

Neurosteroids as potential neuroprotective agents in Parkinson's disease: implications for new therapeutic strategies (NBB Project 741)

Luchetti^{1, 2}, S., Verwer², R., Sluiter², A., Liere³, Ph., Huitinga¹, I., Schumacher³, M., and Swaab², D.

¹Neuroimmunology Research Group, Netherlands Institute for Neuroscience (NIN), Meibergdreef 47, 1105 BA Amsterdam, The Netherlands. ²Neuropsychiatric Disorders Group, NIN, Amsterdam, The Netherlands. ³ISERM University of Paris, France. s.luchetti@nin.knaw.nl

Research question and background

The aim of this project was to analyze if neurosteroid synthesis is affected in Parkinson's disease (PD) before neurodegeneration starts. For this purpose we analyzed levels of neurosteroids in the substantia nigra (SN), which degenerates at earlier stages of PD and the prefrontal cortex (PFC), which degenerates in later stages in order to detect changes of neurosteroids synthesis that occur both very early in the neurodegenerative process and while it is ongoing. Moreover, neuroprotective effects of neurosteroid such as allopregnanolone (AP), dihydroprogesterone (DHP) and dehydroepiandrosterone sulfate (DHEAS) were tested in a human organotypic slice culture for PFC of PD and control subjects.

Methods and tissues used

Human postmortem tissue was obtained from the NBB.

Steroid quantification of 8 frozen samples of SN and PFC from PD patients (BR 4-6) and non-demented controls (CTR; BR0-3) (total of n = 32) have been performed using gas chromatography mass spectrometry (GC/MS) in Dr M. Schumacher/Dr P. Liere lab (University of Paris South, INSERM, Paris, France).

PFC slices (300um thickness) were obtained from 10 PD and 8 CTR subjects. Slices were treated with different concentrations (0.1, 1, 10 and 100 nM) of AP, DHP and DHEAS. After 5 days in culture, RNA was extracted and QPCR experiments were performed to analyze gene expression difference in neuroprotective molecules.

Results and conclusion

Surprisingly the GC/MS analysis didn't show any significant differences in the levels of 26 steroids in the SN and PFC from PD and CTR patients. This might be related to the low number of patients that have been analyzed and the extreme variability in amounts of steroids between samples. The possible cause of this high variability needs future studies. In PFC slices, AP treatment increased the RNA expression of neuroprotective molecules such as BDNF (2,5 fold) and GDNF (2,3 fold), inflammatory molecules such as Alpha B crystallin, IL10 and TNF ($\geq 1,5$ fold) while reduced pro-apoptotic molecule p75 ($\geq 1,5$ fold) in CTR. DHP treatment reduces the RNA expression of p75 cJun and HSP90 while increases GLUL and TNF in CTR. However, in PD slices either AP or DHP treatment showed an increase of transforming growth factor beta (TGF β) an essential factor for the survival and development of midbrain dopaminergic neurons and alpha synuclein (SNCA; $> 1,3$ fold) a protein involved in presynaptic neurotransmission. DHEAS treatment showed a reduction in gene expression for p75 ($> 1,6$ fold) but also neuroprotective molecules such as GLS, APO E and MBP in CTR while in PD DHEAS reduced gene expression of neurotrophic factors such as NGF, GDNF, TGF ($> 1,50$). In summary AP and DHP may induce a chronic neuroprotective effect in human organotypic culture of control but not in PD subjects suggesting that the neurodegenerative process influences the positive outcome of these steroids treatment. DHEAS seems to have a negative influence on neurogenesis in PD patients slice culture.