Oral presentations

SESSION: Neurodegeneration and neuroprotection

The influence of propofol on [^{3}H]-glucose uptake in brain and chosen organs in rats. Studies in an animal model of Parkinson's disease

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Parkinson's disease is a neurodegenerative disorder which results from the death of *pars compacta* dopaminergic neurons of substantia nigra. The observations of patients pointed out that they have serious disturbances of glucose metabolism in brain. Propofol is an anesthetic with an additional biological effects – it decreases intracranial pressure, flow of blood perfusing the brain and the cerebral metabolic rate. It's also suggested that propofol has a neuroprotective properties. The assessment of propofol's influence on dopaminergic transmission was performed by intracerebroventricular treatment with the neurotoxin 6-hydroxydopamine. Three days after birth rats were pretreated with desipramine hydrochloride (20 mg/kg) *ip* and injected bilaterally *icv* with 15 μg of 6-hydroxydopamine or with saline (control). All further experiments were carried out on male offspring after eight weeks. Destruction of the central dopaminergic system disturbed glucose homeostais of the central nervous system which was estimated by uptake of radioactive [6-3H]glucose. It was indicated that propofol decreased glucose uptake by the central nervous system in rats and lesions had no influence on that effect. Propofol didn't affect glucose uptake by rat's peripheral organs. The observed result had no connection with blood glucose level which was normal. It was revealed however that propofol increased insulin releasing in a dose-dependent way and this effect was stronger in the damaged rats. Propofol seems to be a good anesthetic for patients with Parkinson's disease because it decreases brain's energy demand and lesions didn't influence on this effect.

The importance of nitric oxide in the modulation of L-DOPA activity in the 6-OHDA model of Parkinson's disease

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The aim of this study was to investigate the effects of the nitric oxide donor, molsidomine and L-DOPA administration, on rotational behaviour, dopamine (DA) metabolism, the level of soluble guanylyl cyclase (sGC) protein and the number of neuronal nitric oxide synthase (nNOS) immunoreactive neurons in a rat model of Parkinson's disease.

Male Wistar rats were injected unilaterally with a single dose of 6-OHDA (8 μg/4 μl) into the left medial forebrain bundle. Two weeks after surgery, animals were tested for rotational behavior induced by apomorphine. Rats exhibiting more than 100 contralateral turns per hour were treated with molsidomine (2 and 4 mg/kg) and L-DOPA (12.5 and 25 mg/kg) alone or in combination, once daily for 14 days. Rotational behaviour was recorded after the first and penultimate dose of the examined drugs. The animals were killed 1h after the last injections. The level of DA and its metabolites was assayed in the striatal and nigral homogenates by an HPLC method. nNOS and sGC protein levels were determined using immunostaining and immunoblotting techniques, respectively. An unbiased stereological technique was used for cell counting throughout the entire substantia nigra (SN).

Chronic but not acute L-DOPA and molsidomine co-administration resulted in a significant lowering of the number of contralateral rotations in comparison with that observed after L-DOPA treatment alone. Moreover, such combined treatment increased striatal and nigral DA level more distinctly than L-DOPA alone. 6-OHDA lesion evoked significant decreases of sGC level in the ipsilateral striatum and SN. Stereological analysis of nNOS-immunoreactive neurons in the 6-OHDA-lesioned rats revealed a 25% decrease in their number in the ipsilateral SN.

The decrease in the level of sGC and nNOS suggests an abnormality in the nitrergic transmission caused by 6-OHDA lesion. The combined administration of L-DOPA and a nitric oxide donor seems to have beneficial effects by reducing motor complications and increasing DA level in the nigrostriatal system. The latter effect may be associated with facilitated bioavailability of L-DOPA in the brain.

Neuroprotective action of daidzein: a crucial role of G-protein coupled receptor 30

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Daidzein is a plant-derived isoflavone which binds to estrogen receptors with selective estrogen receptor modulator (SERM) properties and may represent an alternative to estrogen for the treatment or the prevention of neurodegenerative disorders. Although neuroprotective effects of estrogen have received much attention, clinical use of estrogen has certain limitations. SERM act as either estrogen receptor agonist or antagonist in a tissue-specific manner distinct from this of estradiol and devoid of estradiol side-effects. Apart from classical estrogen receptors which are ligand-dependent transcription factors, estrogen may exert its effects via newly identified extranuclear G-protein-coupled receptor 30 (GPR30). Thus, a ques-

tion arises whether and to what extent neuroprotection attributed to daidzein is due to interaction with GPR30-mediated signaling? This study aimed to evaluate the role of GPR30 in daidzein effects on glutamate-induced apoptosis in mouse embryonic neuronal cells in primary cultures. Daidzein (0.1–10 μM) inhibited glutamate-induced caspase-3 activity and lactate dehydrogenase (LDH) release in the hippocampal neurons in a time-dependent manner. The biochemical data were confirmed at the cellular level with Hoechst 33342 and calcein AM double staining. Specific GPR30 antagonist G-15 reversed daidzein effects, whereas specific GPR30 agonist G-1 potentiated these effects. Neuronal responsiveness to G-1 was verified electrophysiologically. In addition to glutamate-induced caspase-3 activity and LDH release, daidzein affected GPR30 protein expression in neuronal cells, as indicated with immunofluorescent labeling. Our data point to the crucial role of GPR30-mediated signaling pathway in neuroprotective action of daidzein, which may have implication for treatment or prevention of excitotoxicity at early stages of brain development.

Acknowledgments:
This study was supported by the Polish Ministry of Education and Science grant No. N N401 572138.

Effect of L-DOPA in a rat model of Parkinson's disease induced by the selective proteasome inhibitor lactacystin: behavioral and biochemical studies

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It has been shown that an impaired proteasomal function may play an important role in the pathogenesis of Parkinson's disease (PD). Therefore the aim of the present study was to determine whether L-DOPA would exert a beneficial effect in the lactacystin model of PD. Male Wistar rats were injected unilaterally with lactacystin (2.5 μg/2 μl) or 6-OHDA (8 μg/2 μl) into the substantia nigra pars compacta (SNc). Four weeks after the lesion, the animals were treated with L-DOPA (25 or 50 mg/kg) for two weeks. During L-DOPA treatment, lactacystin-treated rats were tested using multiple behavioral tests for akinesia, rotational behavior, forelimb asymmetry and catalepsy. Only rotational behavior was evaluated in 6-OHDA-lesioned rats. One hour after the last dose of L-DOPA the animals were killed and the levels of dopamine (DA) and its metabolites in the striatum and SN were assayed. Additionally, the binding of the selective radioligands of DA D1 (^{3}H-SCH23390) and D2 (^{3}H-raclopride) receptors was determined. Lactacystin induced akinesia and catalepsy, but also decreased the number of wall contacts of an impaired forelimb during rearing in a cylinder test. L-DOPA attenuated deficits in the akinesia and catalepsy tests, and increased the use of a compromised forelimb in the cylinder test. Furthermore, L-DOPA evoked moderate contralateral rotations in lactacystin-treated animals, but that effect was visible only after repeated L-DOPA administration. In contrast, L-DOPA evoked considerably stronger contralateral rotations in 6-OHDA-treated rats, already visible after the first dose. Both lactacystin and 6-OHDA markedly decreased (> 90%) the levels of DA and its metabolites in the striatum and SN, while L-DOPA partly diminished those deficits. Moreover, both those toxins significantly increased ^{3}H-raclopride binding on the ipsilateral side of the striatum; however, no changes were observed in ^{3}H-SCH23390 binding. Thus, having demonstrated

the efficacy of the standard antiparkinsonian drug L-DOPA in several behavioral tests, our study confirms the usefulness of lactacystin as a model of PD. However, marked differences in the magnitude of rotations after L-DOPA suggest diverse mechanisms of the degeneration of DA neurons, evoked by lactacystin and 6-OHDA.

Rotational behavior and monoamine metabolism in 6-OHDA-lesioned rats treated with L-DOPA and amitriptyline

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There is a controversy regarding the use of antidepressant drugs in the treatment of depression accompanying Parkinson's disease (PD) since application of some of them improves the psychiatric condition of the patients, while others worsen motor symptoms. Moreover, there are no experimental data illustrating how these drugs act when administered in combination with the widely used antiparkinsonian drug L-DOPA.

The aim of the present study was to examine in the rat model of advanced PD, the effects of chronic, combined administration of the classic antidepressant drug amitriptyline and L-DOPA on rotational behavior and monoamine metabolism in the striatum (STR) and prefrontal cortex (PFC). Experiments were performed on several groups of male Wistar rats injected with a single dose of 6-OHDA (16 μg/4 μl) unilaterally into the left medial forebrain bundle. Two weeks after the stereotaxic injection of 6-OHDA, animals were tested for rotational behavior induced by apomorphine (0.25 mg/kg, *sc*). Rats exhibiting more than 100 contralateral turns per hour received amitriptyline (10 mg/kg) and L-DOPA (12 mg/kg) alone or in combination, once daily for successive 21 days. Rotational behavior was recorded after the first and penultimate doses of the examined drugs. Rats were sacrificed 1 h after the last doses of the compounds and the STRs and PFCs were dissected. Noradrenaline, dopamine (DA), serotonin (5-HT) and their metabolites were determined in homogenates using an HPLC method.

