# Endogenous Modulation of Glutamate Transport in Nerve Terminals by Membrane Cholesterol.

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The proper level of extracellular glutamate, which as excitatory neuromediator is involved in many aspects of normal brain functioning and the pathogenesis of neurological disorders, is maintained by a balance between release and uptake. These processes are tightly associated with membrane components of cells. Cholesterol is an abundant constituent of eukaryotic membranes and certain level of membrane cholesterol is very important for normal functioning of a number of membrane proteins involved in synaptic transmission. Taking into account that the ambient glutamate level is critically important for proper synaptic transmission, the main question we asked was weather membrane cholesterol content modulated the processes responsible for maintaining low extracellular glutamate concentration. Using control and cholesterol-depleted rat brain nerve terminals (synaptosomes), the study was focused on evaluation of the ambient glutamate level; contribution of uptake and tonic release in maintaining extracellular glutamate concentration; discrimination of changes in the extracellular glutamate level and tonic release. Confocal imaging of synaptosomes labeled with filipin revealed acute depletion of cholesterol after application of cholesterol acceptor methyl-$\beta$–cyclodextrin (M$\beta$CD). Flow cytrometric analysis showed similarity in cell size and cytoplasmic granularity of control and cholesterol-depleted synaptosomes. Reducing the contribution of glutamate transporters by 200 $\mu$M DL-threo-$\beta$-benzyloxyaspartate (DL-TBOA), a decrease in tonic release of L-[$^{14}$C]glutamate was found that consisted of 10.8 ± 0.6 % of total in control and 8.87 ± 0.6 after cholesterol depletion. The ambient L-[$^{14}$C]glutamate level was increased from 0.193 ± 0.013 nmol/mg of protein in control to 0.282± 0.013 after cholesterol depletion due to inhibition of uptake (the initial velocity was lowered from 3.0 ± 0.3 to 1.77± 0.2 nmol x min$^{-1}$ x mg$^{-1}$ of proteins). We considered that an increase in tonic release might occur when intracellular glutamate was replenishing because of impact of the accumulative ability of synaptic vesicles. An increase in tonic release of L-[$^{14}$C]glutamate in the presence of DL-TBOA from 3.3± 0.6 to 8.88± 0.6 % of total was shown during dissipation of the proton gradient by the protonophore FCCP. The current observations suggested that cholesterol as an endogenous modulator of neurotransmission in the CNS can be extremely important for synaptic plasticity and significant level of cholesterol required for maintaining low extracellular glutamate concentration. An increase in ambient glutamate impairing synaptic transmission may form the potential basis for neurological symptoms in diseases associated with alterations in sterol homeostasis.