NMDA receptors belong to the family of ionotropic glutamate receptors, and are involved in synaptic plasticity, learning and memory. However, overactivation by the agonist glutamate can lead to neuronal death – excitotoxicity. Excitotoxicity is a result of excessive calcium influx into the cell through NMDA receptors, and is associated with many central nervous system (CNS) diseases. Neurosteroids are endogenous compounds capable of NMDA receptor modulation, thus they may have pharmacological potential in the treatment of CNS disorders. The aim of this work was to investigate how pregnanolone sulfate (PA-S) and pregnanolone hemipimelate (PA-hPim) influence somatic calcium and excitotoxicity. We used fluorescence microscopy for recording changes in somatic calcium concentration. We observed that PA-S had no influence on relative somatic calcium concentration. Synthetic analog PA-hPim increased somatic calcium levels slightly. Next, we used oxygen-glucose deprivation (OGD) in vitro to study the influence of neurosteroids on excitotoxicity. Both PA-S and PA-hPim were neuroprotective in the model of acute OGD in vitro. Moreover, PA-S or PA-hPim pretreatment induced ischemic tolerance to a subsequent OGD episode. Our results suggest that neurosteroids PA-S and PA-hPim are potential candidates for the development of therapeutics for the treatment of CNS disorders involving excitotoxicity.