Chronic combined treatment with amitriptyline and L-DOPA resulted in a significant increase in the number of contralateral rotations in comparison to that observed in the group receiving L-DOPA alone. Amitriptyline alone did not induce rotational behavior. Moreover, such joint treatment increased the levels of DA and its metabolites in the ipsilateral STR and PFC. L-DOPA administered alone or jointly with amitriptyline enhanced DA and 5-HT turnover measured as DOPAC/DA, HVA/DA and 5-HIAA/5-HT ratios, respectively, in both examined brain structures. The obtained results suggest that amitriptyline modulates activity of L-DOPA both in the STR and the PFC. The data are discussed in the context of PD therapy.

Effects of ethylene glycol ethers on oxidative stress markers

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Ethylene glycol ethers (EGEs) are a class of chemicals commonly used in the manufacture of a wide range of domestic and industrial products which may result in human exposure and toxicity. The chemical structure of a particular EGE affects its physicochemical properties and in consequence biological effects. 2-Butoxyethanol and 2-isopropoxyethanol showed the most potent hemolytic activity, whereas 2-methoxyethanol and 2-ethoxyethanol have strong gonadotoxic properties. Previously, we found, that 2-butoxyethanol, 2-isopropoxyethanol and 2-phenoxyethanol, but not 2-methoxyethanol, exerted cytotoxic effects on neuronal cells under *in vitro* conditions.

The aim of the present study was to find out whether EGEs, which had cytotoxic effect under *in vitr*o conditions, i.e. 2-butoxyethanol (BE) and 2-phenoxyethanol (PE), affect total antioxidant capacity and lipid peroxidation in the rat hippocampus, frontal cortex or cerebellum.

Male Wistar rats were administered (*sc*, 5 days a week) BE or PE in a dose of 2.5 mmol/kg, b.w./24 h for 30 days. Control rats were injected physiological saline. The rats were decapitated 24 h after the last injection and brain structures were dissected and stored at –80°C. Total antioxidant capacity was measured using the FRAP assay (Ferric Reducing Ability of Plasma).

We have found that BE or PE administration in a statistically significant manner decreased antioxidant capacity in the hippocampus as well as in the frontal cortex, but there were no significant changes in the cerebellum. Analysis of oxidative stress products by measuring malonyldialdehyde (MDA) which is one of the products of lipid peroxidation, revealed an increased concentration of this compound both, in BU- and PE-treated animal, in each studied tissues (frontal cortex, hippocampus and cerebellum).

The obtained results indicate that 2-phenoxyethanol and 2-butoxyethanol reduced antioxidant capacity in the frontal cortex and hippocampus, which can in turn initiate or increase damage of neurons. The results of our previous and present study suggest that neurotoxic effect of EGEs depends on their lipophilicity, which may determined their brain concentration.

Acknowledgments:
This study was supported by the research grant UMO-2011/01/B/NZ7/00136 from the National Science Centre, Kraków, Poland.

Neuroprotective potential of selective modulators of estrogen and aryl hydrocarbon receptors: the effects of raloxifene, daidzein, and 3,3'-diindolylmethane in response to hypoxia

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Due to its high incidence in neonatal pathology, hypoxia appears as a major risk factor which may result in complex cerebral dysfunctions like cerebral palsy or seizure disabilities. Although anti-hypoxic effects of estrogen have been documented, its clinical use has certain limitations. Selective estrogen receptor modulators (SERMs) and selective aryl hydrocarbon receptor modulators (SAhRs) may act as receptor agonists or antagonists in a tissue-specific manner, thus representing novel approach for the treatment or the pre-

vention of various types of neural degeneration and seizures. Raloxifene and daidzein bind to estrogen receptors with SERM properties, whereas 3,3'-diindolylmethane (DIM) exhibits properties of SAhR. However, some actions of SERMs or SAhRs may be independent of selective modulation of receptors acting as ligand-dependent transcription factors. In this study we evaluated the role of raloxifene, daidzein, and DIM in response to apoptotic effects of hypoxia in mouse embryonic neuronal cells in primary cultures. Hypoxic conditions (5% CO_2/95% nitrogen) induced caspase-3 activity and lactate dehydrogenase (LDH) release in the hippocampal neurons in a time-dependent manner. Raloxifene, daidzein, and DIM inhibited the effects of hypoxia by 4–17% in respect to caspase-3 and by 21–56% in respect to LDH. The neuroprotection was observed when the compounds were applied before or simultaneously with hypoxia. Neuroprotective action of daidzein involved newly identified extranuclear estrogen receptor GPR30. The compounds in concentrations of 0.1–10 μM did not cause any effect in neuronal cultures maintained in normoxia. This study demonstrated strong neuroprotective potential of SERMs and SAhR which may represent novel therapeutic tools for brain exposed to hypoxic insults.

Acknowledgments:
This study was supported by the Polish Ministry of Education and Science grant No. N N401 572138 and also by the Polish National Center of Science grant No. 2011/01/N/NZ3/04786.

The effect of 1-benzyl-1,2,3,4-tetrahydroisoquinoline on the metabolism of dopamine and molecular markers of apoptosis in rodent brain structures

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1-Benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ) is an endogenous neurotoxin present in the central nervous system. Acute 1BnTIQ administration induced symptoms of Parkinson's disease (PD) in both rodents and monkeys [Kotake et al., Neurosci Lett, 1996; Abe et al., Brain Res, 2001]. Oxidative stress is involved in apoptosis of dopamine cells in PD and may be the primary cause in the course of this disease. The aim of our study was to investigate whether 1BnTIQ may affect on apoptosis markers (caspase-3 activity, lactate dehydrogenase, LDH) in the hippocampal, neocortical and cerebellar neurons in rodent. Additionally, we measured the concentration of dopamine (DA) and its metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA) after *in vivo* administration of 1BnTIQ (50 mg/kg, *ip*) in rat dopaminergic brain structures by HPLC methodology.

Results: *in vitro* experiments have shown that the highest concentration of 1BnTIQ (500 μM) increased apoptotic cells (with Hoechst 33342 and calcein AM staining), caspase-3 activity and LDH release in mouse hippocampus, and also significantly ($p < 0.001$) intensified glutamate-induced excitotoxicity. On the other hand, 1BnTIQ in the lowest concentration (50 μM) expresses neuroprotective activity. The *ex vivo* study have shown that 1BnTIQ administration increased dopamine metabolism by a strong potentiation of MAO-dependent dopamine oxidation. Such profile of action leads to free radicals production.

Conclusion: the data from *in vitro* and *in vivo* studies have shown that 1BnTIQ demonstrates neurotoxic properties and may be involved in patomechanism of Parkinson's disease.

SESSION: **ModAll**

IF270750: Synthesis and pharmacology of novel $mGluR_4$ positive allosteric modulator

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Background: Glutamate, the most abundant excitatory neurotransmitter in the brain, regulates neuronal firing via ionotropic and eight subtypes of metabotropic glutamate receptors (mGluR). Among mGluRs, $mGlu_4$ primarily found presynaptically in several brain areas and function as an autoreceptors or heteroreceptors: $mGlu_4$ receptor activation inhibits GABA and glutamate neurotransmission. Intervention in glutamatergic neurotransmission through $mGlu_4$ receptor has been pursued intensively for the treatment of vast number of neurological and psychiatric disorders such as anxiety, schizophrenia, epilepsy Parkinson disease, and addiction.

Aim: Identification novel chemical scaffold possessing $mGlu_4$ positive allosteric modulation activity by interaction with transmembrane region of $mGlu_4$ receptor.

Methods: The screening study and activity of potential PAM was determined using forskolin-induced cAMP production, in a HEK-293 T-Rex cell line stably expressing $mGluR_4$, $mGluR_2$, $mGluR_7$ or $mGluR_8$. IF270750 was assayed using HTRF cAMP detection kit (Cisbio, Warszawa, Poland).

Results: We have identified chemical scaffold possessing $mGluR_4$ potential PAM activity. Compound IF270750 alone and in presence of L-Glu decreased the forskolin-induced cAMP production in HEK-293 T-Rex $mGluR_4$ cell line (EC_{50} = 0, 5 uM, Efficacy = 107%). Active compound: induce a leftward-shift of the glutamate concentration-response curve (1, 8 fold). Compound IP270750 do not interact with $mGluR_2$ and $mGluR_7$ receptors up to 30 μM.

Conclusions: Compound IF270750 is a novel positive allosteric modulator of $mGluR_4$.

Acknowledgments:
This study is supported by project UDA-POIG. 01.03.010-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD). http://modall.pl

Point mutation as a strategy for development a new tool in pharmacological research for $mGluR_7$ allosteric modulators

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Background: $mGluR_7$ is widely distributed throughout the CNS, showing its highest concentration in areas involved in emotional reactivity, learning and memory, such as amygdala, hippocampus and locus coeruleus. $mGluR_7$, a G protein-coupled receptor (GPCR), couples to adenylyl cyclase (AC) through Gi signaling and decreases cytosolic cyclic AMP upon receptor activation. Low affinity for L-Glu and very high EC_{50} for L-Glu suggest that this receptor is activated during intense presynaptic activity and act as a presynaptic receptor, inhibiting neurotransmitter release. This receptor can be implicated in schizophrenia, Alzheimer's disease, anxiety, depression, addiction, epilepsy and pain. $mGluR_7$ is a potential target receptor for psychopharmacological research and pharmacology.

Aim: In this study we compeered pharmacological activity $mGluR_7$ with or without mutation (74N-K) in orthostatic ligand binding domain, expressed in T-Rex 293 cell line. Are goal was to achieve mGluR7 mutant that have better pharmacological features, especially higher EC_{50} for L-Glu compared to its natural form.

