E115 Protection of cultured cortical neurons against zinc-induced death by recombinant tissue plasminogen activator

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Recombinant human tissue plasminogen activator (rt-PA) is being used as a thrombolytic agent in brain ischemia. However, rt-PA may be harmful to neurons by potentiating excitotoxic injury. Since zinc neurotoxicity is another key mechanism of ischemic neuronal death, we examined whether rt-PA altered zinc neurotoxicity in mouse cortical culture. Neuronal death that occurred 24 h after 10 min exposure to 300 μM zinc was completely blocked by 10 μg/ml rt-PA added continuously during and after the zinc exposure. This protection was not likely due to the reduction of toxic zinc influx, since fluorescence zinc staining with 6-methoxy-8-quinolyl-para-toluene sulfonamide (TSQ) revealed no difference in intra-neuronal zinc accumulation. Rather, rt-PA attenuated free radical generation and lipid peroxidation, events likely downstream of zinc influx. Consistently, rt-PA also attenuated another oxidative neuronal death induced by iron or H_2O_2 exposure. By contrast, rt-PA did not alter excitotoxin-induced neuronal death. The protective effect of rt-PA was not mimicked by plasmin, the major product of rt-PA; rather, plasmin (30-100 μg/ml) markedly potentiated zinc neurotoxicity. In addition, mild treatment with another protease trypsin also blocked the zinc neurotoxicity, suggesting that proteolytic action may be the basis of the neuroprotection by rt-PA. The present study confirmed that rt-PA can potentiate neuronal injury likely via producing plasmin. However, we have additionally found that rt-PA directly protects against zinc and other oxidative neurotoxicity. These results suggest that certain membrane proteins play important roles in oxidative neuronal injury.

E116 Isolation and Biochemical Properties of Adult Specific Carboxylesterase in *Lucilia illustris*

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Two adult specific carboxylesterases(ASCE-I, ASCE-II) were characterized by their biochemical and enzymatic properties in *Lucilia illustris*. ASCE-I and ASCE-II showed a common optimal temperature, 45℃ regardless of the reacted substrates, α-naphthyl acetate, α-naphthyl butyrate, β-naphthyl acetate. The optimal pH of ASCE-I and II were 7.5. The V_{max} and K_m values of the ASCEs were varied with the reacted substrate ; the V_{max} of ASCE-I was 87.0 μ mol/min/*mg* protein for β-Na, 42.8 μ mol/min/*mg* protein for α-Na, and 33.3 μ mol/min/*mg* protein for α-Nb, whereas ASCE-II exhibited 51.6 μ mol/min/*mg* protein for β-Na, 42.6 μ mol/min/*mg* protein for α-Na, and 33.8 μ mol/min/*mg* protein for α-Nb ; the K_m of ASCE-I was about 1.99×10^{-5}M for β-Na, 1.75×10^{-5}M for α-Na, and 1.81×10^{-4}M for α-Nb, whereas those of ASCE-II were 1.53×10^{-5}M for β-Na, 2.27×10^{-5}M for α-Na, and 9.09×10^{-5}M for α-Nb, respectively. On the otherhand, the denaturation temperatures of ASCE-I and II were of 30℃~40℃ showing their somewhat highly heat-sensitivity, and the enzyme activities were not detectable above 60℃.