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CRAC ION CHANNELS AND AIRWAY DEFENCE REFLEXES IN EXPERIMENTALLY INDUCED ALLERGIC INFLAMMATION

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Calcium ions play the key regulatory role in most cellular processes. Increased knowledge of the calcium metabolism during the last 10 years are due to the discovery of a novel superfamily of channels TRP (transient receptor potential), which contribute to changes in the cytosolic calcium concentration either by transport through the plasma membrane, or via alternative pathways. Calcium activated calcium ion channels (CRAC), which role in airways reflexes is presented, are member of TRP superfamily.
RNA interference screening approaches identified two proteins that are essential for store-operated Ca^{2+} influx and CRAC, Stim and Orai. Transmembrane proteins Stim senses depletion of the Ca^{2+} store in endoplasmatic reticulum and together with Orai form the molecular basis for CRAC activity. CRAC are widely expressed on many cells, e.g. immune system cells, urinary, uterine and airways smooth muscle cells, etc. They are responsible for contractile plateau of smooth muscle cells and secretory functions of immune cells. Their role in reactions of immune system is described in more detail. Less knowledge exists about role of widely distributed plasmalemal CRAC of airways smooth muscle (ASM) cells in airways defence reflexes. The presented studies were focused on possible use of CRAC as therapeutic target in inflammatory airways disorders based on ASM malfunction, e.g. bronchial asthma. The influence of acute (doses 1.3, 1.5, 1.7 $mg.kg^{-1}$ b.w.) and chronic administration (1.5 $mg.kg^{-1}$ b.w., 14 days) of CRAC antagonist (3-fluoropyridine-4-carboxylic acid) on the cough and ASM contractility associated reflexes in guinea pigs with experimental allergic airways inflammation was examined by the following methods: 1, Method of citric acid (c= 0.3 M) induced cough reflex used in measurement of cough response; 2, The measurement of specific airways resistance values as the response on bronchoprovocation induced by citric acid as well as histamine (c= 10^{-6} $M.l^{-1}$) *in vivo* condition; 3, The organ tissue bath method evaluated the response of isolated ASM on cumulative doses of contractile mediators histamine and acetylcholine *in vitro* conditions; 4, The measurements of exhaled NO levels *in vivo* conditions assessed to determine the involvement of NO metabolism in CRAC antagonist induced ASM relaxation. Experimental allergic airways inflammation was elicited by repetitive exposure of guinea pigs to ovalbumin (c=10^{-6} M) adsorbed to $Al(OH)_3$ during 21 days. CRAC antagonist tested under the conditions of experimentally induced allergic inflammation applied intraperitoneally as acute dose significantly and dose- dependently reduced cough response on citric acid. The cough suppressive effect of highest used dose (1.7 $mg.kg^{-1}$ b.w.) exceeded efficacy of codeine. The values of specific airways resistance were reduced in dose dependent manner, which pointed on bronchodilatory activity of CRAC antagonist. These findings were supported by the results of *in vitro* experiments. Furthermore, long- term application of CRAC antagonist resulted in more significant and harmonized

influence on defence reflexes of the airways. The levels of exhaled NO measured on 21st day of sensitization were twofold increased in comparison with unsensitized animals. Both, acute and long-term administration resulted in significant decrease of NO levels. The preliminary histopathological studies evaluated differences in cellular inflammatory pattern on single dose confirmed anti-inflammatory potency of CRAC antagonist. The results confirmed role of CRAC in pathophysiology and symptoms of experimental animal asthma model and could in future extent therapeutic possibilities or design new therapeutic strategy in asthma treatment.