REGULATION OF PHOSPHORYLATION OF DOPAMINE D3 RECEPTORS IN MOUSE STRIATAL NEURONS *in vivo*

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Introduction: Dopamine D3 receptors (D3Rs) are G-protein-coupled receptors. These D3Rs inhibit adenylyl cyclase and the downstream formation of cAMP. Due to their preferential expression in the mesolimbic areas, especially in the nucleus accumbens (NAc), they are known to play a major role in the mesolimbic function. Recently, we found that D3Rs are phosphorylated at serine 229 (Ser229) by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[1]. This phosphorylation is subject to the modulation, and the phosphorylation level controls receptor function. In this study, an effort was made to develop a phospho- and site-specific antibody. Using this antibody, we hypothesized that the modulation of D3R phosphorylation at Ser229 by dopamine D1 receptors would occur in the mouse NAc *in vivo*.

Methods: To detect phosphorylation of D3Rs at Ser229 in striatal neurons *in vivo*, we developed a phospho- and site-specific antibody. A short peptide containing phospho-Ser229 (KRILTRQNpSQCISI) was synthesized and used as an immunogen for producing a polyclonal antibody from rabbits. Upon demonstration of the selectivity of this antibody, we used it in Western blot to monitor changes in Ser229 phosphorylation in striatal neurons in response to dopamine D1 receptor stimulation by a D1 selective agonist SKF81297. Following IACUC approval, adult male mice (CL57/BL6) were randomly divided into 2 groups (n = 3 per group). The animals received an intraperitoneal injection of saline or SKF81297 (1 mg/kg). Animals were sacrificed by cervical dislocation 15 min after drug injection. The NAc was removed and homogenized. Homogenates were centrifuged (1000 *g*, 10 min), and the supernatant was used for Western blot. Densities of immunoblots were measured using optical scanning and the data were analyzed using Student's t-test ($p < 0.05$).

Results: A series of control experiments validated the selectivity of the antibody against phosphorylated D3Rs at Ser229 (pD3R-Ser229). Using this antibody, we found that the level of pD3R-Ser229 in the NAc was significantly increased in mice treated with a systemic injection of the D1 receptor agonist SKF81297 (1 mg/kg, 15 min) compared to mice treated with saline.

Conclusion: Using a phospho- and site-specific antibody against phospho-D3Rs at Ser229, we found that stimulation of dopamine D1 receptors increases phosphorylation

of D3Rs in the mouse NAc *in vivo*.

Discussion: Developing a phospho- and site-specific antibody is necessary for detecting changes in D3R phosphorylation *in vivo*. Using this newly acquired antibody, we found that Ser229 phosphorylation of D3Rs is subject to the modulation by dopamine D1 receptors. This seems to indicate a previously-unrecognized D1-dependent negative feedback control of D3R function given that Ser229 phosphorylation inhibits D3Rs[1]. In a subpopulation of striatal neurons that co-express D3Rs and D1 receptors, dopamine is able to concurrently enhance the D1-mediated phosphorylation of D3Rs to induce a heterologous desensitization of D3Rs after their activation. This regulation is deemed important for maintaining normal homeostasis of D3R function and could be altered to contribute to various neurological disorders.

References:

1. Liu X, Mao L, Zhang G, Papasian C, Fibuch E, Lan H, Zhou H, Xu M and Wang JQ. 2009. Activity-dependent modulation of limbic dopamine D3 receptors by CaMKII. Neuron 61, 425-438.