Doctoral thesis: Study of functional and pharmacological properties of NMDA receptors

ABSTRACT

N-methyl-D-aspartate (NMDA) receptors are a subtype of ionotropic glutamate receptors crucial for synaptic transmission and learning and memory processes. After activation and opening of the ion channel, they are permeable to calcium ions (Ca^{2+}) that mediate further signaling. Hypo- or hyperfunction of NMDA receptors leads to the development of severe neurological and psychiatric diseases. NMDA receptors are modulated by many endogenous and exogenous substances. These include neurosteroids – steroids synthesised *de novo* in the central nervous system and their synthetic analogues, which, depending on their structure, can have positive or negative modulatory effects.

Using electrophysiological and molecular-biological techniques, we have clarified the molecular mechanism of increased sensitivity of NMDA receptors to inhibitory neurosteroids following a transient increase in intracellular Ca^{2+} concentration. The increase in neurosteroid inhibitory activity at NMDA receptors is related to Ca^{2+}-induced depalmitoylation of three cysteines (C849, C854, C871) in the intracellular part of the GluN2B subunit. This simultaneously leads to a change in the receptor's kinetic parameters in favor of the closed state. Furthermore, this work provides new insights into the relationship between the structure and activity of neurosteroids at NMDA receptors, which is important for finding potential new drugs, but also for understanding the function of these receptors. Structural analogues of pregnanolone sulphate (PAS) without the steroid D-ring maintain inhibitory activity, but there is no improvement in effect over PAS. In contrast, the C3-amide substituent increases the inhibitory effect as well as the bioavailability of the substances, leading to the identification of appropriate baseline structures for further studies to find potential drugs for over-activation of NMDA receptors. In the first systematic study investigating the structure-function relationship of neurosteroids with a positive effect on NMDA receptors, we characterized structural analogues derived from the endogenous neurosteroid pregnenolone sulphate with modifications to carbons C3 and C17 in the steroidal ring structure. The potentiating effect is dependent on the length of the C3-hemiester residue in combination with the type of substituent on C17.

This work provides important insights for further studies of the relationship between the structure and effect of neurosteroids on NMDA receptors with the goal of finding potential drugs, and in particular, it clarifies the possible neuroprotective effect of increased neurosteroid inhibitory action under pathological conditions related to increased intracellular Ca^{2+} concentration.