4.3.4. Research Objectives, Activities and Results

Research objective 1 - Glutamatergic pharmaceuticals in clinical practice

Activity 1 - Neurobiology of the antidepressant effect of ketamine in the light of modulation of kynurenine cascade

Depression ranks among serious disorders, with a high lifelong prevalence (on average around 16%), as well as significant debilitating potential and an undesirable society-wide economic impact (Kessler et al., 2003, Kupfer et al., 2012). Considerable costs are generated not only by the treatment of depression, but also by the long period before the selected antidepressant begins to take effect, in connection with the length of the patient's inability to work. However, the weeks to months spent waiting for the therapeutic effect may not always lead to an improvement in the clinical condition. (Rush et al., 2006, Trivedi, 2013). A larger proportion of patients with depression are exposed to adverse neurobiological impacts of the disorder and remain at an increased risk of suicide.

One of the causes of stagnation appears to be the monoaminergic targeting of therapeutic attempts hitherto, which has predominated for more than half a century. However, the monoaminergic hypothesis is not sufficient in order to explain the delay in the onset of the antidepressant effect (rather than side effects) of classic antidepressants, or to clarify the adaptive neuroplastic changes in connection therewith (Heninger et al., 1996). As a result, in recent years we have observed a shift in the paradigm of research into the expected resistance to regular antidepressants in a direction toward alternative pathophysiological mechanisms of neuroplasticity. Glutamate, now considered the primary mediator of

affective disorders (Sanacora et al., 2012), as well as the proportion of immune mechanisms as potential modulators of neuroplasticity, are coming to the centre of interest. The majority of neuroplastic changes take place in glutamatergic neuronal circuits, and clinical evidence of glutamatergic dysregulation as a pathophysiological mechanism of depression has been increasing sharply in recent years – in particular a positive correlation has been demonstrated between the plasmatic level of glutamate and the severity of depressive symptomatology (Kendel et al., 2005, Mitani et al., 2006, Sanacora et al., 2012), as well as a decrease in the serum level of glutamate following successful depression therapy (Kucukibrahimoglu et al., 2009). Spectroscopic studies also confirm an increase in extracellular levels of glutamate in the occipital, frontal and cingulate cortex of depression patients (Sanacora et al., 2004, Hasler et al., 2007, Grimm et al., 2012). A further discussed neurobiological mechanism of depression is pro-inflammatory immune response. Cross-sectional and a number of longitudinal studies have demonstrated signs of activation of the inflammatory response in the peripheral blood and brain tissue of patients with depression. It is possible to present as an example the determined higher levels of TNF-α (Hestad et al., 2013), IL-6 and IL 1-β (Dahl et al., 2014) in the peripheral blood of depression patients, and their reduction through the influence of successful therapy. In their meta-analysis Valkanova et al. demonstrated a correlation between an increased CRP value and depressive symptomatology, and also confirmed a causal influence of the aforementioned IL-6 (Valkanova et al., 2013). Also worthy of note are the results of the research team Zalli et al. from Great Britain and the Netherlands, who in a five-year longitudinal observation of a group of 656 patients demonstrated the influence of the elevation of inflammatory cytokines on the persistence of depressive symptomatology (Zalli et al., 2016). An interesting correlation exists between inflammatory activation and suicidality. An increased expression of cytokines in the orbitofrontal cortex (Tonelli et al., 2008) or higher levels of IL-6 in the cerebrospinal fluid (Lindqvist et al., 2009) was determined in suicide cases. O'Donovan et al. demonstrated a higher inflammatory index (summary score from parameters such as CRP, IL-6, IL-10 and TNF- α) in patients with depression and suicidal thoughts in comparison with depression patients without such tendencies (O'Donovan et al., 2013). These experimental results are closely linked to observation of secondary depressive symptomatology and suicidal thoughts in patients treated with interferons (e.g. Dieperink et al., 2004, Wang et al., 2016).

