ESR-Spin trapping study of NO in neurocyte

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It is desirable to measure endogenous nitric oxide (NO) free radicals in neurocytes directly, because NO plays very important roles in pathological and pharmacological processes, as well as in physiological process. It can be realized with ESR-spin trapping method, using DETC (diethyldithiocarbamate) and Fe^{2+}–citrate as spin traps. They format a stable adduct, NO-Fe-$(DETC)_2$, which can be detected with a conventional ESR spectrometer. In normal situation, the amount of endogenous NO is too low to be measured. We used extraction method with ethyl acetate as an organic solution that collected NO-Fe-$(DETC)_2$ product from water phase to ethyl phase, resulting in an increase the sensitivity of ESR measurement at room temperature.

PC12 cells, which have been differentiated with nerve growth factor (NGF) and present obvious dopamine (DA) metabolism, are frequently used as a neurocyte model to study the mechanisms of neurodegeneration diseases.

We studied the spin trapping method and found the concentration of spin traps, incubating time and temperature affected the amount of NO trapped in PC12 cells. A better spin trapping system is as following: DETC (10mmol/L) and Fe^{2+} (2mmol/L) incubated with PC12 cells at 37°C for 0.5h. The addition of higher Fe^{2+} could increase the NO signal intensity, but excessive Fe^{2+} induced additive damage to cells, even the NO signal in control system was more than in induced system where some cell factors added in. A NO supplement, 250 μmol/L S-nitrosoglutathione (GSNO) was incubated with PC-12 cells overnight, getting standard spectrum parameters of NO-Fe-$(DETC)_2$ complex in cells. It has a triplet signal: g_1=2.046, g_2=2.037 and g_3=2.028, hyperfine splitting constant a=12.45 Gauss. The effects of INF-γ, TNF-α and insulin on the formation of endogenous NO in cells were studied. Addition of INF-γ(100u/ml) alone can induce to increase the generation of NO under existing arginine (1mmol/L), but coinduction of both INF-γ and TNF-α(500u/ml) produced more NO in cells. The insulin decreased the production of NO in cells. It is related to the concentration of insulin and different cell lines. For PC12 line, NO signal decreased 50% when interaction with 10 μg/ml of insulin for 15h. R2, rat cerebellum cell line was also studied. It is more sensitive to the formation of NO in R2 than in PC12 when induced by above cell factors.

We are conducting an experiment to check the NOS express for clarifying the mechanism of NO production in cells, using immunocytochemical method.