DESENSITIZATION OF TRPV1 RECEPTORS IS SENSITIVE TO TRPA1 CHANNEL ACTIVITY AND CALCIUM IN RAT DORSAL GANGLION NEURONS

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Abstract

Vanilloid receptors of type 1 transient receptor potential (TRPV1) are ligand-controlled cationic channels, which were found mainly in the nociceptive neurons of the peripheral nervous system. Desensitization of TRPV1 channel is thought underlies the paradoxical analgesic action of capsaicin. The aim of our studies was to establish the ways involved in strengthening and weakening of the desensitization of TRPV1 receptors. The activities of TRPV1 channels, their sensitivity to selective activator capsaicin (Caps), especially their interaction with TRPA1 channels were studied. As well we studied the influence of intracellular calcium of the desensitization of TRPV1 channels. The methods of microfluorescent microscopy with calcium sensitive dye fura- 2AM and molecular biological methods were used. Researches were conducted on cultured DRG neurons. Application of Caps on soma of DRG neurons resulted in an increase in intracellular calcium. Consistent repeated Caps applications resulted in a significant reduction in the amplitude of calcium transients (desensitization of TRPV1 channels), which accounted 20,7% of initial value. Further application of selective TRPA1 channel agonist resulted in restoration of sensitivity to Caps (resensitization TRPV1 channels). It is proved, that this effect was observed in neurons which co-expressed both TRPV1 and TRPA1. We have shown that the desensitization of capsaicin channels are increased as a result of increasing intracellular calcium both under the influence of successive capsaicin applications, and under the influence of depolarizing concentration of KCl. Thus, we have established that TRPA1 channel activity removed the TRPV1 receptor desensitization, whereas increase of intracellular calcium concentration produced the TRPV1 desensitization.

Keywords: TRPV1-channels; TRPA1-receptors; calcium; DRG-neurons; calcium signaling; capsaicin; allyl isothiocyanate.

Methods

Culturing of dorsal root ganglia neurons of rat

In the experiments we used a one-day culture of dorsal ganglia neurons isolated from Wistar rats aged 9 days. All animal work was performed in strict compliance with all applicable international and local regulations for the proper use of animals in research. After rat decapitation, allocated spinal ganglia were transferred into a Tyrode solution containing enzymes collagenase (type IA) at a concentration of 1 mg/ml and protease (type XIV) at a concentration of 2 mg/ml, and were kept for 30 minutes, at 37 °C. To remove the remainders of the enzymes and to prevent their further action, the ganglia were incubated in DMEM solution with 10% fetal calf serum (FBS) for 10 minutes at the same temperature with repeated replacement of the solution for the complete inactivation of enzyme with FBS proteins. For getting the suspension of cells, the ganglia were pipetted using the Pasteur pipettes of different diameters in 0,5 ml culture medium (DMEM with FBS and antibiotics). The obtained suspension was placed on the pre-fat-free glass coverslips in the sterile Petri dishes which were put to the CO_2 incubator. The neurons were used in the experiments on the next day after platting.

RNA isolation, reverse transcription and PCR

Excretion of RNA was performed from neuronal DRG cultures grown on glass coverslips coated with polylisine. Culture medium was removed from the experimental Petri dishes, where the coverslips with cells were located, and 1 ml TRIzol (TRIzol® Reagent, Ambion®) was added. The further RNA isolation procedure was performed as described by the manufacturer.

The excreted total RNA was converted to cDNA using a set of reagents "Revert Aid H Minus First Strand cDNA synthesis Kit" (Thermo Scientific), as described by the manufacturer. From the resulting cDNA PCR assay was fulfilled with adding the reagents for the reaction "2x PCR Master Mix" (Fermentas), as described by the manufacturer. The primers with final concentration of 0.25 mM were added to the reaction mixture. To identify the expression of genes encoding proteins of TRPV1 channels the primers with sequence F 5'-AGC GAG TTC AAA GAC CCA GA-3 'and R 5'-TTC TCC ACC AAG AGG GTC AC-3' were used with the size of the product 300 base pairs. For TRPA1 channels the primers with sequence F 5'-CCC CAC TAC ATT GGG CTG CA-3 'and R 5'-CCG CTG TCC AGG CAC ATC TT-3' were used with the product size of 500 base pairs. Polymerase chain reaction took place in thermal cycler PeqSTAR 96 Universal Gradient (PeqLAB Biotechnologie GmbH).

All amplicons were included into agarose gel with dye (6×DNA Loading Dye, Fermentas). Matching the size of the product was determined by the marker (Gene Ruler 100 bp Plus DNA Leader, Fermentas). All of the products after electrophoresis were photographed under ultraviolet light (254 nm) with MicroDOC System with UV Transilluminator (DUOVIEW254/312 (Cleaver Scientific Ltd, UK) under the same conditions.

Measurement of changes in Ca^{2+} concentration with the microfluorescent method.

