4.2. RESEARCH AIM 1- SYNTHESIS OF SUBSTANCES INFLUENCING GLUTAMATERGIC NEUROTRANSMISSION

4.2.1. Abstract

Searching for novel drugs potentially useful for therapy of central nervous systém (CNS) diseases belongs to most investigated topic in contemporary pharmacology and neuroscience. Our previous results and patents demonstrated that dual compounds modulating glutamatergic with cholinergic or GABA-ergic systém or kynurenic pathways inhibitors and neuroactive steroids can modulated glutamatergic neurotransmission in special manners. The proposed project is based on the study of biological properties of newly developed drugs and evaluating of their pharmacotherapeutical potential.

A number of recent studies have demonstrated the important role played by glutamate in the pathogenesis of several CNS diseases. Therefore, in a close collaboration of participating institutions we have started development and testing of the new specific classes of the glutamatergic drugs. A number of papers demonstrated ability of glutamatergic drugs mainly NMDA receptor (NMDAR) antagonists. Nonetheless, their therapeutic potential is rather limited due to high coincidence of negative side effects. Regarding the fact, that NMDAR are very abundant in the CNS, their general antagonism may impair signal transmission between nerve cells and thus to impair many CNS functions. On the other hand, a large divergence of NMDAR offers a possibility to search for drugs selectively binding only to a subset of NMDARs, which may have therapeutic potential and lower coincidence of negative side effects. This branch of research gives rise to the possibility of obtaining the drugs with neuroprotective properties and minimal side effects, i.e., with more favorable benefit/risk ratio.

The executive members of the team of experts are as follows:

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The guarantor of the research is RNDr. Karel Valeš, PhD.

The research programme will be implemented during the entire term of the Project (from 1. 1. 2018 to 31. 12.2022).

Principal investigator

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Partner entity

- Institute of Physiology ASCR

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4.2.2. Current state of knowledge

In the field of glutamatergic neurotransmission dysfunction in the CNS, the research was mainly focused on mapping its role in the pathophysiology of a wide range of neurodegenerative and neuropsychiatric diseases. The effect of glutamatergic neurotransmitters is already clinically used in the therapy of Alzheimer's disease (memantine), epilepsy (lamotrigine) and in the therapy and prophylaxis of bipolar affective disorder (lamotrigine). Preclinical and clinical monitoring has shown that substances that increase neurotransmission mediated by ionotropic NMDA (N-methyl-D-aspartate) receptors positively treat the symptoms of post-traumatic stress disorder. In connection with the perspective of clinical use in the treatment of posttraumatic stress disorder, anxiety disorders, depressive disorders and schizophrenia, substances modulating the function of metabotropic glutamatergic receptors are tested. In pathophysiology, schizophrenia plays a very important role in the dysfunction of NMDA-mediated cortical glutamatergic neurotransmission. Research seeks to clarify this role and the possibility of medically influencing pathophysiological mechanisms. The use of glutamatergic drugs would allow the therapeutic effect of schizophrenia to affect not only the positive symptoms but also the mechanism responsible for cognitive deficits and negative symptoms.

Glutamate is the main building block in protein synthesis. When used as a neurotransmitter in the CNS, it is converted from glutamine. Glutamine is converted into glutamate via the mitochondrial enzyme glutaminase in neurons. Glutamate is stored in synaptic vesicles for later release during neurotransmission. A large portion of the glutamate released into the synaptic cleft is trapped by glial cells that adjacent to the neuron. In the case of glutamate neurons, the neighboring cells of glie glutamine provide a precursor for conversion to the neurotransmitter glutamate. Glutamate is first converted to glutamine in the glial cell with the enzyme glutamine synthetase. Glutamine is then transported to the neuron for conversion to the neurotransmitter glutamate (or GABA). Because of its charge, glutamate cannot diffuse through the cell membrane. Nano endings and glial cells have a high affinity uptake system. The effect of glutamate is not discontinued by enzymatic decomposition of the substance as in other neurotransmitter systems. From the synaptic cleft glutamate is removed by two transport pumps, the so-called glutamate transporters, which bind to the guanine nucleotide, called G proteins. Subsequent changes of secondary messengers generate slower synaptic transmission compared to ionotropic receptors. The high availability of glutamate, as a neurotransmitter in combination with a widespread distribution of metabotropic receptors, is the core of this central modulation system of secondary messengers in the CNS.