Furthermore, it is being shown that these two hypotheses are mutually linked. Pro-inflammatory cytokines interact with glutamate on a number of levels, especially in the reduction of expression of glutamate transporters, the release of glutamate from astrocytes with its subsequent action on extrasynaptic NMDA receptors, down-regulation of BDNF and an increase in excitotoxicity, or also inhibition of the activity of glutamine synthetase with a resultant accumulation of glutamate concentration (Ida et al., 2008, Miller et al., 2009). However, even more interesting is the finding that pro-inflammatory cytokines are capable of triggering an alternative path of degradation of tryptophan, in which instead of the duction of serotonin, a kynurenine cascade is triggered through induction of the enzyme indoleamin 2.3-dioxygenase (Dantzer et al., 2008). The two end products of this cascade are quionlinic and kynurenic acids, both with numerous effects on neurotransmission (Schwarcz et al., 2012). Kynurenic acid is generated from kynurenine (through the effect of the enzyme kynurenine aminotransferase), it is produced by astrocytes and has the properties of an NMDA antagonist, and as a result its psychotomimetic effect and role in the pathogenesis of schizophrenia is being discussed (Linderholm et al., 2012). However, through the influence of kynurenine 3-hydroxylase, kynurenine is converted into quinolinic acid, which by contrast is an NMDA agonist, and is produced by microglial cells. Quinolinate is a significant excitotoxin of the CNS, selectively antagonising the NMDA receptors in the hippocampus, striatum and neocortex, where it leads to an alteration of neuronal functions or even apoptosis (Guillemin, 2012). Preclinical (quotation Kubešová,

Horáček) and clinical findings supporting the activation of kynurenine cascade in psychiatric disorders are multiplying, and in the case of depression and suicidal patients it appears that it is the shift in the direction of the formation of quinolinate that is decisive. For example, a higher concentration of quionlinate has been demonstrated in the area of the anterior cingulate cortex in the brains of depression patients following their suicide in comparison with a control group of brains of individuals without any psychological disorder (Steiner et al., 2011). The authors Erhardt et al. demonstrated a significantly increased level of quinolinic acid in the cerebrospinal fluid of patients with an anamnesis of a TS attempt in comparison with healthy controls, but in addition determined a correlation with the level of IL-6, depressive symptomatology according to the MADRS scale and also stated a significant drop in quinolinic acid in the cerebrospinal fluid 6 months after a suicide attempt, together with a reduction of depressive symptomatology (Erhardt et al., 2013). A further team of authors replicated the finding of a higher level of quinolinate in the cerebrospinal fluid of suicide cases in comparison with healthy controls, and furthermore pointed to a correlation between the amount of quinolinate and the severity of depressive symptoms (Bay-Richter et al., 2015). An increase in the level of quinolinate in serum and in the cerebrospinal fluid of depression patients was regularly accompanied by a reduction in concentrations of kynurenic acid, in which a negative correlation between the level of kynurenic acid and depressive symptomatology was clinically determined according to MADRS (Bay-Richter et al., 2015). This reciprocal relationship was experimentally confirmed – for example under the influence of quinolinic acid on the cellular cultures of astrocytes, their apoptosis occurs (Guillemin et al., 2005), with the attendant impossibility of formation of kynurenic acid by these cells. A reduced level of kynurenic acid evidently also finds its application in the pathogenesis of depression. A hypothesis exists concerning its participation in dopaminergic neurotransmission (Scwarcz et al., 2012) within the context of depression and impairment of the dopamine neuron response to reward (Tye et al., 2013, Bay-Richter et al., 2015), but the precise mechanisms are not yet known. [JH3]

One of the turning points in research into depression was the finding of the prompt antidepressant effect of molecules of ketamine, an NMDA antagonist approved by the FDA as an anaesthetic since the 1970s. This effect in humans was first described by the authors Berman et al. in the year 2000, who thus triggered a cascade of studies devoted to the rapid antidepressant effect of this molecule. Over the course of the following 17 years the significant antidepressant and anti-suicidal effect of ketamine was replicated several times (Reinstatler et al., 2015, Xu et al., 2016), and in fact it appears that its anti-suicidal effect need not be dependent on its antidepressant effect (Price et al., 2009). New observations concerning the molecular action of ketamine provide a further reason for the integration of glutamatergic and inflammatory theories of depression. The application of ketamine immediately generates a number of molecular processes leading to neuroplastic changes: presynaptic de-inhibition of glutamatergic neurons, activation of AMPA receptors (Zarate and Manji, 2008; Koike et al., 2011) and blockade of extrasynaptic NMDA receptors (Tizabi et al., 2012) and postsynaptic activation of signal pathways incorporating BDNF (Garcia et al., 2008) and mTOR (Li et al., 2010), leading to neuroplastic processes. Last but not least, however, there is talk of a correlation between neuroplasticity and the immunomodulatory effect of ketamine. Thus by rats the antidepressant effect of ketamine is linked with the inhibition of microglia and with down-regulation of pro-inflammatory cytokines in the hippocampus (Wang et al, 2015, Chen et al., 2017). A correlation was also found between the antidepressant effect of ketamine and a decrease in serum levels of IL-6, and in fact it was demonstrated that initial levels of IL-6 are predictive for the antidepressant response to ketamine (Yang et al., 2015). An interesting finding was presented for example by the study by the authors Walker et al., focusing on molecular mapping of the mechanism of the antidepressant effect of ketamine. They started out from the fact that lipopolysaccharide induces

