POST-TRANSLATIONAL REGULATION OF THE P2X$_7$ RECEPTOR THROUGH GLYCOSAMINOGLYCAN CHAINS OF CD44 PROTEOGLYCANS

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The P2X$_7$ is a purinergic receptor, member of the ATP-gated ion channels P2X family; and is an important element of inflammation, proliferation, differentiation and cell death processes. These receptors act in complexes and with functional association with adhesion molecules, the integrins. Since proteoglycans control the integrin activity, we decided to investigate the glycosaminoglycans (GAGs) influence on expression, distribution and modulating activity of P2X$_7$ in CHO-K1 cells and its mutant cell line CHO-745, defective in GAGs biosynthesis. The P2X$_7$ receptors were analyzed by microfluorimetry, flow cytometry, immunoprecipitation, confocal microscopy and molecular dynamics. The GAGs presence on the CHO cell surface greatly increases sensitivity to low concentrations of ATP and changes the main P2X$_7$ kinetic parameters EC$_{50}$, Hill coefficient and E$_{\text{max}}$.

In the absence of ATP, even the highest heparin concentrations tested did not elicit discernible P2X$_7$ activation. The allosteric inhibition of the P2X$_7$ receptor current through extracellular Mg$^{2+}$ was mitigated in the presence of GAGs. These data suggest the allosteric sensitization of the receptor through GAGs. In addition, the formation, recruitment, and dilation of the P2X$_7$ pore augmented in the presence of GAGs as demonstrated through the acceleration of cellular uptake of the large molecule propidium iodide and molecular dynamic simulations. Increases in E$_{\text{max}}$ of [Ca$^{2+}$]$_{\text{cyt}}$ and acceleration of propidium iodide influx confirmed the potentiating effect of GAGs on native P2X$_7$ receptors. Consequently, wild-type CHO-K1 cells were 2.5-fold more sensitive to cell death induced through P2X$_7$ agonists compared with mutant CHO-745.

Also, we provide the first evidence that the P2X$_7$ receptor interacts with the soluble ectodomain of CD44 proteoglycan (sCD44) on the CHO-K1 cell surface. In the present study, the data demonstrated that GAGs positively modulate the P2X$_7$ receptor, and sCD44 is a part of a regulatory positive feedback loop linking P2X$_7$ receptor activation for the intracellular response mediated through P2X$_7$ receptor stimulation.

Keywords: P2X$_7$ Receptor, Glycosaminoglycans, Allosteric Modulation

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