The complexity of expression of glutamate receptors and the possibility of their modification under physiological and pathological circumstances are factors that make it difficult to assess their role in the pathogenesis of neurological and psychiatric illnesses. On the other hand there is a rising interest of scientific community on glutamatergic drugs. This trend can be demonstrated in following examples.

In the pathophysiology of schizophrenia, dysfunction of glutamatergic neurotransmission occurs. Hypothesis of glutamatergic hypofunction implies hypofunction of brain glutamatergic neurons in schizophrenia. The most important consequence of this hypothesis is the therapeutic effect of schizophrenic symptomatology on drugs that increase glutamatergic neurotransmission. A hypotension of glutamatergic hyperfunction has also been formulated, based on the ability to influence schizophrenic symptoms with glutamate leaching agents. The effect of certain substances affecting the glutamatergic

system is clinically tested in patients with schizophrenia. It is not only agents specifically agonistic or antagonistic to ionotropic or metabotropic glutamate receptors, but also substances affecting the transport of these receptor agonists and presynaptically reducing glutamate release. NMDA receptor agonists Glycine, D-serine. In recent years, the therapeutic use of agonists acting on the glycine modulating site of the NMDA receptor, glycine and D-serine, is of interest. Glycine as an inhibitory neurotransmitter influences motor and sensory functions primarily in the brain stem and spinal cord. As a co-promoter of the NMDA receptor, it also mediates excitatory neurotransmission. Glycine, which binds to the glycine binding site of the NMDA receptor, is predominantly derived from glial cells and is formed by sequential conversion from L-serine. D-serine, a selective full agonist at the glycine site of the NMDA receptor, is composed of L-serine with the enzyme D-serine frames. The pharmacokinetic properties of these agonists are different. D-serine penetrates better than the glycine blood-brain barrier. Peripherally administered D-serine has central effects. Peripheral administration of glycine leads to increased cerebrospinal fluid levels. Metabolism of D-serine and glycine in the CNS and their relationship to modulation of glutamatergic neurotransmission in CNS in schizophrenia is the subject of research. Interestingly, there was a finding of significantly reduced serum serum D-serine and serine fractions in schizophrenic patients compared to healthy controls.

D-serine in adjuvant treatment of schizophrenic patients treated with classical and atypical antipsychotics (except for combination with clozapine) has been shown to improve negative, positive and cognitive symptoms. Also, glycine treatment in adjuvant therapy significantly reduces the intensity of the negative symptom. The effect of glycine on positive, negative and cognitive symptoms has been demonstrated in adjuvant therapy in patients with schizophrenia treated with both classical and atypical antipsychotics. D-Cycloserine at the glycine modulating site of the NMDA receptor, it acts as a partial agonist D-cycloserin, which also passes through the blood-brain barrier. D-cycloserine increases the response of the NMDA receptor to endogenous glutamate without directly activating the receptor. In addition, as a high-dose partial agonist, it interferes with NMDA receptor activation, thereby reducing the risk of hyperglutamatergic neurotoxic and convulsive effects. In a double-blind, placebo-controlled study with a neuroleptic group treated in patients with chronic schizophrenia with a predominance of negative symptoms, D-cycloserine significantly reduced negative symptoms and significantly improved cognitive performance. The effect did not occur with too low or high doses of D-cycloserine. The therapeutic effect on the negative symptoms was later demonstrated in D-cycloserine in the adjuvant treatment of schizophrenic patients treated with both classical and atypical antipsychotics. The dose-effect profile is consistent with the pharmacodynamic properties of partial agonists ("volcanic addiction"). The effect of glycine in the CNS also affects its transport. Two so-called glycine transporters, which belong to a large group of sodium and chloride ion-dependent neurotransmitter carriers, have been identified. They were named GLYT-1 and GLYT-2. Both transporters have similar kinetic and pharmacological properties, but their distribution is different. GLYT-1 provides transport in glial cells, while GLYT-2 is the primary neuronal transporter. The glycine receptor distribution is close to the distribution of mRNA encoding GLYT-1. Both the swelling and the back-absorption of glycine into the glia are directly influenced by the GLYT-1 transporter. In this context, the interest in GLYT-1 activity and thus the extracellular glycine level in the CNS increased. The so-called GLYT-1 inhibitors stimulate hypofunctional NMDA glutamatergic receptors and modulate dopaminergic dopaminergic neurotransmission. GLYT-1 inhibitors include, for example, glycyldodecylamide (GDA) or ALX5407. The subject of the clinical trial is primarily sarcosin (N-methylglycine). Sarkosine, in addition to GLYT-1 inhibition, modulates the function of transporters involved in the A-system of transporters, which also modulate the transport of glycine and glutamine. Only at the

outset is research into the modulation of extracellular levels of D-serine in the CNS by inhibiting its transport to glial cells.

