Research objective 3 - Multireceptor activity of neuroactive steroids

Activity 1 - Neurosteroids with positive allosteric effect at NMDA receptors

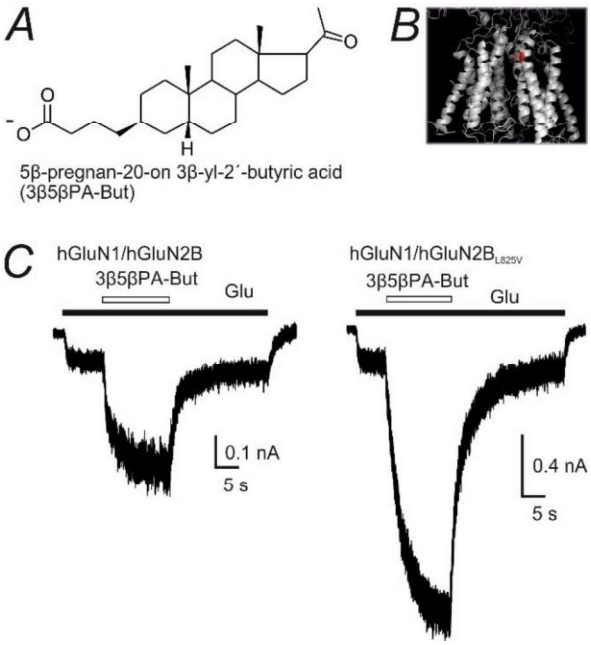
The aim of this activity is the synthesis of new steroidal compounds with positive allosteric effect at NMDA receptors. Specific steps are:

- Organic synthesis and testing of structural analogues of the naturally occurring neurosteroid pregnenolone sulfate. The main focus will be directed toward structural requirements leading to biological activity, e.g. hemiester substituents at the position C-3 and the position of the double bond in A or B ring. Additionally, plasma-stable analogues will be prepared.
- Detailed analysis of the steroid interaction with the NMDA receptor. In this step, patch-clamp technique will be used to study effects of newly synthetized steroids to assess their potency to increase responses to NMDAR agonists of the WT hGluN1/hGluN2B receptors.
- Biophysical analysis of mutated hGluN1/hGluN2 receptors. These will be selected based on mutations found by gene sequencing in psychiatric and neurological patients (these are published in selected Journals, specific databases (PubMed https://www.ncbi.nlm.nih.gov/clinvar/) and/or provided by specialized departments on clinical genetics (such as Department of Paediatric Neurology, Second Faculty of Medicine, Charles University and Motol University Hospital with which we have recently established a colaboration). Molecular biology methods will be used to generate these mutations and patch-clamp analysis of mutated receptors will reveal which NNMDAR function is impaired (affinity for glutamate, glycine, Mg2+, Ca2+-permeability, probability of opening, receptor trafficking).
- Analysis of the steroid interaction with the mutated NMDA receptor. Here we will assess the relative potency of the steroid to potentiate mutated receptors. At this point structural analysis of steroids at mutated receptors will be performed to make rational steps in the development of new pharmaceutical compounds for personalized medicine.
- Based on the above results we will order GMO mice carrying a specific mutation. This mouse will be subsequently phenotypically and pharmacologically tested.

  We have shown previously that pregnenolone sulphate (PE-S), a naturally occurring steroid, enhances responses mediated by NMDARs (Horak et al., 2004) (Horak et al., 2006) (Petrovic et al., 2009). Based on mathematical analysis and kinetic modeling, we have proposed that the affinity of the NMDAR for PE-S depends on receptor activation, being high for non-activated receptors but low for activated receptors, and further that the action of PES is dependent on receptor subunit composition (Horak et al., 2006).

Recent human genetic studies show the existence of multiple alterations in NMDAR subunit genes in several common brain diseases, such as intellectual disability, autism spectrum disorders (ASD), or epilepsy (Burnashev and Szepetowski, 2015) (Hu et al., 2016). Data already published together with our preliminary data indicate that de-novo single-point mutations in GluN1, GluN2A, and GluN2B may result in receptor hypofunction that likely contributes to the observed clinical symptoms. This clearly shows the need for compounds that could compensate for the loss of NMDAR function. Among these compounds, neurosteroids with a positive allosteric effect may prove to be particularly useful.

Our preliminary data show striking differences between wild-type human GluN1/GluN2B receptors (hGluN1/hGluN2B) and receptors with a missense mutation found in autism hGluN2B(L825V) (Tarabeux et al., 2011). Biophysical analysis indicates smaller currents induced by glutamate in HEK293 cells expressing hGluN1/hGluN2B(L825V) receptors than in the WT. The reason for this is the lower probability of opening (Po) in mutated receptors (~1%) compared to the WT hGluN1/hGluN2B receptors (8%). No change in the glutamate/glycine affinity or surface expression of WT vs. mutated receptors was observed. To test for steroid potential in the treatment of NMDAR channelopathies we have used the 5β-pregnan-20-on 3β-yl-2´-butyric acid (3β5βPA-But) (newly synthesized steroid that has a positive allosteric affect at rat native and recombinant receptors; unpublished data) and analyzed its effect at hGluN1/hGluN2B and hGluN1/hGluN2B(L825V) receptors. Figure 1 shows that 3β5βPA-But at the concentration of 100 µM potentiated the hGluN1/hGluN2B receptors 5-fold while the hGluN1/hGluN2B(L825V) receptors were potentiated 10-fold. This suggests therapeutic potential for steroids with a positive allosteric effect at NMDA receptors in the treatment of channelopathies associated with NMDAR hypofunction.



Action of 3β5βPABut at mutated human NMDA receptor associated with autism. ***A.*** Chemical structure of 5β-pregnan-20-on 3β-yl-2´-butyric acid. ***B***. Homology model of the membrane domains of human GluN1/GluN2B receptors (hGluN1/hGluN2B). The position of the alteration in hGluN2B(L825V) is indicated in magenta ***C.*** Response of di-heteromeric human GluN1/GluN2B receptors (hGluN1/hGluN2B) and those with a mutation found in autism (hGluN1/hGluN2B(L825V)). Mutated receptors were potentiated by 3β5βPABut (100 µM) two-fold more than WT.

Institutions/researchers involved

- Institute of Physiology CAS (IPHYS): Prof. Ladislav Vyklicky, MD, PhD, DSc,
- Institute of Organic Chemistry and Biochemistry (IOCB): RNDr. Eva Kudová, PhD.
- (There has been more than 16 years of fruitful cooperation between IPHYS and IOCB in the research of steroid-induced modulation of NMDAR activity - numerous papers were published in prestigious international journals and five patents accepted. Agreement on the cooperation between both laboratories is supplemented *20160324_laboratore_Vyklicky.pdf*)
- In addition, we have recently established cooperation with MUDr. Milos Petrovic, PhD. from University of Central Lanceshire.

References

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