Methods: GRM7 cDNA was genetically modificated by point mutation in the N-terminal extracellular domain were the glutamate binds. cDNA for both form of $mGluR_7$ was cloned into the T-Rex 293 cell system. Expression of the receptors was analyzed by means of RT-PCR and Western blot and flow cytometer. For the functional characterization of mGluR7 we determined quantity of cAMP in T-Rex293 cells by HTRF assay (Cisbio, Warszawa, Poland).

Results: We obtained $mGluR_7$ mutant with, a higher affinity for glutamate and EC_{50} for L-Glu 10 – times lower than naive $mGlu_7$ receptor.

Acknowledgments:
This study is supported by the Ministry of Science and Higher Education [Grant N405 055737] (to A.P.).

The quest for allosteric binding sites in mGluRs group III

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Background: Regulation of metabotropic glutamate receptors activity through allosteric modulation represents an yet to explore alternative for conventional orthosteric receptor stimulation since it addresses an unmet need for selective ligands. Expression of metabotropic glutamate receptors in heterologous mammalian cells cells is a method of choice to study ligand binding and receptor activation. We transiently overexpress mGluR4 in HEK293 cells, either native type of protein or with single amino acid substitution within transmembrane receptor portion. Cells are exposed to known allosteric ligands and/or glutamate, next the intracellular cAMP is directly quantified based on competitive immunoassay, where native cAMP produced by cells displaces labeled cAMP form its complex with specific antibody, followed by drop in FRET signal (Homogenous Time-Resolved Fluorescence).

Aim: Our goal is to identify amino acid residues within mGluR4, 7 and 8 transmembrane domains (TMs), crucial for selective positive and/or negative allosteric modulation of the receptor activity. The experimental data will support in silico receptor structure prediction, molecular modeling and will contribute to the design of new positive and negative allosteric agonists (PAMs and NAMs).

Results: The coding sequence for mGluR4 variants with single amino acid substitutions were generated by site-directed mutagenesis and verified by DNA sequencing. Native and mutated receptors were transiently expressed in HEK293 cells. Seven receptor variants with different amino acid substitutions within TMs were tested. The preliminary results show that substitution of tryptophan with lysine in position 798 (numeration according to NCBI reference sequence NP_000832.1) resulted in increased sensitivity VU01-55041 and PHCCC (mGluR4 PAMs).

Acknowledgments:
This study is supported by project UDA-POIG.01.03.010-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD). http://modall.pl

Cell synchronization as a tool to optimize protein expression level of mGlu receptors in applying the inducible expression system

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Background: The metabotropic glutamate receptors (mGluRs) are class C GPCRs that play important neuromodulatory roles throughtout the brain, as such they are involved in a number of psychiatric and neurological disorders like anxiety, depression, Parkinson's disease and schizophrenia. Expression of metabotropic glutamate receptors in heterologous mammalian cells is a method for their functional characterization, and tool to study the agonist; antagonist and allosteric effects witch are very attractive targets for therapeutic intervention.

Aim: Our goal is to optimize protein expression level in HEK293 cells containing T-Rex expression system by means of synchronization of cell cycle.

Methods: GRMs were cloned into genome of HEK293 T-Rex cells**.** Stable transfected cells were arrested at the beginning of S phase by using a double thymidine block. The extent of synchronization was controlled by flow cytometric analysis of DNA (propidium iodide-stained cells). Expression of the receptors was analyzed by RT-PCR, Western Blot or Flow Cytometry.

Results: Asynchronous stable transfected HEK293 T-Rex cells exhibit overexpression our receptors, but not always sufficient to use forskolin-induced cAMP accumulation method for their functional characterization. Therefore we tested protein expression level after cells synchronization, and compered with protein expression level asynchronously growing cells. The results so far showed no significant difference in protein expression level, and the modification of inducible expression system by cell synchronization can be insufficient.

Acknowledgments:
This study is supported by project UDA-POIG.01.03.010-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD). http://modall.pl

The searching of novel PAM of mGluR III by Virtual Screening of commercial chemical databases

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In recent years the Virtual Screening (VS) has become increasingly popular, as an alternative approach to HTS in the pharmaceutical and academic researches, especially in hit discovery and lead optimization [Shoichet, Nature, 2004; Vyas et al., Sci Pharm, 2008]. This *in silico* technology uses high-performance computing to analyze large database of chemical compounds in order to identify possible new ligands of a given target (top-ranked hits) for biological evaluation [Hou and Xu, Curr Pharm Design, 2004].

Here, we show the implementation of multistep virtual screening workflow to the searching of potentially new Positive Allosteric Modulators (PAM) of mGlu receptors family III. To their construction, a broad range of computational techniques (i.e., 2D fingerprints, 1D molecular descriptors, pharmacophore similarity search, docking and scoring, clustering), machine learning (support vector machines, SVM) and statistical (i.e. PCA and data fusion) methods were applied. The protocol was employed to screen the largest chemical databases (i.e., Enamine, ChemBridge, ChemDiv, UORSY and Vitas-M), containing approximately 5.5 million of tangible compounds.

To improve the global performance parameters of VS, such as efficiency, accuracy and hit rate level, a great effort is being made to develop and validate new tools and methods. Additionally, a web-based interface to the database linking results of different research teams will be shown. Detailed aspects and initial results of this study will be presented.

Acknowledgments:
This study is supported by project UDA-POIG.01.03.010-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD). http://www.modall.pl

Design and synthesis of novel metabotropic glutamate receptor allosteric modulators

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Metabotropic glutamate receptors (mGluRs) are members of the group C family of G-protein-coupled receptors (GPCR) and play important roles in a broad range of central nervous system functions, having therapeutic potential in a variety of neurological and psychiatric disorders, such as Alzheimer's disease, Parkinson's disease, anxiety, depression, and schizophrenia [Conn et al., Ann Rev Pharmacol Toxicol, 2010; Urwyler et al., Pharmacol Rev, 2011].

The mGluRs are categorized into three classes (Group I – III), based on their sequence homology, signal transduction profile and ligand binding specificity [Nieswender et al., Curr Top Med Chem, 2009]. Due to the lack of selectivity and physiochemical properties of mGluR orthosteric ligands, a significant effort has been made to identify compounds that can act as active sites' allosteric modulators. Among all mGluRs, group III further divided into subtypes: mGluR4, mGluR6, mGluR7 and mGluR8 still remains the least characterized and explored.

Herein we present the approach to design as well as further structure development of new synthesized molecules as potential innovative allosteric modulators of group III mGlu receptors. Ligands with known

mGluR activity described in recent literature were collected into a constantly supplemented database, and served as a model compounds for elaboration of new structures supported by pharmacophore models generation.

Acknowledgments:
This study is supported by project UDA-POIG.01.03.010-12-100/08-00 "Allosteric modulation – new strategy in pharmacotherapy. Identification of psychotropic properties of glutamatergic receptor ligands group III" co-financed by European Union from the European Fund of Regional Development (EFRD); http://www.modall.pl

SESSION: **Addiction**

Endocannabinoid level in the brain structures in rats following cocaine self-administration and the drug extinction

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Some recent preclinical reports indicate that the endocannabinoid system may play a role in cocaine addiction [Arnold, Pharmacol Biochem Behav, 2005], especially it is involved in the reinstatement of cocaine-seeking behavior [Adamczyk et al., J Physiol Pharmacol, 2009]. The endocannabinoid system consists of: 1) endocannabinoids, such as anandamide (AEA) or 2-arachidonoylglycerol (2-AG), which are synthesized "on demand" and act as retrograde messengers; 2) enzymes responsible for the endogenous ligands degradation; 3) G-coupled cannabinoid receptors [Oz Murat et al., J Neurochem, 2010].

The aim of this project was to evaluate the concentrations of AEA and 2-AG in several brain regions in rats underwent cocaine self-administration and extinction training.

Male Wistar rats (280–300 g) were trained to self-administered cocaine (0.5 mg/kg/infusion); some rats underwent also extinction training with cocaine withdrawal. A yoked-triad-procedure was employed to generate control groups [Frankowska M. et al., Pharmacol Rep, 2009]. After completion of behavioral experiments the following brain structures were isolated: the nucleus accumbens (NAC), dorsal striatum (STR), prefrontal cortex (PFC), frontal cortex (FC), hippocampus (HIP) and cerebellum (CER).

We found a statistically significant ($p < 0.05$) decrease of the AEA level in the FC and CER in animals self-administered cocaine while yoked cocaine controls showed decreases in the endocannabinoid level in the NAC and CER. During maintenance of cocaine self-administration a reduction in the 2-AG levels was seen in the STR and HIP and an increase in the CER while animals passively administered cocaine displayed increases in the 2-AG levels in the HIP and FC. Following a 10-day extinction, there were potent decreases in the AEA levels in almost all limbic and subcortical areas in rats previously self-administered cocaine; less potent decreases in these brain areas were seen in the "yoked" cocaine group. During extinction, the levels of 2-AG either increased (in the NAC and PFC of rats self-administered cocaine, or in the FC in the "yoked" cocaine group) or decreased (in the STR and CER of rats self-administered cocaine, or in the CER in the "yoked" cocaine group).

To summarize, the present findings on the endocannabinoid levels in rats addicted to cocaine support a role of this neurotransmitter system in motivational action of cocaine intake and cocaine withdrawal. Further studies focusing on other endocannabinoids/endovanilloids as well as CB1 receptor proteins allow to better explain the role of the endocannabinoid system in the mechanism of drug addiction.