Another option for the therapeutic effect of dysfunction of the glutamatergic system in schizophrenia is the use of substances positively modulating the functions of ionotropic AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate) receptors, called ampacines. In a clinical study, the effect of ampicillin CX516 on the augmentation of atypical antipsychotic therapy was studied in patients with schizophrenia. In patients with schizophrenia treated with clozapine, significant improvements in cognitive function, particularly attention and memory, have been reported with CX516.

Glutamate leaching inhibitors may reduce the hyperglutamatergic consequences of NMDA receptor dysfunction. Flushing of glutamate may be inhibited by sodium and calcium channel blocking agents, potassium-lowering agents, toxins that prevent fusion of vesicles with presynaptic membrane and presynaptic glutamatergic metabotropic receptor agonists. One of the neuronal membrane stabilizing agents that inhibits the cortical leaching of glutamate is lamotrigine (3,5-diamino-6- [2,3-dichlorophenyl] - 1,2,4-triazine) which is antiepileptic III. Generation, whose efficacy has been demonstrated in focal epilepsy therapy with secondary generalization. The results of other studies demonstrate efficacy in the therapy and prophylaxis of certain affective disorders, particularly in the treatment of bipolar affective disorder. The main mechanism of action of lamotrigine is the inhibition of glutamate and aspartate release by stabilizing the presynaptic membrane through inhibition and blockade of presynaptic sodium channels dependent on the level of depolarization stress. Lamotrigine has a stabilizing effect on neuronal membranes by inhibition and blockade of voltage-dependent P and N-type calcium channels and effects on potassium channels.

Last but not least example of glutamatergic drugs are neurosteroids that rapidly alter neuronal excitability through interaction with ligand-gated ion channels. Neurosteroid compounds can modulate the function of membrane receptors for various neurotransmitters, namely $GABA_A$ receptors, NMDA receptors and sigma1-opioid receptors. These mechanisms are likely responsible for their psychopharmacological effects and may account for their anticonvulsant, anxiolytic, neuroprotective and sedation effects as well as for their positive influence upon learning and memory functions. Neurosteroid action on NMDARs is complex. Sulfated neurosteroids can either positively or negatively modulate NMDAR activity, depending on the steroid structure and receptor subunit composition (Sedlacek et al., 2008) (Korinek et al., 2011). *In vivo*, neurosteroids are synthesized from cholesterol in mitochondria. Cytosolic sulfotransferases catalyze the addition of a sulfate group onto the steroid, giving rise to pregnanolone sulfate (20-oxo-5β-pregnan-3α-yl sulfate; PA-S), a steroid with an negative allosteric effect (NAE) at NMDARs; and pregnenolone sulfate (20-oxopregn-5-en-3β-yl sulfate; PE-S), a steroid with a positive allosteric modulators effect (PAE) at NMDARs (Fig. 1). Recent studies suggest that the synthetic machinery for neurosteroids is mainly expressed in excitatory neurons in many brain regions, including hippocampal and neocortical pyramidal neurons[7].

Our recent work was focused on understanding how SIENs access the NMDAR and on identifying their site of action at the receptor (Vyklicky et al., 2015) (Borovska et al., 2012). In addition we have shown that neurosteroids with NAE at NMDAR preferentially act at tonically activated receptors (typical for pathological processes leading to acute neurodegeneration) while having a smaller or no effect at receptors phasically activated during synaptic transmission (Vyklicky et al., 2016) . The neuroprotective activity of NAEs was confirmed in behavioral experiments. Importantly, even at high doses, NAEs were devoid of psychomimetic symptoms characteristic for voltage-dependent NMDAR inhibitors such as MK801 (Mony et al., 2009) (Rambousek et al., 2011). We have synthetized about 300 steroids and a characteristic structural feature of SIENs was the pregnanolone (“bent”) structure (Fig. 1). In an attempt to prepare metabolically

stable and more potent pregnanolone analogues with a neuroprotective potential we have coincidentally identified steroids with a bent structure that, instead of having an inhibitory effect, potentiated NMDAR responses.

