GDP, however, it was inhibited by ATP and glibenclamide. Kir6.1 and SUR2A were predominant in the RT-PCR examinations.

(Human detrusor)

Levcromakalim also induced a long-lasting outward current which was abolished by glibenclamide. In the RT-PCR examinations, SUR2B was predominantly expressed.

Interpretation of results

This is the first report of a single channel activity of K_{ATP} channels in the detrusor smooth muscle cells. The K_{ATP} channels with unitary conductance of 12pS are activated by levcromakalim and GDP, while they are suppressed by glibenclamide and ATP, however, neurotransmitters had little effect on them. The findings of the RT-PCR examinations suggested a predominant expression of SUR2A and SUR2B in pigs and humans, respectively. This unique SUR isoform of pig detrusor cells may therefore account for the uncoupling of the cAMP-signal pathways of the K_{ATP} channel activity.

Concluding message

These results suggest that K_{ATP} channel openers selective for SUR 2B isoform may have tissue-specific therapeutic potential for overactivity bladder.

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