

## Change in expression of ubiquitin ligase in the hippocampus of stress-maladaptive mice

Hiroko Miyagishi<sup>1,2</sup>, Yasuhiro Kosuge<sup>1</sup>, Minoru Tsuji<sup>2</sup>, Hiroshi Takeda<sup>2</sup>, Kumiko Ishige<sup>1</sup>

<sup>1</sup>Lab. Pharmacol., Sch. Pharm., Nihon Univ., <sup>2</sup>Dept. Pharmacol., Sch. Pharm., IUHW

Stress is thought to be a risk factor for psychiatric disorders, such as major depression. Previously, we reported that mice exposed to repeat excessive restraint stress showed emotional abnormality. Corticosterone (CT) elevated by repeated stress have been reported to increase the ubiquitination in the brain. The present study was designed to investigate the levels of expression of ubiquitin ligase proteins in the prefrontal cortex and hippocampus of stress-maladaptive mice and to examine whether CT alters the proteins expression levels in a mouse hippocampal HT22 cells.

Male ICR mice were chronically exposed to inadaptable stress, i.e. repeated restraint stress for 240 min/day for 14 days. After the final exposure to stress, brains of mice were rapidly removed and the hippocampus was dissected. HT22 cells were cultured in DMEM media supplemented with 10% FBS. Cell viability was measured by using MTT Assay.

Western blot analysis revealed that no change in the level of expression of E3 ubiquitin ligase Nedd4-2 was observed in among all mice. In contrast, a significant decrease in the expression level of phosphorylated Nedd4-2 (p-Nedd4-2) was observed in the hippocampus, but not the prefrontal cortex, of stressed mice. Although CT had no effect on cell survival at concentrations of less than 3  $\mu$  M, pretreatment with 3  $\mu$  M CT down-regulated p-Nedd4-2 in HT22 cells. These results suggested that CT is an important effector for activation of Nedd4-2 in hippocampal neurons, and Nedd4-2 plays a pivotal role in the emotional abnormality in stress-maladaptive mice.