Acknowledgment:
This study was supported by grant K/PBW/7000782 by the Ministry of Science and Higher Education (Warszawa, Poland) and by the statutory funds (K/ZDS/001295).

Effects of adenosine $(A)_{2A}$ receptor ligands on locomotor responds to nicotine-repeated treatment

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The abuse and addictive features of psychoactive substance nicotine is linked to the enhancement of dopaminergic (DA) neurotransmission and indirect activation of DA D2 receptors in the brain mesolimbic system. Literature data indicate antagonistic interactions at the molecular, neurochemical and behavioral levels between D_2 and adenosine $(A)_{2A}$ receptors that occurs in the striatum [Ferré Set et al., Neuroscience, 1994]. This interaction may have a significance to control behavioral effects induced by nicotine [Castañé et al., Neuropharmacol, 2006].

We examined the role of A_{2A} receptors in the locomotor effects to acute and repeated treatment with nicotine in wild-type rats and transgenic animals overexpressing A_{2A} receptors. Furthermore, the effects of the A_{2A} receptor agonist CGS 21680 on the development and expression of nicotine sensitization in wild-type rats were investigated. Sensitization to nicotine was developed by repeated, intermitted drug injections (days 1–5 and day 10). During development of nicotine sensitization wild-type animals received the following injections: vehicle or CGS 21680 (0.2-0.4 mg/*kg, ip*) in combination with vehicle or nicotine (0.4 mg/kg, *sc*), while transgenic animals were given vehicle or nicotine (0.4 mg/kg, *sc*). On 10 day, all rats received challenge dose of nicotine (0.4 mg/kg, *sc*). During expression of nicotine sensitization the animals were given vehicle or nicotine on days 1–5, and following a 5-day withdrawal they were pretreated by CGS 21680 (0.1–0.2 mg/kg) before nicotine (0.4 mg/kg) challenge dose. Measurements of locomotor activity began immediately after last injection and were recorded individually for each animal for 60 min.

The results indicate that the challenge dose of nicotine (0.4 mg/kg) after 5-day withdrawal to its repeated administration increased (ca. 2-fold) locomotor activity in wild-type as well as transgenic animals. In acute studies administration of CGS 21680 in doses of 0.2 and 0.4 mg/kg reduced nicotine locomotor activity. CGS 21680 in dose of 0.4 mg/kg, but not of 0.2 mg/kg given repeatedly with nicotine during development of sensitization produced a significant decrease in the locomotor activity to the nicotine challenge dose. Pretreatment with CGS 21680 (0.1–0.2 mg/kg) in a dose-dependent manner decreased the locomotor activity of the nicotine challenge in rats repeatedly treated with nicotine. The present results show that the pharmacological stimulation of adenosine A_{2A} receptors reduces both the development and expression of nicotine sensitization in wild-type animals, which may offer a therapeutic potential of A_{2A} receptor agonists in the treatment of nicotine dependence. These date mirror our previous result on cocaine addiction [Filip et al., Brain Res, 2006].

Acknowledgment:
Supported by statutory funds from the Institute of Pharmacology, Polish Academy of Sciences, (Kraków, Poland).

Modeling co-existence of depression and cocaine addiction in rats: the effects of imipramine and N-acetylcysteine on the cocaine-priming and discrete contextual cues induced relapses

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Several clinical reports indicate a high comorbidity between depression and drug (e.g. cocaine) abuse. Patients suffer from depression initiate drug-taking behavior to self-medicate the symptoms associated with the existing psychiatric disorder. Chronic use of abused drugs, however, may exacerbate the symptoms of mental disorders and subsequently increase drug-taking behavior. Cocaine dependence is characterized by relapses to drug-seeking and -taking behavior following periods of abstinence and drug detoxification. It was recognized that the biggest challenge of successful treatment of co-existing depression and cocaine addiction is preventing craving and relapse.

The present study was performed to characterize co-existence of depression and cocaine intoxication with special focus on drug seeking behavior. To this end, we employed bulbectomized (OBX) rats (an animal model of depression) and cocaine self-administration. Additionally, the effects of imipramine (IMI) and N-acetylocysteine (NAC) on cocaine seeking behavior in OBX and SHAM-control animals were studied.

Male Wistar rats that underwent intravenous catheter implantation and the olfactory bulbs removal were trained for 10 days to self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio 5 schedule of reinforcement. SHAM controls were treated similarly except that the olfactory bulbs were not removed. Then, forced extinction procedures were instituted and lasted for 10 days. Later on, reinstatement was induced by injection of cocaine (10 mg/kg, *ip*) or contextual cues (tone + light) previously paired with cocaine self-administration. The effects of pretreatment with IMI (10–30 mg/kg, *ip*) or NAC (25–100 mg/kg, *ip*) on cocaine-seeking behavior were studied in rats.

Compared with SHAM control rats, the active-lever pressing was higher in OBX animals during the first 3 days of extinction. Administration of cocaine (10 mg/kg) as well as re-exposition to the cue significantly enhanced reinstatement of seeking-behavior seen in both OBX and SHAM rats however, the number of active lever presses in OBX rats was much higher than in control animals. The cocaine-seeking behavior was affected by pretreatment with IMI (20–30 mg/kg) or NAC (50–100 mg/kg).

These findings indicate that OBX produces different behavioral responding during extinction training and reinstatement to cocaine- and cocaine-associated cues compared with SHAM control group. Both pharmacological intervention used effectively reduced drug seeking behavior.

Acknowledgments:
This research was supported by the Operating Program of Innovative Economy 2007-2013, grant No. POIG.01.01.02-12-004/09 (Poland) and the statutory funds of the Institute of Pharmacology, Polish Academy of Sciences, Kraków.

Effects of serotonin (5-HT)$_{1B}$ receptor ligands on amphetamine-induced reinstatement of amphetamine-seeking behavior in rats

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Recent research has demonstrated involvement of 5-HT, especially its 5-HT$_{1B}$ receptors, in the behavioral effects of the psychostimulants [Miszkiel et al., Pharmacol Rep, 2011]. Among other things, it has been established that 5-HT$_{1B}$ receptor ligands affect the maintenance of amphetamine [Miszkiel et al., Eur J Pharmacol, 2012] and cocaine [Przegaliński et al., Eur J Pharmacol, 2007] self-administration as well as the reinstatement of cocaine-seeking behavior in rats [Przegaliński et al., Pharmacol Rep, 2008]. Since there are no preclinical evidence concerning role of 5-HT$_{1B}$ receptors in amphetamine reinstatement, in our experiment, we employed an extinction/reinstatement model to examine the effect of 5-HT$_{1B}$ receptor ligands on the amphetamine-induced seeking behavior in rats.

Rats were trained to self-administer amphetamine (0.12–0.06 mg/kg/infusion) under a fixed ratio (FR) 1–5 schedule of reinforcement. Subsequently, the animals were subjected to extinction procedures. After stabilized extinction (ca. 10 days), reinstatement of drug seeking was provoked by the amphetamine priming dose (1.5 mg/kg, *ip*) while a selective 5-HT$_{1B}$ receptor antagonist (SB 216641), an agonist (CP 94253), or both, were given as a pretreatment.

The results of the present study showed that the 5-HT$_{1B}$ receptor antagonist SB 216641 (5 mg/kg, but not 2.5 mg/kg, *ip*) attenuated the amphetamine-seeking behavior manifesting itself as reduction in the number of active lever presses. An inhibitory effect on amphetamine-induced reinstatement was also observed after pretreatment with the 5-HT$_{1B}$ receptor agonist CP 94253 (1.25–5 mg/kg, *ip*); however, the effect induced by its dose of 2.5 mg/kg was not blocked by SB 216641 (2.5 mg/kg).

Our findings indicate that tonic activation of 5-HT$_{1B}$ receptors is involved in the amphetamine-induced reinstatement of amphetamine-seeking behavior. The attenuation of the amphetamine-seeking behavior caused by the 5-HT$_{1B}$ receptor agonist does not seem to be related to 5-HT$_{1B}$ receptor activation.

Acknowledgment:
Supported by the grant no. 0508/09/36 from the Ministry of Science and Higher Education (Warszawa, Poland).

Cocaine-induced adaptive changes in metabotropic glutamate receptors mGlu5 in brain structures of rats underwent cocaine self-administration, extinction training and drug-induced relapse

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Studies of cocaine addiction in the past few years have focused on the glutamatergic system, because of its interference with drug seeking behavior [Kalivas et al., Neuropharmacology, 2009] as well as acquisition and consolidation of extinction learning [Cleva et al., Behav Neurosci, 2011]. Out of 8 metabotropic glutamatergic receptors, the metabotropic glutamate receptors type 5 (mGluR5) have been shown to modulate the neurochemical and behavioral effects of abused drugs, and they are currently considered as a potential target for improved treatment of drug abuse and dependence. As shown, blockade of the mGluR5 reduces cocaine self-administration in rats, probably by weakening the function of the reward system [Kenny et al., Psychopharmacology, 2005] while mGluR5 knockout mice do not acquire cocaine self-administration response [D'Ascenzo et al., PNAS, 2007].

The aim of this study was to investigate changes in mGluR5 density in brain structures of rats underwent cocaine self-administration, extinction training and drug-induced relapse.

