Exocytosis and Na^+-dependent Glutamate Transport in Nerve Terminals after Membrane Cholesterol Depletion by Methyl-beta-cyclodextrin.

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Role of membrane cholesterol in exocytosis and direct and reversed function of Na+-dependent glutamate transporters was investigated. Recently we have demonstrated that depletion of membrane cholesterol by methyl-beta-cyclodextrin (MeCD) resulted in a dose-dependent significant reduction of the L-[^{14}C]glutamate uptake by synaptosomes. Treatment of synaptosomes with 5 and 15mM MeCD caused a decrease in the velocity of L-[^{14}C]glutamate uptake by 20±4% and 49±4%, respectively ($P \leq 0.05$). The intracellular level of L-[^{14}C]glutamate accumulated inside of synaptosomes did not change as a result of cholesterol depletion despite of drastic decrease of glutamate uptake activity. Depletion of membrane cholesterol did not change the tonic outflow of preloaded L-[14C]glutamate. The depolarization stimulated Ca^{2+}-independent glutamate release occurred via reverse functioning of glutamate transporters decreased insignificantly for 1 min from 8.0±0.4 % to 6.7±0.4 % of total accumulated synaptosomal label after MeCD treatment. Further analysis over the next 6 minutes exhibited the increased similarity of the L-[^{14}C]glutamate release between control and MeCD treated synaptosomes that consisted of 14.0±2.0 %. Depletion of membrane cholesterol resulted in a reduction of the depolarization evoked exocytotic release from 8.0±1.0% to 4.2±1.0% of total synaptosomal label. 1 microM ionomycin (calcium ionophore) induced the release from synaptic vesicles that decreased from 20.0±1.9 % to 14.0±1.9 % after cholesterol depletion. We examined the effects of [Na^+] on the L-[^{14}C]glutamate outflow to evaluate the balance of uptake and reversed transport. Control and MeCD–treated synaptosomes demonstrated the similar increase in the extracellular level of glutamate in NMDG-supplemented media. Synaptosomes were treated with MeCD-cholesterol complex to determine MeCD-induced effects to be a result of cholesterol depletion. Thus, cholesterol depletion was found to decrease significantly the Na^+-dependent uptake and exocytotic release of glutamate.