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THE ROLE OF PKC PHOSPHORYLATION IN REGULATING THE METABOTROPIC GLUTAMATE RECEPTOR mGluR5 SIGNALING AND ENDOCYTOSIS

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We previously have shown that serine 839 on mGluR5 is phosphorylated by PKC and thereby mGluR5 elicits a characteristic oscillatory calcium transient. More than this, we observed several major phosphopeptide spots from *in vitro* phosphopeptide map, so we searched for a new PKC phosphorylation site on mGluR5 C-terminus and its function in receptor endocytosis. Using *in vitro* phosphopeptide mapping technique combined with site-directed mutagenesis, we identified a novel PKC phosphorylation site on mGluR5, serine 901. We used a 1st one-third of mGluR5 C-terminus GST-fusion protein (GST-mGluR5-Cprox) to identify the phosphorylation sites by PKC because most PKC phosphorylation sites seem to be present on 1st one-third of mGluR5 C-terminus from a our previous study. Upon S901A mutation on GST-mGluR5-Cprox, a major phosphopeptide spot disappeared on phosphopeptide maps. We raised phosphorylation site-specific antibody against S901 on mGluR5. This antibody recognized PKC phosphorylated wild-type GST-mGluR5-Cprox, and did not recognize PKC phosphorylated GST-mGluR5 Cprox (S901A). We tested the effect of the PKC phosphorylation of S901 on agonist-induced mGluR5 internalization. After treatment of glutamate, mGluR5 wild-type internalized while mGluR5 S901A mutant maintained its cell surface expression level. These suggest that PKC phosphorylation of serine 901 is important in regulating mGluR5 endocytosis.

Key Words: mGluR5, Phosphorylation, Endocytosis, Calcium oscillations