

ABSTRACT

N-methyl-*D*-aspartate (NMDA) receptors are glutamate-gated calcium permeable ion channels that play a key role in excitatory synaptic transmission and plasticity, and their dysfunction underlies several neuropsychiatric disorders. The overactivation of NMDA receptors by tonically increased ambient glutamate can lead to excitotoxicity, associated with various acute and chronic neurological disorders, such as ischemia, Alzheimer and Parkinson's disease, epilepsy or depression. On the opposite, NMDA receptor hypofunction is thought to be implicated in autism, schizophrenia, or intellectual disability. Recent DNA screening for neurological and psychiatric patients revealed numerous mutations in genes encoding for NMDA receptor subunits. The activity of NMDA receptors is influenced by a wide variety of allosteric modulators, including neurosteroids that could both inhibit and potentiate the activity of NMDA receptors, which makes them promising therapeutic targets. In this thesis, we describe new classes of neurosteroid analogues which possess structural modifications at carbons C3 and C17 of the steroidal core, and analogues without D-ring region (perhydrophenanthrenes). We evaluated the structure-activity relationship (SAR) for their modulatory effect on recombinant GluN1/GluN2B receptors. Our results demonstrate that: (i) endogenous neurosteroid pregnanolone sulphate (**PA-S**) and its analogues, substituted at C3 with a carboxylic acid moiety of varying length, are effective antagonists of tonically activated NMDA receptors with a smaller or no effect on phasically activated NMDA receptors and that the difference in potency between tonic and phasic inhibition increased with the length of the C3-residue. (ii) We further demonstrated that all tested compounds with modifications at C17 (compounds **3.1-51.1**) are more potent inhibitors of NMDA receptors (with values of IC_{50} , the concentration of compound that produces 50% inhibition, varying from 84.0 nM to 5.4 μ M) than the endogenous **PA-S** ($IC_{50} = 24.6 \mu$ M). (iii) For perhydrophenanthrene analogues (compounds **1.2-10.2**) we showed that they act as inhibitors of NMDA receptors and compound **9.2** ($IC_{50} = 15.6 \mu$ M) was assessed as a more potent inhibitor of NMDA receptor currents than the endogenous **PA-S**. (iv) We also tested analogues of **PA-S** and pregnanolone glutamate (**PA-G**) with C3-residue connected by amide (compounds **1.3-4.3**). We found that inhibitory effect of compounds **3.3** and **4.3** on NMDA receptors was significantly improved ($IC_{50} = 1.0$ and 1.4μ M, respectively) as compared with endogenous **PA-S** and its neuroprotective analogue **PA-G** ($IC_{50} = 51.7 \mu$ M). (v) Further, we tested the sensitivity of mutated human NMDA receptors to steroids that potentiate the activity of NMDA receptors. We showed that responses induced in mutant

hGluN1/hGluN2B(V558I; W607C; V618G; and L825V) receptors (with diminished probability of channel opening by ~10-fold) were potentiated by 59-197% and 406-1647%, respectively, when recorded in the presence of endogenous pregnenolone sulphate (**PE-S**) and its analogue androstene hemisuccinate. (vi) In case of **PE-S** analogues (compounds **2.4-24.4**) we found out that they potentiate the activity of NMDA receptors (EC_{50} values, corresponding the concentration producing half-maximal potentiation, varying from 1.8 to 151.4 μ M and E_{max} values varying from 48 to 452 %) and 10 of them were more potent NMDA receptor modulators than endogenous **PE-S** ($EC_{50} = 21.7 \mu$ M). Moreover, the SAR study revealed a relationship between the length of the residues at C3 of the steroid molecule and the potentiating effect at GluN1/GluN2B receptors for various D-ring modifications. Our results provide an opportunity for the development of new therapeutic drugs based on neurosteroids that can be potentially used in the treatment of neuropsychiatric disorders involving hyperfunction or hypofunction of NMDA receptors.

KEYWORDS: NMDA receptor, neurosteroid, structure-activity relationship, pregnane analogues, pregnene analogues, human mutations