In this experiment we involved a yoked-triad procedure in which animals were either self-administered cocaine, given passive cocaine injection (yoked cocaine) or delivered passive saline injections (yoked saline). This procedure allows to distinguish pharmacological and motivational effects of the psychostimulant. Brain structures (the hippocampus, dorsal striatum and nucleus accumbens) were taken at the end of maintenance phase (after 12 cocaine self-administration sessions), at 10-day extinction training period and following cocaine-induced reinstatement of seeking behavior. Biochemical assays included the saturation analyses performed with [^{3}H]MPEP as a radioligand.

Our findings showed no differences in mGluR5 density in the maintenance phase in all analyzed brain structures. After extinction training procedure mGluR5 density was importantly increased in the dorsal striatum ($p < 0.05$) only in animals passively exposured to cocaine, while in the nucleus accumbens we observed a significant decrease ($p < 0.05$) in mGluR5 density in both active and passive cocaine groups. After the drug-induced relapse we found an important decrease ($p < 0.01$) of mGluR5 density in the hippocampus limited to rats previously self-administered cocaine.

Our results indicate the importance of mGluR5 in repeated exposure to cocaine and in controlling drug seeking behavior.

Acknowledgment:
This study was supported by grant K/PBW/769 by the Ministry of Science and Higher Education (Warszawa, Poland) and by the statutory funds (K/ZDS/001295).

Diversity of frequency-modulated 50-kHz vocalization response to intermittent amphetamine treatment in Sprague-Dawley rats

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Vulnerability to drug dependence is highly diversified. This diversity, which is related to intrinsic neurobiological factors, is pivotal both for the understanding of the mechanisms that underlie the emergence of addictions and for the development of therapies. Drug dependence in laboratory rodents can be assessed by sensitization of behavioral (locomotor or ultrasonic vocalization) responses to repeated exposure to psychoactive substances. We sought inter-individual differences in the propensity for sensitization of ultrasonic vocalization response to amphetamine. Rats were first tested for their anxiety, pain sensitivity (in a hot-plate test) and responses to novelty and a natural reward. Next, they were subject to the so-called two-injection protocol of sensitization followed by two weeks of daily amphetamine treatment, two-week withdrawal, and final amphetamine challenge. The development and progress of sensitization were monitored by recording post-drug frequency-modulated 50-kHz vocalization. Three patterns of the response to repeated exposure to amphetamine, which were identified using the two-injection protocol, persisted after completion of the extended treatment. The pattern showing true sensitization was found in but a minor subset (~23%) of the study rats. In summary, the propensity for sensitization of the ultrasonic vocalization response to amphetamine shows large inter-individual diversity, but also a prominent intra-individual stability that cannot be overcome with repeated drug treatment. High propensity for this sensitization is associated with lower sensitivity to pain and longer latency of the vocalization response to first drug exposure.

Effects of adenosine A_{2A} receptor ligands on cocaine self-administration and relapse in rats

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Cocaine is one of the most known addictive substances in humans and its dependence is characterized by high risk of relapse following periods of abstinence. It is well-established that the brain dopaminergic pathways play a key role in the behavioral effects of cocaine use and seeking [Koob et al., Neuropsychopharmacol, 2001]. Literature data point that adenosine $(A)_{2A}$ receptors are well positioned to influence the responses to repeated dopaminergic stimulation in psychostimulant addiction [Filip et al., Brain Res, 2006]. Till now separate reports indicate that A_{2A} receptor stimulation attenuates cocaine maintenance [Knapp et al., Psychopharmacol, 2001] and relapse [Wydra et al., Eur Neuropsychopharmacol, 2011].The present study investigated the effects of adenosine A_{2A} receptor ligands (including the antagonist KW 6002 and the agonist CGS 21680) in cocaine self-administration and cocaine-induced seeking behavior in rats.

Male Wistar rats were trained to self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio 1 sche-

dule of reinforcement. Then forced extinction procedures were instituted on following 10 days. Later on, reinstatement was evoked by the priming dose of cocaine (10 mg/kg, *ip*) or by the cue (tone and light)-induced. Given systemically (*ip*) KW 6002 at doses of 0.25–1 mg/kg did not alter cocaine self-administration. In direct contrast, CGS 21680 at doses of 0.2–0.4 mg/kg dose-dependently reduced the cocaine-evoked active lever presses and produced a downward shift in the cocaine (0.125–0.5 mg/kg/infusion) dose-responses curve. During reinstatement of cocaine-seeking behavior administered alone KW 6002 at doses of 0.25–0.5 mg/kg (but not 0.0625 and 0.125 mg/kg) dose-dependently induced reinstatement. Moreover, in a combination experiment, inactive doses of KW 6002 (0.0625 mg/kg) and cocaine (2.5 mg/kg) provoked increases in the number of active lever presses. On the other hand, pretreatment with CGS 21680 at doses of 0.1–0.2 mg/kg significantly attenuated the reinstatement of active lever presses evoked by cocaine (10 mg/kg, *ip*), while this agonist at dose of 0.1 mg/kg attenuated the cue-induced relapse.

Our findings indicate that tonic activation of A_{2A} receptors is not necessary for the rewarding properties of cocaine in rats while their pharmacological stimulation plays a inhibitory control over cocaine reward. During cocaine-seeking behavior, activation of A_{2A} receptors reduced relapse, evoked by either cocaine- or cocaine-associated cues. As such, A_{2A} receptor agonists may represent a novel target for the prevention of relapse to cocaine seeking.

Acknowledgment:
Supported by the grant no. N N401 019635 (Warszawa, Poland) and the statutory funds of the Institute of Pharmacology (Kraków, Poland). No potential conflict of interest.

SESSION: **Neuroinflammation, brain metabolism and varia**

Streptozotocin-induced diabetes increases sfingomielin hydrolysis in the brain of rats

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Insulin insufficiency is associated with disturbances of brain activity. We hypothesized that ceramides may constitute an important contribution to the diabetes-linked brain dysfunction. Our previous results showed that levels of ceramides increase in the brain of rats with streptozotocin (STZ)-induced diabetes. Myriocin, the inhibitor of serine palmitoyltransferase, enzyme of ceramide *de novo* synthesis pathway, reduced ceramide generation in hyperglycemic brains. The main goal of this work was to determine the changes of ceramide concentration in the hippocampus, prefrontal cortex and cerebellum of rats after administration of STZ. Additionally, we verified if the sphingomyelin hydrolysis is important for the brain ceramides generation in STZ-treated rats. We found, that ceramide content markedly increased in prefrontal cortex and cerebellum of rats after 2 two weeks of diabetes induced by STZ. Independently on duration of diabetes (two or four weeks) ceramide level significantly augmented in prefrontal cortex. STZ-induced diabetes elevated ceramide generated from sphingomyelin in all studied structures. The above results suggest, that hydrolysis of sphingomyelin to ceramides in the brain is an important mechanism by which functions of the cerebellum, prefrontal cortex and hippocampus of diabetic rat can be impaired.

Acknowledgments:
This work was supported by the Medical University of Bialystok grant No. 113-185-81LM.

AICAR increases the production of toxic molecules and affects the profile of cytokines release in rat primary microglial cultures

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AICAR (5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside, Acadesine, AICA riboside) is an activator of AMP-activated protein kinase (AMPK). The results of recent studies suggest that AICAR, in addition to its application for treating metabolic disorders, may also have therapeutic potential for treating neuroinflammatory diseases where reactive microglia play an etiological role. However, the molecular mechanisms of action by which AICAR exerts its anti-inflammatory effects still remain unclear or controversial. For this reason we attempt to evaluate the effects of AICAR on non-stimulated and LPS-activated rat primary microglial cell cultures. Our evidence supports the conclusion that AMPK activated by

AICAR is involved in regulation of ROS and cytokine production (IL-1 beta, TNF-alpha (6 h), IL-10 and TGF-beta) as well as arginase I and PGC-1alpha expression. Furthermore, we found that the effects of AICAR on IL-6 and TNF-alpha (12, 24 h) release and on the expression of iNOS and NF-kappaB p65 are not AMPK-dependent because the pre-treatment of LPS-activated microglia with compound C (a pharmacological inhibitor of AMPK) did not reverse the effect of AICAR. The results of the our study provide additional data about AMPK-dependent and -independent mechanisms whereby AICAR may modulate inflammatory response of microglia.

Acknowledgments:
The work was supported by grant No NN 401 072139 from the State Committee for Scientific Research.

The influence of tryptophan-deficient diet on liver cytochrome P450

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Tryptophan is one of essential amino acid of protein construction and a precursor of serotonin. Within the central nervous system (CNS), serotonin is a neurotransmitter located in serotonergic neurons. Whereas blood platelets and, to a much lesser extent, enterochromaffin cells of the intestine, represent a major storage site for serotonin outside the CNS. Our previous study showed the involvement of the serotonergic system in the regulation of liver cytochrome P450 (CYP) [Kot and Daniel, Pharmacol Res, 2011]. The aim of the present study was to demonstrate simultaneous responsiveness of liver CYP, as well as the peripheral and brain serotonergic systems to the tryptophan-free diet, during three days, one week and three weeks of ingestion.

Three days of tryptophan-free diet increased serotonin content in the hypothalamus (but not in the brain stem or plasma). After one week serotonin level was not changed in the brain whereas it incredibly increased in plasma. Three weeks of tryptophan restriction significantly reduced the concentration of serotonin in the brain and plasma. Changes in CYP2C6 and CYP2C11 maintained at a similar level throughout the time of experiment (an increase and a decrease, respectively), while those concerning other CYP isoforms (CYP1A, CYP2A, CYP2B and CYP3A) were varying, usually leading to a gradual increase within three weeks.

