## NEUROPROTECTIN D1 (NPD1) ATTENUATES BRAIN DAMAGE INDUCED BY TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS THROUGH TRPC6/CREB PATHWAYS

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**Background & Objective:** Neuroprotectin D1 (NPD1) could serve as an endogenous neuroprotective role in brain ischemia injury, yet the underlying mechanism is poorly understood. We aim to investigate whether intracerebroventricular (ICV) injection of NPD1 is neuroprotective against transient focal cerebral ischemia. We also sought to verify the neuroprotective mechanisms of NPD1.

**Experimental approaches:** Rats subjected to 2 h ischemia followed by reperfusion were treated with NPD1 at 2 h after reperfusion. PD98059 or KN62 was administrated 20 minutes prior to the operation. Western blot analysis was performed to detect the protein levels of calpain-specific aII-spectrin breakdown products of 145kDa (SBDP145), TRPC6 and phosphorylation of cAMP/Ca2+-response element binding protein (p-CREB) at 12, 24 and 48 h after reperfusion. The immunoreactivity of p-CREB and TRPC6 were measured by Quantum Dots-based Immunofluorescence analysis. Infarct volume and neurologic scoring were evaluated at 48 h after reperfusion.

**Results:** NPD1, when applied at 2 h after reperfusion, significantly reduced infarct volumes and increased neurologic scores at 48 h after reperfusion accompanied by elevated TRPC6 and p-CREB activity and decreased SBDP145 activity. When mitogenactivated protein kinase kinase (MEK) or calcium/calmodulin-dependent protein kinase (CaMKIV) activity was specifically inhibited, the neuroprotective effect of NPD1 was attenuated and correlated with decreased CREB activity.

**Conclusion:** Our results clearly showed that ICV injection of NPD12 h after reperfusion improves the neurological status of MCAO rats through the inhibition of calpain-mediated TRPC6 proteolysis and the subsequent activation of CREB via the Ras/MEK/ERK and CaM/CaMKIV pathways.