We have shown previously that 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 the mathematical analysis and kinetic modeling, we have proposed that the affinity of the NMDAR for PE-S depends on the receptor activation, being high for non-activated receptors but low for activated receptors, and further that the action of PES is dependent on the receptor subunit composition (Horak et al., 2006). Our experimental analysis of the effect of structural analogues of PA-S and PES (~300 newly synthetized compounds) as well as others (Weaver et al., 1997) indicates that steroids with the bent structure at the A/B ring interface (introduced by the βH at the carbon C5; 5β-pregnane) are inhibitory while steroids with the flat structure (introduced by the αH at the carbon C5, or a double bond) potentiate NMDAR responses. We have synthetized and tested for their activity of approximately 300 steroidal compounds of which some had NAE, some PAE and some mixed PAM/PAE (citace). Our recent data indicate that the binding inhibitors some potent Neuroactive steroids.

The most prominent targets of glutamatergic drugs are NMDA receptors (NMDARs). The activity of NMDARs is modulated by different classes of endogenous compounds including endogenous neurosteroids and their synthetic analogues (neuroactive steroids) or kynurenines.

NMDAr are glutamate-gated ion channels widespread in the CNS, which have long fascinated neuroscientists for their distinct biophysical properties and their critical roles in neuronal communication and plasticity. These receptors are also involved in numerous neurological and psychiatric disorders (Traynelis et al., 2010) and therefore constitute targets of potential therapeutic interest. *In vivo*, NMDARs exist in multiple subtypes which differ in their molecular, anatomical, functional and signalizing properties. Such complexity raises key questions about the **physiological relevance of this subtype plurality** and its impact on circuit and brain function. This diversity derives from a set of receptor subunits, encoded by seven genes classified into three subfamilies (GluN1, GluN2A-D, GluN3A-B) (Fig. 1).

