

Conference abstract PO-19

Functionability of NMDA-Receptors in an *in vitro* model of the Blood-Brain-Barrier

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A diversity of disease processes and conditions such as Alzheimer's disease, schizophrenia, epilepsy and stroke – although they are caused by different mechanism – share one common target: the NMDA-receptor [1]. Recent studies reported the presence and the participation of this glutamate-gated ion-channel in major regulatory mechanisms at the blood-brain barrier (BBB). In order to show the functionability of this receptor and his effects on the BBB, we used an *in vitro* model based on human cell line ECV304 [2].

The NMDA-receptor consists of combinations of NR1- and NR2A-D –subunits. The presence of the NR1 subunit, which is necessary to form a functional receptor, was verified by immunofluorescence microscopy and western blotting in our model. The activation of the NMDA-receptor leads to a permeation of calcium ions into the cell. Consequently, one of our aims was to measure this calcium influx as the first functional parameter of the activation of the NMDA-receptor by a fluorescence method using a 96-well plate system. Different concentrations of glutamate, NMDA and NMDA-inhibitors were applied to the cells in order to evaluate the *in vitro* model and to simulate pathological conditions leading to such a controlled calcium influx. Furthermore, methods as western blotting, immunofluorescence microscopy and the measurement of the transendothelial electrical resistance (TEER) were used in order to investigate the influence of overstimulation or inhibition of the NMDA-receptors on specific BBB properties.

In summary, a 96-well plate calcium influx assay was established which may be used as a first screening method to find possible substrates targeting the BBB and regulating the calcium influx. Methods as western blotting and TEER measurement could be applied in order to prove the functional relevance of these calcium influx modulating drugs. Moreover, the adverse effects of glutamate on BBB properties could be confirmed by the *in vitro* model used.

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