depressive syndrome in mice by means of activating a kynurenine cascade, with the formation of the above-mentioned NMDA agonist quionlin at e. ln their experiment they administered a low dose of ketamine to mice shortly before the application of depressogenic lipopolysaccharide , and determined that after such pre-medication lipopolysaccharide no longer triggered depressive behaviour in animals, although induction of cytokines was evident in the brain tissue, and other induced signs of inflammation (in addition to depression) were clinically evident . Furthermore, upon application of the AMPAR antagonist NBQX, the effect of lipopolysaccharide on depressive behaviour was once again renewed, even despite the administered ketamine . The study therefore inclines toward the independent action of ketamine within the region of the NMDA receptor in competence with quinolonic acid, in this case even without its influence on the initiation of a kynurenine cascade.

On the basis of the above-mentioned observati ons, it is possible to infer that metabolites of the kynurenine cascades are the central generators of depressive and suicidal symptomatology, which interconnect the inflammatory and glutamate theories, and that ketamine may be the key to a more detailed understanding of the neurobiological mechanisms of depression, as well as the possibilities of its effective influencing.

ln the planned placebo controlled trial, we would like to focus on the correlation between the antidepressant effect of a one-off infusion of a subanaesthetic dose of ketamine with the level of metabolites of the kynurenine cascade and further examine the dependency of these processes on the sex and hormonal status of patients. To date there has been very little investigation into the relationship between the therapeutic effect of ketamine and sex, although the share of sex hormones in the pathophysiology of depression and the effect of antidepressants is indisput able. Affective disorders are generally more frequent in pre- or perimenopausal women, and studies also demonstrate the facilitation of hippocampal NMDA signalling through the influence of oestrogen (Woolley, McEwen, 1994, Smith et al., 2009) . ln animal experiments it has been determined that female mice are more sensitive to the acute effects of ketamine , although the length of its antidepressant effect was greater in males (Franceschelli et al., 2015). The role of sex hormones is supported by further findings from animal studies, in which the higher sensitivity of female rats to ketamine receded after ovariectomy, and again increased following hormone supplementation (Carrier, Kabbaj, 2013).

We intenci to include 30 patients in the study, with medium-severe to severe depression and without psychotic symptoms, who will be administered a one-off application of a subanaesthetic doses of ketamine. The patients will be randomised, and a placebo infusion will be cross-administered after an interval of 14 days (can this be an active placebo? For example BZD? Perhaps in this way we could level out the accessory "placebo" effect of the ketamine trip and thus better focus on neurobiology. ln total, each subject will therefore receive 2 infusions (ketamine and placebo) during the course of the experiment , with an interval of two weeks. Examination of the vital functions of the subject during the course of the experiment will take place in the form of checking pulse and blood pressure by a trained nurse and doctor, both before and then every 15 minutes throughout the course of the entire experiment . After the end of infusion, each subject will be observed for a further 3 hours, with a check of the circulatory parameters each 30 minute s. Psychopathology will be evaluated in the form of a clinical rating with the aid of the BPRS (Brief Psychiatrie Rating Scal e), MADRS (Montgomery-Ásberg Depression Rating Scale), BDI (Beck depression inventory) and CGI (Clinical Global lmpression) at a period of O, 1, 3 and 7 days from the commencement of application of the infusion. During each infusion, samples of venous blood will be taken

in order to determine the level of ketamine and norketamine, the level of sex hormones, the basic biochemical parameters, CRP and kynurenic acid in serum (IL-6 unstable... can you think of anything else?).

Samples will be taken at a period of 0, 1h, 24h and 7 days from the beginning of infusion (these times are only a preliminary proposal). In the case that a regression of psychopathology (50% and more according to the MADRS scale) occurs between the first (evaluated as one day before each of the infusions) and second infusion, the patient will be indicated as a responder to the first infusion and will not receive a second.