

ABSTRACT

N-methyl-D-aspartate (NMDA) receptors are a subtype of receptors for major excitatory neurotransmitter glutamate in the central nervous system. Their activity is regulated by variety of allosteric modulators, including endogenous neurosteroids and their synthetic analogues. NMDA receptor dysfunction is implicated in various forms of neurodegeneration and inhibitory neurosteroids have unique therapeutic potential to act as neuroprotective agents. The aim of this work is to investigate relationship between structure and function of neurosteroids with modifications in the D-ring region, using whole-cell patch clamp recording at recombinant GluN1/GluN2B receptors. In this work, we characterised inhibition effect of 19 neurosteroid analogues on NMDA receptor activity and found several of them to be potent NMDA receptor inhibitors. According to our results, there is a linear relationship of IC_{50} and lipophilicity of a neurosteroid compound, suggesting the plasma membrane plays an important role in neurosteroid access to NMDA receptor. Indeed, using capacitance recording configuration in combination with amphipathic molecule gamma-cyclodextrin, we were able to separate the kinetic of neurosteroid membrane binding from receptor binding. Moreover, these experiments showed that neurosteroid accumulation in the membrane is critical for its inhibition effect. Our results are consistent with previously suggested model of inhibitory neurosteroid action at NMDA receptors.