The observed alterations in liver CYPs suggest the involvement of both central and peripheral serotonin in the regulation of liver CYP expression *via* different mechanisms.

In conclusion, wrong balance of tryptophan in an incorrect diet may be responsible for very serious metabolic food-cytochrome P450 and food-drug metabolism interactions. This type of interaction may also refer to drugs acting *via* serotonergic system.

Acknowledgment:
This study was supported by a statutory funds obtained from the Institute of Pharmacology, Polish Academy of Sciences (Kraków, Poland) and by Grant MniSW nr NN405055737.

Kynurenic acid: an unknown mechanism of valproate action?

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Valproate (VPA) is a commonly used antiepileptic drug. The mechanism of action of VPA has not been fully elucidated. Kynurenic acid (KYNA) is a metabolite of tryptophan (TRP) degradation and it is synthesized from L-kynurenine (KYN). Similarly to GABA, KYNA is one of the most important endogenous inhibitory neuroactive agents. The present study was designed to determine whether kynurenine pathway plays a role in the action of VPA. We investigated changes in the concentrations of KYNA, KYN and the some amino acids in the brain and plasma following acute and chronic VPA administration to PTZ-kindled and non-kindled rats (*in vivo* and *in vitro*). We studied also l-tryptophan 2,3-dioxygenase (TDO) activity, a key enzyme of tryptophan (TRP) catabolism. We found a potent increase in KYNA, KYN and TRP levels accompanied by an increase in KYNA and an decrease inTRP in plasma after VPA administration. Furthermore, VPA significantly increased of glutamate, glycine and GABA and decreased of aspartate concentration in the brain. In plasma, administration of VPA evoked an increase of glutamate and a decrease of aspartate, glutamine and alanine. We also observed the U-shaped time response curve in TDO activity after administration of VPA. The current study strongly suggests that kynurenine pathway contributes to the mechanism of action of VPA. Moreover, because of the inhibitory role of KYNA on the neuronal activity it is likely that the antiepileptic and neuroprotective effects of VPA, at least in part, may be a consequence of changes in the brain KYNA concentration.

Influence of tianeptine on LPS-induced BDNF expression in brain structures of chronically-stressed female rats

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Stress plays an important role in etiology and progression of psychiatric and neurological disorders what in part, may be a result of an augmentation of neuroinflammatory process that is considered as a crucial mechanism leading to CNS injury [Anisman, J Psychiatry Neurosci, 2009]. It is known that women are more vulnerable than men to stress-related psychopathologies [Kessler, Annu Rev Psychol, 1997]. Recent studies suggest that brain-derived neurotrophic factor (BDNF) that promotes cell survival and positively modulates neuroplasticity is disturbed in stress and depressive states [Naert et al., Neurosci, 2011]. The aim of this study was to investigate the influence of tianeptine given chronically on the effect of lipopolysaccharide (LPS) on BDNF expression in brain structures of female rats subjected to chronic social instability stress. LPS is an endotoxin applied in experimental studies to induce inflammatory process in the CNS. Female Sprague-Dawley rats (initial weight 180 ± 20 g) were subjected to 4-week stress, including phases of isolation and crowding, in an unpredictable manner [Herzog et al., Neurosci, 2009]. Tianeptine (10 mg/kg/2 ml), an atypical antidepressant with antistress properties or saline was administered *ip.* once daily. On the last day of experiment, LPS (1 mg/kg/2 ml, *ip*) or saline were injected to rats being at the

same phase of estrus cycle. Six hours later the rats were decapitated and the amygdala, hippocampus, hypothalamus and pituitary were rapidly isolated. qRT-PCR experiments were performed using TaqMan Gene Expression Assays with ABI PRISM 7700 Sequence Detection System. In LPS-treated rats subjected to chronic stress, BDNF mRNA levels were decreased in the all studied brain structures. Tianeptine reversed this effect in the amygdala, hippocampus and hypothalamus but enhanced suppression of BDNF mRNA in the pituitary. We did not observe any significant effect of stress or LPS alone. We concluded that the modulation of BDNF expression induced by tianeptine may contribute to its therapeutic activity.

Biological effect of pentapeptide Any-GS a potential antagonist of some insect neuropeptides

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Several insect neuropeptides of different aminoacid sequence and of different length of their aminoacid chain exert antinociceptive effect in rats. This effect of some insect neuropeptides is mediated by central opioid receptors. Two recently discovered insect neuropeptides decapeptide MAS MT-I and pentacosapeptide poneratoxin also induced antinociceptive effect in rats. However this effect was not mediated by central opioid receptors. It was demonstrated that prior administration of naloxone hydrochloride an antagonist of opioid receptors did not block antinociceptive effect of neither MAS MT-1 nor of poneratoxin. Looking for the mechanism of antinociceptive effect of MAS MT-1 or of poneratoxin there were undertaken investigations of an effect of pentapeptide Any-GS on analgesia induced in rats by intracebroventricular (*icv*) administration of MAS MT-1 or of poneratoxin. Pentapeptide Any-GS (H-Asp-Ile-Leu-Arg-Gly-NH2), isolated from wild silkmoth Antheraea yamamai acts as a growth suppressor. Synthetic Any-GS suppresses activity of rat hepatoma cells. Biological study on Tenebrio mollitor proved that Any-GS shows a strong cardioinhibitory effect, opposite to that of cardiostimulatory effect of MAS MT-1. This fact prompted us to use Any-GS to block antinociceptive effect of MAS MT-1 as well as of poneratoxin. The study was performed on adult Wistar rats, which a week before experiments were implanted with polyethylene cannulas into the lateral brain ventricle (*icv*). On the day of experiment both synthetic investigated peptide were applied *icv* to unanaesthetized rats and antinociceptive effect was determined by a tail immersion test. It was found a significant antinociceptive effect of MAS MT-1 and of poneratoxin. Prior *icv* administration of Any-GS inhibited antinociceptive effect of both peptides: MAS MT-1 and of poneratoxin. This result suggests conclusion that Any-GS may be a potential antagonist of biological effects of other neuropetides. We hope that the further study may display the mechanism of antagonistic effect of this interesting peptide.

mGlu4-dependent reversal of the MK-801-induced cognitive impartment involves 5-HT1A receptors

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The present study was designed to evaluate the role of the serotonergic 5-HT1A receptors in the mechanism of action of the selective mGlu4 positive allosteric modulator (PAM) Lu AF21934, on the deficit in working memory induced by MK-801. The working memory was assessed by the delayed spatial alternation task (SAD), which is based on natural tendency of rats to explore two arms of the T-maze sequentially and in succession. Thus, the paradigm consists of a choice situation, in which the animals must remember their initial response to select an alternative response when re-exposed to an apparatus in the second run. Interposing a delay between the first and second run makes this a delayed alternation task. In the initial studies we found that MK-801 (0.1 mg/kg) impaired the SAD behaviour, i.e. significantly decreased the number of alternations, and this effect was effectively reversed by standard antipsychotic drugs, such as risperidone (0.5 mg/kg), olanzapine (2 mg/kg), aripiprazole (5 mg/kg) and cariprazine (0.065 mg/kg). Similarly, the MK-801-induced deficit in the SAD task was dose-dependently reversed by Lu AF21934 (0.5–10 mg/kg), which had no effect in animals not challenged with MK-801. The attenuation of the MK-801-induced deficit in SAD test by Lu AF21934 was antagonized by pre-treatment with 5-HT1A antagonist WAY100635 (0.1 mg/kg). Concomitantly, the combined administration of non-effective doses of AF21934 (0.5 mg/kg) and 5-HT1A agonist, 8-OH-DPAT (0.01 mg/kg), effectively reversed the MK-801 induced deficit. The results clearly indicate an involvement of serotonergic 5-HT1A receptors in the action of Lu AF21934 in the SAD paradigm.

SESSION: **Depression**

Involvement of cerebral α1-adrenergic receptors in responsiveness to chronic mild stress and imipramine treatment

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We investigated how the chronic mild stress (CMS) procedure that induces depression-like symptoms (anhedonia) in rodents affects the α1-ARs and whether imipramine interferes with the CMS-induced effects at the level of receptors' expression. Rats were subjected to either 3-weeks or 8-weeks CMS and were given imipramine (IMI) for five weeks. The total α1-AR density was analyzed by [^{3}H]prazosin autoradiography and mRNA expression of their α1A, α1B, and α1D subtypes was measured by RT-qPCR. In the hippocampus of CMS-reactive rats the mRNA expression of only α1B was increased after 3-weeks-CMS (*vs*. sham and stress-nonreactive groups). In rats subjected to 8-weeks CMS that did not respond to IMI treatment, the expression of α1A-, α1B- and α1D-AR mRNAs was increased (*vs*. sham-sal and stress-IMI groups). Similar direction of changes was seen in the α1-AR density.

Opposite change was found in the thalamus of CMS reactive rats where the α1B and other two receptor subtypes (α1A and α1D) mRNA were decreased (vs. sham and stress-nonreactive rats) and α1-AR total density was attenuated as well. In rats responding to IMI the mRNA expression of only α1A was increased whereas there was no change in the receptors of IMI-non-responders,

Our results suggest that α1-ARs play a role in mediating the behavioral effects of CMS. All subtypes of hippocampal α1-AR are importantly involved in the phenomenon of resistance of depressive rats to IMI treatment and the α1B-AR seems to be specifically engaged in responsiveness to CMS action.