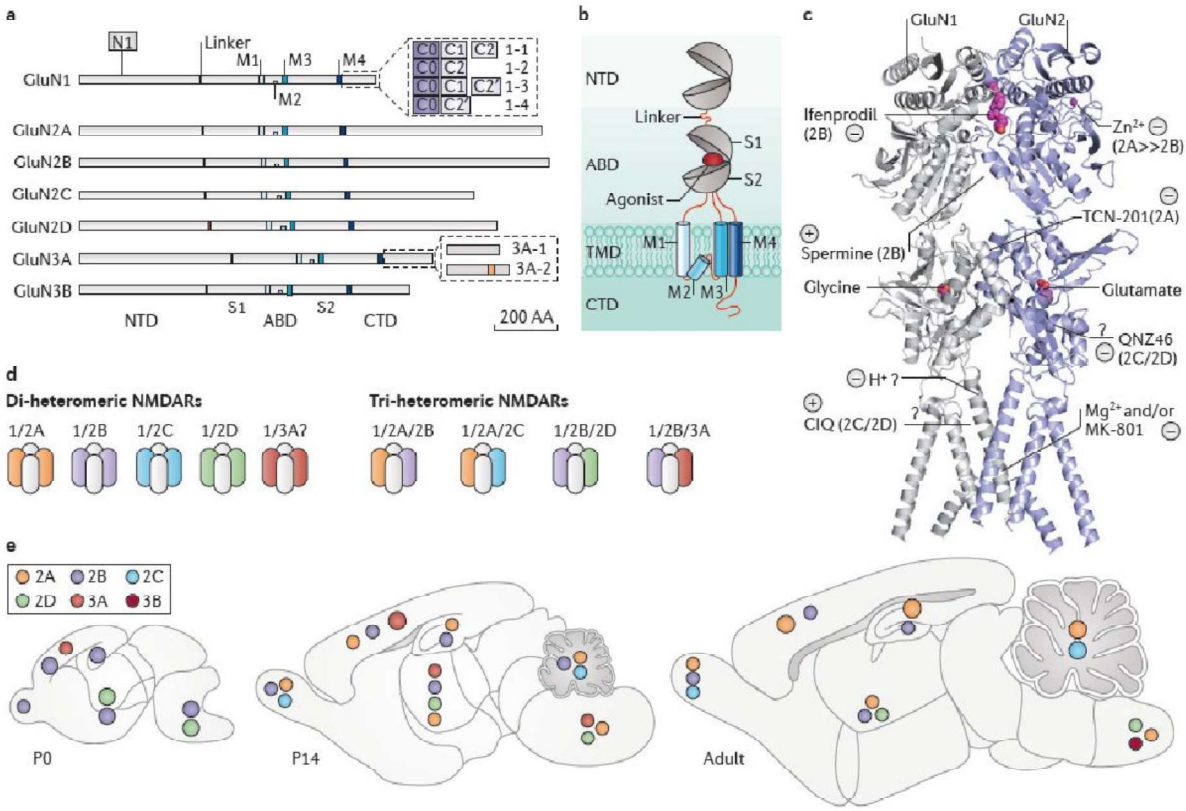

Figure 1: NMDAR subunit diversity, structure and expression. Excerpt (Paoletti et al., 2013).

NMDA receptor structure

The majority of native NMDARs are GluN1/GluN2 heteromers composed of two obligatory GluN1 subunits binding either glycine, or D-serine and two GluN2 subunits binding glutamate, for which extensive knowledge is available (Paoletti et al., 2013). Hence, each GluN2 subunit endows NMDARs with distinct biophysical and pharmacological properties. Moreover, while GluN1 is ubiquitously expressed at all stage of development, the four GluN2 subunits show strikingly difference expression profile (for review, see (Paoletti, 2011)). In the embryonic and early postnatal brain, GluN2B and GluN2D predominate. In contrast, GluN2A is the most abundant subunit in the adult brain, with GluN2B expression mostly restricted to forebrain regions, GluN2C to the cerebellum, whereas GluN2D drops to very low levels.

In line with the large number of subunits and their overlapping expression in several brain regions, many different NMDAR subtypes coexist in the CNS. Taken into account the various GluN1 splice variants, at least a dozen functionally distinct NMDAR subtypes have been described (Paoletti et al., 2013, Traynelis et al., 2010), but the exact number may be significantly larger. All NMDARs are thought to combine two copies of the obligatory GluN1 subunits and two copies of GluN2. The two non-GluN1 subunits can be identical or different, giving rise to so-called diheteromeric and triheteromeric receptors, respectively. Diheteromeric GluN1/GluN2B and GluN1/GluN2A receptors represent an important fraction of juvenile and adult NMDARs. Triheteromeric GluN1GluN2A/GluN2B receptors also populate many regions in the adult brain, particularly in the hippocampus and cortex, where they represent a sizable fraction (~50%) of the total receptor population with important implication for synaptic plasticity (Paoletti et al., 2013).

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4.2.3. Link to the current research of the Applicant and Project partners

A key role in the solution of research objective 1 will be played by the National Institute of Mental Health (NUDZ), which has been engaged over the long term in research into the neurobiology of mental disorders in preclinical experiments and the issue of the synthesis of substances influencing the activity of the CNS. In this research, emphasis is placed on a translational approach. The proposed research activities are targeted at a whole spectrum of psychological disorders, but above all concentrate on research into psychotic disorders, in particular schizophrenia, affective disorders, anxiety disorders and dependencies. In human experiments we focus on the study of neurobiological correlates in the brain with the aid of neuro-display methods, such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) or their simultaneous registration, and a subsequent analysis with the aid of advanced methods (connectivity, entropy etc.). At the same time the project studies parameters of neurocognitive functions, correlated with biochemical biomarkers, analysis of polymorphisms etc. We are an entirely unique centre in the Czech Republic and one of a few centres worldwide to conduct clinical trials on healthy volunteers and patients with the use of glutamatergic pharmaceuticals, primarily ketamine, both with the aim of understanding the neurobiology of the disorder and with a therapeutic objective. Preclinical experiments then enable us to study the neurobiology of mental disorders from a molecular-genetic level, via a microscopic and biochemical level up to complex behavioural paradigms, advanced electrophysiological experiments and neuro-display studies with MRI. The project shall follow on from the research grants listed below, which have been or are being realised in our centre, and expands upon them in a fundamental manner. The table below presents an overview of some of the most significant of these:

