Effects of an endogenous dopaminergic neurotoxin, 6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline (norsalsolinol), on dopamine secretion and its intracellular dynamics in rat pheochromocytoma PC 12 cells

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Naturally occurring neurotoxins, 6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinolines (DHTIQs), have been thought to be the causative agents of parkinsonism. DHTIQs, including norsalsolinol, have been found in the mammalian central nervous system. Norsalsolinol can be formed in parkinsonian patients. However, the effects of DHTIQs on the secretion of dopamine, as well as other neurotransmitters, have not been elucidated. In this study, the effects of norsalsolinol on dopamine secretion from nerve growth factor-differentiated PC 12 cells were investigated. Norsalsolinol (1-100 μM) pretreatment suppressed both ATP (100 μM) -and K^+ (50 mM) -induced dopamine secretion from PC 12 cells in a concentration-dependent fashion but did not affect basal dopamine secretion. In β-escin-permeabilized PC12 cells, norsalsolinol pretreatment suppressed Ca^{2+} (pCa= 4-8) -induced dopamine secretion but did not inhibit secretagogue-induced change in intracellular Ca^{2+}concentration. These results suggest that norsalsolinol causes inhibition of secretagogue-induced dopamine secretion from PC 12 cells without altering intracellular Ca^{2+}concentration. Next, the uptake of norsalsolinol into PC 12 cells was studied. The compound was actively taken up by PC 12 cells, and its rate was dependent on the concentration of extracellular Na^+ with a Km value of 176.2±4.5 μM and a maximum velocity (V_{max}) of 55.6±3.5 pmol/min/mg protein (n = 4). The uptake of norsalsolinol was sensitive to two dopamine transporter (DAT) inhibitors, GBR-12909 and reserpine, but was less sensitive to desipramine, a noradrenaline transporter inhibitor. Dopamine, an endogenous DAT substrate, inhibited norsalsolinol uptake into PC 12 cells. The Km and Vmax values of the uptake of norsalsolinol in the presence of 100 μM dopamine were 241.1± 13.9 μM and 47.6±5.1 pmol/min/mg protein, respectively (n= 4). The kinetic parameters suggest that the inhibition was competitive. The Ki value of dopamine was estimated to be 271.2±61.6 μM (n= 4). These results suggest that norsalsolinol is taken up mainly by the dopamine transporter into PC 12 cells. Finally, the intracellular dynamics of norsalsolinol in PC 12 cells was studied. I found by using the sucrose density gradient method that dopamine and norsalsolinol are co-localized in the secretory granule layer in norsalsolinol-treated PC 12 cells. Norsalsolinol was actively taken up into the isolated secretory vesicle fraction from PC 12 cells with a Km value of 41.5±6.8 μM (n= 4). The uptake of 10 μM of norsalsolinol was sensitive to reserpine (1 μM), an inhibitor of the vesicular dopamine transporter, and dopamine, an endogenous substrate, but insensitive to GBR-12909, an inhibitor of the dopamine transporter on the plasma membrane. In norsalsolinol-treated

PC 12 cells, exposure to 50 mM KCl or 100 μM ATP resulted in simultaneous release of norsalsolinol and dopamine. The time courses of release of dopamine and norsalsolinol evoked by 50 mM KCl or 100 μM ATP coincided to each other. These releases were dependent on the concentrations of secretagogues. These findings suggest that norsalsolinol is taken up with dopamine into secretory vesicles via the vesicular catecholamine transporter. These findings may also be related to postural abnormality in Parkison's disease.

Mechanism of generation of reactive oxygen species with accumulation of copper in the liver of Long-Evans Cinnamon (LEC) rats and its relationship to the onset of acute hepatitis

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Long-Evans Cinnamon (LEC) rats accumulate excess copper (Cu) in the liver in a manner similar to patients with Wilson's disease (WD), and they spontaneously develop acute hepatitis with severe jaundice at 3 ~ 5 months after birth. Reactive oxygen species (ROS) generated as a consequence of the accumulation of excess Cu are thought to be responsible for acute hepatitis in LEC rats. However, the mechanisms by which the excess Cu generates the ROS and induces hepatitis have not yet been revealed in this animal model. In this study, the author investigated the mechanisms of ROS generation accompanying Cu accumulation in the liver of LEC rats and their relationship to the onset of acute hepatitis.

1) The author investigated the mechanism of increased lipid peroxidation (LPO) in the liver using an in vitro incubation system. The levels of LPO in the liver S-9 from hepatitic LEC rats were increased by incubating the liver S-9 in the presence of NADPH-generating system (NADPH-gs). This increase was inhibited by EDTA, butylated hydroxytoluene, and catalase (CAT), suggesting that the hydroxyl radicals (·OH) generated by the Fenton-type reaction between H_2O_2 and free Cu, are involved in this increase in LPO. H_2O_2 and ·OH are known to be generated one after another during Cu-catalyzed GSH oxidation. Electron spin resonance study revealed a marked increase in ·OH generation in the liver cytosol from hepatitic LEC rats in the presence of GSH and H_2O_2. The cyclic regeneration of GSH from GSSG by NADPH-dependent glutathione reductase in the presence of NADPH-gs may cause sustained generation of ·OH in the presence of excess free Cu.

2) In vivo ·OH production in plasma and liver of hepatitic LEC rats was quantified by trapping ·OH with salicylic acid (SA) as 2,3-dihydroxybenzoic acid (2,3-DHBA). The ratios of 2,3-DHBA/SA were significantly higher in plasma and liver of hepatitic LEC rats and acute Cu-overload Wistar rats than in those of Wistar rats and LEC rats showing