Acknowledgments:
Supported by statutory funds of the Institute of Pharmacology and POIG.01.01.02-12-004/09-00 financed by European Regional Development Fund.

Hormonal, metabolic and behavioral consequences of prenatal stress

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A high prevalence of depression in patients with diabetes suggests the involvement of similar factor/s in pathogenesis of these disorders. Since both depression and hyperglycemia are often accompanied by hyper-

activity of the hypothalamic-pituitary-adrenal (HPA) axis, it has been suggested that an enhanced action of glucocorticoids may be a causative link between these disorders.

The aim of the present study was to find out whether there are any changes in glucose, glycogen, insulin and corticosterone concentrations in the hippocampus and frontal cortex in prenatally stressed rats, an animal model of depression.

Pregnant Sprague-Dawley rats were subjected to three stress sessions per day from 14th to 21st day of pregnancy. After weaning, male rats were housed for 3 months and next the forced swim test (Porsolt test) was performed. Two days after the Porsolt test, the animals were killed and the brain structures were quickly dissected. The concentrations of glucose, glycogen, insulin and corticosterone were assayed.

It has been found that prenatally stressed rats had statistically significantly higher levels of immobility behavior in the forced swimming test than control animals, i.e. they showed depression-like behavior. Our biochemical study showed that prenatally stressed rats had higher level of glycogen, glucose, insulin and corticosterone in the frontal cortex than control animals.

The obtained results confirmed the previous data indicating that prenatal stress evoked depression-like behavior. Increased glycogen, glucose, insulin and corticosterone contents in the frontal cortex suggests that glucocorticoids acting during perinatal period evoked long-term changes not only in corticosterone concentration, but also in metabolic sources, which may be in turn responsible for impairment of neuronal and glia cell function in depression. The increase in cortical insulin level suggests that prenatal stress may lead to brain glucose-resistance.

Acknowledgments:
This work was supported by the Operating Program of Innovative Economy 2007–2013, grant No. POIG.01.01.02-12-004/09.

Zinc deficiency as a possible model of depression – behavioral evaluation

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Background: There is much evidence supporting a link between serum zinc level and mood disorders [McLoughlin and Hodge, Acta Psychiatr Scand, 1990; Maes et al., J Affect Disord, 1994, Maes et al., Biol Psychiatry, 1997, Siwek et al., J Affect Disord, 2010]. It has been also reported that zinc deficiency induced by zinc – deficient diet elicits behavioral disturbances in animals**,** e.g., enhanced depressive – like behavior was found after dietary zinc deprivation in the forced swim test (FST) [Tamano et al., Neurochem Int, 2009; Whittle et al., Amino Acids, 2009]. Since there is a relationship between zinc homeostasis and depression in humans and since zinc deprivation induces depressive – like behavior in animals it seems interesting to evaluate if procedure of zinc deprivation might be useful on preclinical level as a model of depression. The aim of the present study was to examine behavioral changes induced by zinc deficiency as a possible model of depression.

Methods: Male Sprague Dowley rats were fed the control (30 mg Zn/kg) or zinc deficient (3 mg Zn/kg) diet for 4 weeks. Behavior of the control and zinc deficient rats was assessed in the forced swim test (FST) and the open field test. Locomotor activity was also measured.

Results: Zinc deficiency resulted in a significant increase in immobility time and decreased swimming and climbing in the FST. The spontaneous locomotor activity was significantly increased in zinc deficient rats compared to control animals. Four weeks of zinc deprivation seems to influence also exploratory activity of rats (the number of rearing was decreased in zinc deficient rats while the number of line crossing

were not different between the control and zinc deprived animals).

Conclusions: This data indicated that dietary-induced zinc – deficiency leads to the development of depressive – like behavior and that experimentally induced zinc – deficiency might be a useful model of depression.

The effect of prenatal stress on the expression of insulin receptor substrate family: an implication for depression

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A novel function of insulin and insulin receptor in the central nervous system (CNS) is related to their role in pathology of psychiatric disorders. In recent years, it has been documented that disturbances in circulating insuline levels can negatively affect the CNS and increase the risk of depression. Insulin exerts the biological effect by binding to specific receptors which is a tetrameric membrane protein. Insulin binding triggers autophosphorylation of the receptors followed by tyrosine phosphorylation of the insulin receptor substrate (IRS) proteins. The activated IRS binds to various effector molecules and activates intracellular kinase pathways. The aim of the present study was to find out if the prenatal stress, which is an animal model of depression, can influence the insulin signaling pathway in the hippocampus and frontal cortex of adult male rats. Pregnant Sprague-Dawley rats were subjected daily to three stress sessions from day 14 of pregnancy until delivery. At 3 months of age, the control and the prenatally stressed male rats were tested for behavioral changes. The animals were killed by rapid decapitation two days after the behavioral test and the brain areas were quickly dissected out. The expression of insulin, insulin receptor (IR) and insulin receptor substrate family IRS1 and IRS2 was determined by Western blot, ELISA and two-step real-time quantitative RT-PCR methods. Possible changes in the phosphorylation of IRS1 and IRS2 were also determined. The obtained results confirmed our preliminary study and showed that prenatally stressed animals displayed depression-like behavior, i.e. increased levels of immobility behavior in the forced swimming test (Porsolt test). It was found that the level of insulin in the frontal cortex of rats with depression-like behavior was significantly increased in comparison with control adult rats. Furthermore, the decrease in expression of the total and phosphorylated IR receptor subunits was detected in the hippocampus as well as in the frontal cortex ELISA studies showed the tissue-dependent changes in the level of IRS1 and IRS2. Additionally, the expression of phosphorylated IRS1 was enhanced in the frontal cortex and hippocampus of prenatally stressed animals. On the other hand, we did not find the changes in expression for IRS1 mRNA and IRS2 mRNA in both regions under study. In summary, the presented results have shown that behavioral changes in an animal model of depression are accompanied by insulin signaling dysfunction. Furthermore, it can be suggested that prenatal stress may be involved in the modulation of not only the insulin level, which we showed in our previous study, but also the expression and phosphorylation of insulin receptor and insulin receptor substrate family. The changes in this pathway may be one of probable causes by which early stress precipitates the onset of depression in adult life.

Acknowledgments:
This work was supported by the Operating Program of Innovative Economy 2007–2013, grant No. POIG.01.01.02-12-004/09.

1,2,3,4-Tetrahydroisoquinoline as the potential antidepressant compound in forced swimming test in rat

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1,2,3,4-Tetrahydroisoquinoline (TIQ) is an exo- and endogenous amine present naturally in mammalian brain and may be the natural regulator of monoaminergic systems with a visible neuroprotective potency [Antkiewicz-Michaluk et al., J Neurochem, 2006]. In our study we tested the potential antidepressant properties of TIQ in comparison with a classic antidepressant drug, imipramine by using forced swimming test in rats (FST). Further, we measured the levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT), and their metabolites, as well as the rate of monoamines metabolism in different rats brain structures by HPLC methodology with ED. The locomotor activity test was used to check motor function of rat after investigated drugs administration. All experiments were performed on male Wistar rats weighing 220–240 g.

Results: FST has shown that TIQ (10, 25, 50 mg/kg, *ip*) significantly reduced immobility time similarly to imipramine (30 mg/kg, *ip*). TIQ significantly elevated swimming activity ($p < 0.01$) while imipramine increased climbing time ($p < 0.01$). Additionally, TIQ (25 mg/kg, *ip*) and imipramine (15 mg/kg, *ip*) injected simultaneously decreased immobility time, increased the swimming and did not affect the climbing activity. The biochemical analysis showed that TIQ increased the levels of monoamines: DA, NA and 5-HT in rat brain structures. Moreover, the factor of DA re-uptake inhibition, calculated as the ratio [3-MT]/[DOPAC], was significantly elevated by TIQ administration. The rate of serotonin metabolism was strongly decreased ($p < 0.01$) while, the rate of noradrenaline metabolism was increased ($p < 0.05$) after injection of TIQ and imipramine. TIQ did not change the locomotor activity in rats.

Conclusions: The obtained data indicate that TIQ produced antidepressant-like effect in FST with potency comparable to imipramine. Thus, in that light and taking into account its neuroprotective potential of action in the brain TIQ may be useful in clinical practice for therapy of depression.

Chronic MTEP treatment, like antidepressants, induces adaptive changes in mGlu5 receptors in the rat brain

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The data of recent years suggests the involvement of glutamatergic transmission in the pathophysiology of depression and antidepressant activity. The experiments of chronic mild stress have shown increased expression of metabotropic glutamate receptors (mGluR5) in areas CA1 and decreased in the CA3 region in the hippocampus [Wierońska et al., Pol J Pharmacol, 2001]. The elevation of mGluR5 protein levels in the hippocampal CA1 region in response to stress was also described [Riedel et al., Neuropharmacology, 2000]. Cowen et al. [J Pharmacol Exp Ther, 2005] reported a significant reduction in mGluR5 receptor gene expression within the olfactory tubercles. Literature data have indicated that

mGluR5 antagonist are effective in some tests and animal models that are predictive of antidepressant-like activity [Pałucha et al., Pharmacol Biochem Behav, 2005].

The present studies were designed to examine the effects of antidepressants (escitalopram 10 mg/kg, reboxetine 10 mg/kg, milnacipran 10 mg/kg and imipramine 30 mg/kg) and the mGlu5 receptor antagonist MTEP (1 mg/kg) on the density of mGluR5 in rat hippocampus and cerebral cortex.