15-08577S	Affective response to visual art: a linkage of the fine arts and neuroscience perspective
P304/12/G069	Project for excellence in the sphere of the neurosciences
16-32696A	Utilisation of machine teaching in data analysis from magnetic resonance imaging for the purpose of improving timely diagnosis of schizophrenia and bipolar disorder
15-29900A	Efficacy and functional changes in the brain in treatment of depression using translational direct current simulation (tDCS) in comparison with venlafaxine
15-33250A	Prediction of therapeutic response in patients with depression with the aid of new methods of EEG analysis
15-28998A	Endophenotypes of psychotic disorders
15-29370A	Genetic and functional study of NMDA receptors focusing on the potential diagnosis and treatment of schizophrenia
7F14236	Physis in the sphere of improving human cognitive capabilities
NT13897	An animal and human serotonergic model of schizophrenia: validity assessed with the aid of qEEG and fMRI.
NS10375	The role of metabotropic mGlu2/3 and AMPA glutamate receptors in the neurobiology of schizophrenia
NR8792	The glutamatergic theory of schizophrenia in an animal and human model of disconnection, tested by a comparison with a population of schizophrenia patients
VG20122015080	Creation of standards for determining the degree of influence on drivers following the use of cannabis based drugs: an assessment of the level of cannabinoids in blood with respect to the time of use, psychomotor performance, vigilance and influence on brain activity
7E12038	Development of a Novel FGL Therapy and Translational Tests for Regenerative Treatment of Neurological Disorders
NT13403	The role of mTOR (mammalian target or rapamycin) signal pathway in the antidepressant effect of ketamine and antidepressants in patients with depression: a translational study
NS10379	Changes in QEEG cordance and EEG connectivity following the application of a subanaesthetic dose of ketamine in patients with depression – a randomised, double-blind, placebo-controlled cross-over trial
1M0517	Centre for neuropsychiatric studies of the CNS

The project realisation shall fundamentally improve the quality of the centre's research potential for realising experiments, and shall similarly expand the analytical facilities of the laboratories and enable a significant increase in the effectiveness of the existing methods, as well as a qualitative and qualitative intensification of co-operation with the project partners and subjects in the application sphere.

However, a further fundamental contribution shall be the realisation of adjustments and alterations, leading to the introduction of our own production line for the preparation of substances of a standard of good manufacturing practice (GMP) and the testing of their resulting quality, which shall be interconnected with a research programme of the Academy of Sciences entitled “Preclinical Testing of Potential

Pharmaceuticals", which is classified within the Strategy of the Academy of Sciences of the Czech Republic AV21: "Top quality research in the public interest". This advance is of absolutely key importance, since it enables an entirely unique development of the centre in a direction toward the preparation and subsequent testing of new candidate molecules for clinical assessments of phase I-II, which cannot otherwise be prepared by a standard method. Thanks to this, the centre shall become a **unique subject of international significance**, which shall be "on demand" and within a clinical context will conduct research directed toward the development of entirely new therapeutic procedures, experimental and psychopharmacological studies, as well as studies focusing on understanding the neurobiology of neuropsychiatric disorders in clinical experiments. With regard to the extensive analytical basis, it shall subsequently be possible to provide a service to third parties, which shall enable coverage of operation and obtaining of further resources for experimental research and development. Within the framework of adjustments, there shall be an adaptation of certain areas for the manufacturing process of category A of the GMP regime, the creation of conditions for stability studies and certified analytical procedures within the GMP regime.

4.2.4. Research Objectives, Activities and Results

Research objective 1 - Biological and pharmacological properties of new NMDAr inhibitors

Activity 1 – Neuropharmacology of potential medicaments with dual modulation of the cholinergic and glutamatergic system

The objective of this activity is the design, synthesis and biological testing of structurally different groups of compounds with a dual effect that can be utilised in research into neurodegenerative disorders. In particular it concerns the following individual activities:

- Using the method of organic synthesis, preparation and analysis of new dual compounds targeted at the CNS with appropriate physical-chemical properties. We envisage the preparation of three new ranges of these substances. The design of the substances shall be based on the simultaneously obtained data and a structural-activity evaluation thereof
- Screening *in vitro* evaluation of the prepared compounds, description of the mechanism of effect on NMDA receptors and acetylcholinesterase
- Subsequent *in vivo* evaluation of the most promising candidates. In addition to classic tests for potential medicaments (acute toxicity and bioavailability), the pharmacodynamics of the selected candidates will be studied.
- Evaluation of the relationships between biological activity and structure (SAR) and feedback for the design of new dually acting substances.