Fura-2AM as a calcium indicator dye was used to study the changes in $[Ca^{2+}]_i$ in sensory neurons of rat dorsal ganglia (Lukyanetz, Stanika, Koval, and Kostyuk, 2003). Neurons were incubated for thirty minutes at 37 °C in Tyrode solution with dye. Then cells were washed with Tyrode solution and remained there for 20 minutes for the completion of Fura-2AM de-esterification. Loading with the dye and further de-esterification were conducted in the absence of light. Then coverslips were transferred into the experimental chamber, which was fixed on the inverted microscope Olympus IX-71 (Japan). During the experiments, the chamber was constantly washed with fresh Tyrode solution. Excitation of the fluorescent probe was fulfilled at a wavelength of 340 (F1) and 380 (F2) nm using a xenon lamp and the appropriate optical filters. The filter operation and data recording were carried out using the system and software Cell^M (Olympus, Germany). Registration of the probe emission was carried out in the range of 480-570 nm with a peak of 510 nm. Evaluation of changes in the intracellular Ca^{2+} concentration was fulfilled with the ratio (R) of the intensity of the fluorescence probe at two wavelengths R= F1/F2. Before the measurements of fluorescence the background was measured and accounted for the subsequent calculations.

All reagents for experiments were obtained from the Sigma-Aldrich, USA.

Statistics

Statistical analysis was performed using the software OriginPro 8 (OriginLab Corporation, USA). Data were presented as mean ± SEM. Statistical differences between groups were determined using two-way ANOVA.

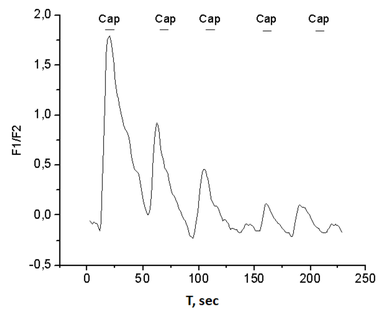


Fig.4. Desensitization of TRPV1 channels. Ca2+ transients evoked by repeated Caps applications are shown. The arrows and lines point moments of applications.

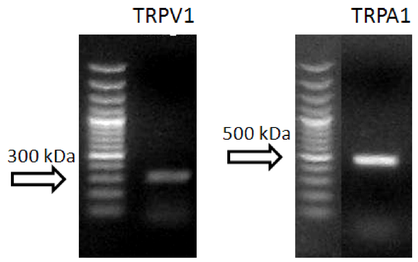


Fig. 1. Expression of TRPV1 and TRPA1 channels in DRG neurons. Agarose gel electrophoresis of PCR products confirmed the expression of TRPV1 and TRPA1 mRNA in DRG neurons.

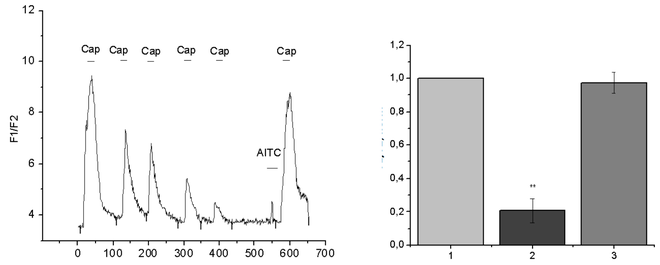
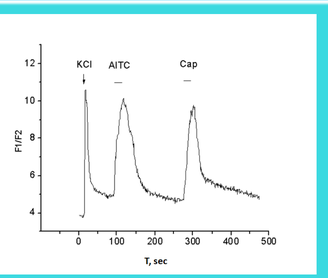


Fig. 5. Resensitization of TRPV1 channels caused by activation of TRPA1 channels. The Ca2+ transients evoked by repeated applications of Caps and AITC are shown. The arrows and lines point moments of applications.

Fig. 6. The amplitude of Ca2+ transients during desensitization and resensitization of the TRPV1 channels. The average values of the Ca2+ transient amplitude induced by the application of Caps at the beginning of the experiment (1), during desensitization (2) and after resensitization (3) of TRPV1 channels (after preliminary AITC application) are presented; n = 10. ** P <0,05 compared to the amplitude of Ca2+ transient induced by Caps at the beginning of the experiment.



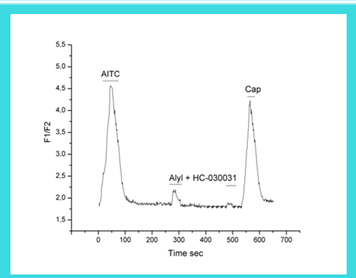


Fig. 2. Calcium transients evoked by TRP channel agonists. Calcium transients caused by membrane depolarization (KCl) and application of agonists - TRPV1 (Caps) and TRPA1 (AITC) channels. The arrows and lines point moments of applications.

Fig.3. Effects of TRPA1 channel blocker on calcium transients evoked by the action of TRPA1 channel agonist. Ca2+ transients evoked by AITC application and its effect in the presence of blocker HC-030 031 are shown. The Ca2+ transients induced by capsaicin (Cap) activation of TRPV1 channels also is shown. Moments of applications are shown by lines.

Conclusions

Taking into account polymodality of the TRPV1 channels, we can assume that depending on the type of stimulus (temperature, pH, or agonists) the different mechanisms of TRPV1 and TRPA1 receptor interaction may be involved. Accordingly, the results of our studies together with the data of other authors (Akopian, Ruparel, Jeske, and Hargreaves, 2007;Staruschenko, Jeske, and Akopian, 2010) indicate the presence of interaction between TRPA1 and TRPV1 channels, which are expressed in nociceptive neurons. The results indicate the ability of the TRPA1 receptor to resensitize TRPV1 receptors. Further study of the interaction between TRPA1 and TRPV1 channels will reveal the mechanisms of such interactions.