Receptor adaptive changes, after two-weeks administration (*ip*) of antidepressants and MTEP, were determined in rats hippocampus and cerebral cortex (neocortex) using saturation analysis with [3H]MPEP as a radioligand.

The results of our study showed a significant increase in the density of mGluR5 receptors in the hippocampus after two weeks treatment with imipramine ($p < 0.01$), escitalopram ($p < 0.01$) and MTEP ($p < 0.01$). In the cerebral cortex we observed statistically significant up-regulation of mGluR5 after chronic treatment with imipramine ($p < 0.01$), escitalopram ($p < 0.01$), reboxetine ($p < 0.01$), milnacipran ($p < 0.05$) and MTEP ($p < 0.01$).

The present data indicate that chronic treatment with antidepressants up-regulate brain mGluR5. These data indicate some similarities in mechanism of antidepressant action between MTEP and antidepressants.

Acknowledgment:
We thank Lundbeck Company for supplying with a free sample of escitalopram.

Effects of acute and repeated administration of imipramine and N-acetylcysteine on the level of endocannabinoids in different rat brain structures

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Depression as one of the major lifestyle diseases of the twenty-first century is a serious therapeutic problem in modern pharmacotherapy. Different mechanisms of action of antidepressant drugs suggest that these drug interaction with the direct target molecule is not responsible for the therapeutic efficacy but rather neuroadaptive mechanisms have a significance. In recent years there has been highlighted the potential participation of the endocannabinoid system in the pathogenesis of depression. The endocannabinoid system seems to be a part of forging bind of previously accepted theories of depression.

The aim of this study was to investigate the effect of imipramine (IMI), classical antidepressant and of N-acetylcysteine (NAC), showing in preclinical studies antidepressant activity, on the level of endocannabinoids anandamide (AEA) and 2-arachidonylglicerole (2-AG) in different rat brain structures.

Male Wistar rats received drugs intraperitonerally chronically (daily for 14 days) and on day 14th, followed by 13 days of saline injections, while the control group received only solvent (saline) for 14 days. Twenty four hours after the last administration of drugs the animals were decapitated and the tissue levels of AEA and 2-AG were determined using the liquid chromatography mass spectrometry (Applied Biosystem: Agilent 1100 and API 2000, column: Thermo Scientific). Data were analyzed by using one-way ANOVA followed by the Dunnett's test.

Administered acutely IMI (15 mg/kg) resulted in increased levels of AEA in the hippocampus and prefrontal cortex and of 2-AG in the frontal cortex and nucleus accumbens. At the same time, IMI decreased AEA and 2-AG in the frontal cortex and cerebellum, respectively. After the chronic IMI (15 mg/kg) administrations increases in AEA levels in the hippo-

campus and striatum and in 2-AG in the frontal cortex and the striatum as well as a decrease of 2-AG in the cerebellum were reported. Single administration of NAC (100 mg/kg) increased the AEA levels in the striatum and nucleus accumbens and 2-AG levels in the striatum; in the prefrontal cortex, nucleus accumbens and cerebellum decreases in 2-AG levels were seen. Chronic administrations of NAC (100 mg/kg) resulted in increases of AEA levels in the frontal cortex, prefrontal cortex and hippocampus and of 2-AG in the frontal cortex and striatum, while 2-AG levels in the prefrontal cortex and cerebellum were reduced.

Our preliminary data may suggest the significance of the endocannabinoid system in the mechanism of antidepressant drug action, but this issue requires further investigation.

Effects of repeated administration of imipramine or N-acetyl-L-cysteine on selected parameters of oxidative stress in different brain structures in animal model of depression

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Depression is a life-threatening disease, and highly hindering the functioning of a society; the problem of this disease refers particularly to developed countries. The cause of depression is not clear and the effectiveness of antidepressants is not yet sufficient. Recently the oxidative stress has been implicated in the pathophysiology of depression. Among antioxidants, N-acetyl-L-cysteine (NAC) (common mucolytic agent) was shown to scavenge free radicals and supplements the deficiencies of glutathione. The preclinical behavioral studies [Ferreira et al., Behav Pharmacol, 2008; Miszkiel et al., Pharmacol Rep, 2011] showed antidepressant-like effects of NAC in rodents, while a separate clinical trial supported usefulness of NAC in depressive patients [Berk et al., Biol Psychiatry, 2008].

The aim of this paper was to examine the influence of NAC on selected parameters of oxidative stress in the bulbectomized rats (BULB), which served as an animal model of depression.

Male Wistar rats were separated into groups underwent bulbectomy (removal of the olfactory bulbs) or exposed to the SHAM surgery (olfactory bulbs were left undestroyed). Additionally imipramine (IMI), a classical antidepressant (10 mg/kg; *ip*) was used as reference drug. After 14-day recovery the animals were given chronic (10x) injections of NAC (50–100 mg/kg, *ip*) or IMI (15 mg/kg, *ip*) or their solvent (saline; *ip*). Following completion of behavioral tests (locomotory activity, forced swimming test) the animals were decapitated and biochemical tests (the total antioxidant capacity (FRAP) and the superoxide dismutase activity (SOD)) were performed in homogenates of animals brains. Data were analyzed by using one-way ANOVA test followed by Dunnett test.

The olfactory bulbectomy caused a decrease of FRAP in the nucleus accumbens and cerebellum, while SOD activity falled in the hippocampus, frontal cortex, dorsal striatum and cerebellum. After chronic administration of IMI in SHAM-operated rats FRAP increased in the cortical areas only, whereas in BULB rats increases in FRAP in the hippocampus and in the SOD activity in the hippocampus, frontal cortex, dorsal striatum and cerebellum were seen. After chronic administration of NAC in BULB rats we found an increase in FRAP (the frontal cortex and cerebellum) and SOD activity (the hippocampus, frontal cortex and dorsal striatum).

Summarizing, in bulbectomized rats FRAP and SOD decreased in some brain structures and administration of IMI and NAC reversed these effects. Based on the well-known antioxidant properties of NAC, the results confirm the hypothesis that the oxidative stress is a key component of depression.

Influence of acute peripheral administration of lipopolysaccharide on the level of IGFBP4 in an animal model of depression

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Some data suggest that weaker activity of Insulin like growth factor 1(IGF-1) may play a role in the pathogenesis of depression. Among many factors, proinflammatory cytokines and a family of six binding proteins (BP1-6) are the critical ones, regulating IGF axis. It has been found that the presence of BP in the circulation and in extravascular fluids prolongs IGF's half-life, and modulates their interactions with receptors. One of the less known is IGFBP4, e.g. IGF-1 proteolysis is stimulated by it's binding with IGFBP4.

The aim of this study was to investigate if acute lipopolysaccharide (LPS) administration may affect the level of IGFBP4 in hippocampus and frontal cortex in rats that underwent the procedure of prenatal stress which is an animal model of depression.

Pregnant Sprague-Dawley rats were subjected daily to three stress sessions from day 14 of pregnancy until delivery. After weaning, male offspring was divided into 4 experimental groups: control, control + LPS, prenatally stressed, prenatally stressed + LPS. LPS was injected once *ip* and 4 hours later animals were killed by rapid decapitation and the brain tissue were dissected out and stored at –80^{o}C. Levels of IGFBP4 were measured in hippocampus and frontal cortex by ELISA and western blot assays.

We have found that in frontal cortex LPS injections increased IGFBP4 expression in control animals. Moreover this effect was potentiated in prenatally stressed rats. On the other hand in hippocampus no significant alterations were detected.

Obtained results suggest that proinflammatory cytokines may affect IGF levels indirectly by altering the levels of IGFBP4. Thus changes in IGF system may be of importance in pathogenesis of depression.

Acknowledgments:
This work was supported by the Operating Program of Innovative Economy 2007–2013, grant No. POIG.01.01.02-12-004/09.

Corticosterone-induced changes in the expression of alpha-2 GABA-A receptor subunits and glucocorticoid receptors in the brain of rats selected for low and high anxiety in conditioned fear

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The aim of the experiment was to assess the effect of an acutely administered corticosterone on the expression alpha-2 subunits of GABA-A receptors, and the glucocorticoid receptor in the brain structures of high (HR) and low (LR) anxiety rats (divided according to their conditioned fear-induced freezing response), subjected to the second conditioned fear session (one week after fear conditioning). We found that corticosterone (20 mg/kg, *sc*) given to rats prior to conditioned fear session significantly enhanced a decrease

in fear expression in HR group, an effect occurring between the first test session and second test session of conditioned fear. The behavioural effect of fear was accompanied by increased expression of alpha-2 subunits in the basolateral amygdala (BLA) and dentate gyrus of the hippocampus (DG) of the HR group, as well as and lower expression of glucocorticoid receptors in the M2 area of the prefrontal cortex and in the DG (immunocytochemistry). In HR rats, corticosterone potentiated the effect of fear on alpha-2 subunits expression in the BLA, DG and the M2 area and significantly increased the expression of glucocorticoid receptors in the DG and M2 area to the level similar to that in LR animals. These results suggest that animals that are more responsive to fear stimuli (as HR group) differ in the intracellular mechanisms that control GABA-A receptor alpha-2 subunit fear-induced expression in the prefrontal cortical area and limbic structures, and implicate glucocorticoid-related mechanisms in this phenomenon. These findings may help to explain the neurobiological basis of a propensity to anxiety disorders appearing in some people, and provide some insight into the mechanisms of effects of cortisol in the extinction-based therapy of anxiety disorders.