With regard to the numerous etiology of neurodegenerative disorders, in recent years research in the field of the development of pharmaceuticals has been focused on such structures that are capable of hitting a number of targets simultaneously. A combination of pharmaceuticals in clinical practice is not a new phenomenon. In particular MMT (multiple-medication therapy) is used, which is a combination of a number of medicaments used for a specific disorder with a different mechanism of effect, and "multiple-compound medication" (MCM), in which a number of different medicaments are incorporated into a single pharmaceutical form. From the perspective of the development of new pharmaceuticals, an interesting strategy is based on the assumption that a single compound would be capable of hitting a number of targets simultaneously. This concerns **multi-target-directed ligands** (MTDLs). One of the most commonly

In the case of CNS drugs, it is essential that the manufacturer of this substance in a pharmacopoeia quality also has a right to handle OPLs. No drug manufacturer in the Czech Republic has this right, in the EU only one company (THC Pharm), which specializes only in cannabinoids. This greatly limits the possibilities of advanced phases of clinical trials that cannot be dealt without GMP. NIMH, as one of the few workplaces that is licensed to handle OPLs, is the ideal place to build your own lab under good manufacturing practice. This will facilitate the shift of research from academic to practical use.

The aim of the project here is to build and equip the laboratory with the requirements of SVP for the preparation of medicaments with classical synthetic equipment, the size of batches in tens to hundreds of grams. The amount produced is sufficient for both preclinical and clinical studies. Part of the project is the development of the procedure for the preparation of the organic synthesis of selected drugs, the preparation of 3 batches (necessary for SÚKL approval) and the obtaining of certification for the production of these CNS drugs

Institutions involved

- National Institute of Mental Health - Jakub Rak, PhD

Milestones research aim 1

- Milestone 1: 09/2018: Completion of molecular modeling of the first series of drugs at Objectives 1-3; development and validation of analytical methods, preparation of first systems for transport of substances to CNS; preparation of GMP laboratory, equipment selection, consultation with SIDC
- Milestone 2: 09/2019: In vitro and in vivo characterization of candidates from the 1st series of substances at Objectives 1-3; Development of a biomarker panel and testing; In vitro and in vivo characterization of the candidate system (s); Development of the synthesis of 1 substance, initiation of the process for approval of production by SIDC
- Milestone 3: 12/2020: In vitro characterization of the 2nd series of substances in activities 1-3; Biophysical analysis of selected mutations of NMDAR subunits associated with CNS diseases (autism, epilepsy, schizophrenia, etc.); Completion of the production process of SÚKL 1. substances, production of 1. substances for clinical studies, development of synthesis of the 2nd substance, initiation of the process for approval of production by SIDC
- Milestone 4: 12/2021: In Vivo Characterization of the 2nd Series of Substances and Completion of the 3rd Series of New Substances in Activities 1-3; Analysis of the effect of neurosteroids on excitatory synaptic transmission, selection of suitable mutations for the preparation of GMO mice; Preparation of the GLP panel of biomarkers and development of advanced statistical methods, completion of the production process of SIDC 2. substance
- Milestone 5: 12/2022: In vitro and in vivo characterization of candidates from the 3rd series of substances; SAR analysis of the influence of neurosteroids on mutated receptors, phenotyping and pharmacological characterization of GMO mice; Use of a laboratory for the preparation and certification of other pharmaceuticals, protection of intellectual property; Preparation of patent applications and establishment of cooperation with the subjects of the application sphere

Expected outcomes of the research aim 1

- physically prepared substances including biological data.
- 16 impacted publications (Jimp) as a result of the project solution
- for substances with confirmed unique biological activity, the patent application (min 1)
- Presentation of results at expert conferences
- Building and certifying a GMP laboratory for production and quality control, we assume as outputs of this activity also patent applications and utility models that protect the developed processes of synthesis and formulation of pharmaceuticals. At least three utility models or patent applications are expected to be available throughout the project
- establish cooperation with the subjects from the application sphere on the further development of proof-of-concept and submission of international grant projects

Results and outcomes of activities	Target Value of the Project Implementation
Indicator 2 02 11 Publications created by supported entities (selected types of documents)	**16**
Indicator 2 02 16 Publications in co-authorship with researchers from abroad created by the supported entities (selected types of documents)	**5**
Other result that is not reflected in indicators: possible partial outcomes of implementing the activities are the results, which are defined according to the Definitions of types of research and experimental development results for the RIV database. (Please give the type of result of planned target value)	**3**
Other result, which is not reflect in indicators. (Please give the type of result of planned target value)	**1**