Disturbed P2X7 Receptors Ca2+ Kinetics in GAG-Deficient CHO-745 Cells

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Proteoglycans and integrins mediate cell attachment to various extracellular ligands. These molecules physically bridge the ECM and cytoskeleton, and act as transducers of "outside-in" and "inside-out" signaling. Due to this, a myriad of cellular functions such as differentiation, proliferation, migration, apoptosis, and inflammatory response can be affected. It has been shown that the purinergic P2X7 receptors binds integrin-laminin complex and its activation not only promote a cytosolic influx of Ca^{2+}, but it also results in rearrangement of cytoskeleton. Here, we report on the effects of glycosaminoglycans (GAGs) deficiency on P2X7 Ca^{2+} kinetics in CHO cells, indicating a novel role for GAGs in cell calcium physiology. CHO-K1 and its defective mutant on the biosynthesis of GAGs, CHO-745 cells, were examined for their cellular responses to the P2X7 receptor agonists. Ratiometric measurements of the cytosolic Ca^{2+} concentration were performed in both CHO cell lines, using a high-speed UV confocal laser scanning microscope and agonist stimulation. We verified that in CHO-K1 cells ATP was also agonists but were less potent than BzATP, while UTP, ADP and (α,β) methylene-ATP were inactive to cause a rapid and transient (10-20 s) accumulation of Ca^{2+} in cytoplasm at concentrations up to 100 μM. These results strongly suggested that CHO-K1 cells express an endogenous P2X7 receptor which can be activated by ATP. Interesting, Ca^{2+} influx stimulated by ATP was severely depressed in GAG-deficient CHO-745 cells or CHO-745 cells occasionally failed to generate Ca^{2+} influx upon ATP stimulation. Also, aberrant Ca^{2+} stead-state levels were observed in CHO-745 cells, in non-stimulated CHO cells the basal levels of cytosolic Ca^{2+} in CHO-745 cells was 50% smaller than the basal levels of CHO-K1 cells. The results presented here strongly indicate the involvement of GAGs in the control of P2X7 Ca^{2+} kinetics in CHO cells.

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