SUMMARY OF Ph.D. DISSERTATION

School Fundamental Science and Technology	Student Identification Number	SURNAME, First name HOMMA, Kohei

Title

Effects of intracellular Ca^{2+} signaling on neurite extension and retraction via the regulation of actin cytoskeleton.

Abstract

Intracellular calcium ion (Ca^{2+}) involves a variety of physiological processes, including neural development, degeneration, and synaptic plasticity, through multiple signal transductions. Curiously, similar increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$.) could induce neurite outgrowth or retraction by different stimulations and/or in different neural cell types. However, the mechanism for the opposite effects induced by increases in $[Ca^{2+}]_i$ has not been elucidated. We demonstrated that serotonin enhances NGF-induced neurite outgrowth, while ATP induces transient neurite retraction using PC12 cell for studying neural differentiation and neurite outgrowth. In both cases, increase in $[Ca^{2+}]_i$ is essential for the events, and Ca^{2+}-dependent signal transductions for these effects are discussed.

Chapter 1 describes the physiological function of several neurotransmitters and Ca^{2+} signaling.

Chapter 2 describes the fluorescent imaging techniques for investigation of intracellular molecules: measurement of intracellular ions concentration using fluorescent indicators, visualization of dynamics of proteins using fluorescent tags, and measurement of interaction between proteins using fluorescence resonance energy transfer (FRET).

Chapter 3 discusses the effect of serotonin on neurite outgrowth via Ca^{2+}-dependent signal transductions in PC12 cells. Serotonin induces increases in $[Ca^{2+}]_i$ in differentiated PC12 cells, and the increases in $[Ca^{2+}]_i$ were amplified over the course of the NGF-induced cells differentiation. Serotonin-induced increases in $[Ca^{2+}]_i$ were inhibited by MDL 72222, a selective 5-HT_3 receptor antagonist, and nifedipine, an L-type calcium channel blocker, but not by ketanserin, a 5-HT_2 receptor antagonist, thapsigargin, a specific inhibitor of endoplasmic reticulum Ca^{2+}-ATPase. Furthermore, the neuritogenic effect of serotonin was suppressed by MDL 72222, nifedipine, trifluoperazine, a calmodulin inhibitor, and cypermethrin, a calcineurin inhibitor. These results indicate that serotonin-induced increases in $[Ca^{2+}]_i$, that are mediated via 5-HT_3 receptors and L-type calcium channels in PC12 cells, and subsequent activation of calmodulin and calcineurin enhance NGF-induced neurite outgrowth.

Chapter 4 discusses the regulation of actin cytoskeleton by ATP-induced increases in $[Ca^{2+}]_i$. PC12 cells express purinergic P2X and P2Y receptors, and activation of these receptors increases in $[Ca^{2+}]_i$. We observed ATP-induced formation of cofilin rods especially in neurites. In co-transfection experiments of Cofilin-Venus and Cerulean-actin, interaction of cofilin rods with actin filaments was confirmed by using FRET measurement. ATP-induced formation of cofilin rods were not observed in Ca^{2+}-free medium. UTP (a specific P2Y receptor agonist) did not induce the formation of cofilin rods. Pre-treatment of suramin or thapsigargin that inhibits P2Y receptor-derived increases in $[Ca^{2+}]_i$ did not inhibit ATP-induced formation of cofilin rods. Furthermore, trifluoperazine and cypermethrin inhibited ATP-induced formation of cofilin rods. From these results, it is concluded that ATP-induced formation of cofilin rods depends on Ca^{2+} influx through P2X receptors, and activation of calmodulin and calcineurin.

Chapter 5 discusses the intracellular Ca^{2+} signaling for neurite extension and retraction via the regulation of actin cytoskeleton, and gives the conclusions from this study.