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INVOLVEMENT OF THE MODULATED-NEURONAL NITRIC OXIDE SYNTHASE ACTIVITIES THROUGH INTERACTIONS OF PROTEIN KINASES IN LEAD NEUROTOXICITY.

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This work aimed to identify neuronal cell toxicity induced by decrease of physiological NO production by differential phosphorylation of constitutive neuronal NO synthase (nNOS), which can be mediated by Ca2+-dependent PKC and/or CaM-KII activities activated by metals. We used Pb to modulate Ca2+-dependent PKC and/or CaM-KII activities, which were also controlled by pretreatment of the kinase specific inhibitors such as Ro-31-8220 and KN-93, respectively. CATH.a cells, a dopaminergic cell line that produce NO spontaneously by the constitutive nNOS expression were cultured in RPMI1640 media, and then treated with high concentration of lead (Pb, 100 mM) for 24 h. Cell viability and levels of phosphorylation of nNOS were determined by MTT assay and Western-blot with phospho-nNOS antibody, respectively. The nNOS reductase activity was also assessed to compare the phosphorylated site-specific activity of nNOS. Activities of each enzyme (nNOS and tyrosine hydroxylase) and dopamine contents were determined, and the survival capacity of neuronal cells were also compared in Western-blot by using of BDNF (brain-derived neurotrophic factor) antibody. In CATH.a cells, Pb treatment increased the phosphorylation of nNOS and PKC inhibitor (Ro-31-8220) alone protected the phosphorylation. However, the phosphorylation of nNOS by CaM KII inhibitor (KN-93) alone was clearly enhanced. Interestingly, when the cells were treated with Pb plus Ro-31-8220 or Pb plus KN-93, the nNOS was phosphorylated strongly. The levels of nNOS phosphorylation were oppositely correlated with expression levels of BDNF. Furthermore, protein expression and the catalytic activities of TH and nNOS were also altered after treatment of Pb or Pb plus each inhibitor. In addition, cell viability was significantly decreased after Pb treatment, but restored by low concentration of NO donor (0.1

mM). These results demonstrate that physiological NO production in dopaminergic neuronal cells can be decreased by nNOS phosphorylation mediated by activation of PKC and/or CaM KII, and thus Pb neurotoxicity may be induced by modulation of NO production.

Keyword : Pb, Neurotoxicity, Nitric oxide, NOS,