Oral presentations

SESSION: Pharmacology of anxiety

Are mu-opioid receptors (MORs) involved in the anxiety-like effect of ethanol withdrawal in rats?

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Withdrawal from alcohol leads to a negative emotional state including an increase of anxiety, hyperirritability or depressed mood. The withdrawal-induced anxiety has been reported to be a risk factor for relapse [Lucht et al., Eur Addict Res, 2002]. Strong evidence indicates that enkephalins, endogenous opioid peptides with delta-opioid receptors (DORs) affinity, reveal anxiolytic-like effects [Perine et al., Br J Pharmacol, 2006] and reduce withdrawal-induced anxiety-like behavior in the ethanol dependent rats [van Rijn et al., J Pharmacol Exp Ther, 2010]. A cyclic analog of enkephalin, cyclo[N^{ε},N^{β}-carbonyl-D-Lys^{2}, Dap^{5}] enkephalinamide (cUENK6) showed predominant affinity to MORs and, to a lesser extent to DORs *in vitro* [Pawlak et al., J Pept Sci, 2001]. The aim of the present study was to determine whether cUENK6 could affect an anxiety-like behavior measured during withdrawal from chronic ethanol administration in male Wistar rats. Ethanol dependence was induced according to the previously established method [Kotlinska and Bochenski, Eur J Pharmacol, 2008]. Our study indicated that ethanol decreased the time spent by rats in the open arms. This effect was reversed by intracerebroventricular (*icv*) administration of cUENK6 (0.0625, 0.125 and 0.25 nmol) at the doses of 0.0625 and 0.25 nmol. Our study suggests that MORs could participate in an anxiety-like behavior during withdrawal from chronic ethanol administration.

Assessment of amphetamine withdrawal anxiety-like behavior in the elevated plus-maze test in rats

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Drug taking cessation often implicates many negative affective symptoms. The dysphoric state, such as depression, anhedonia and anxiety-like behaviors is observed in early amphetamine abstinence. These symptoms certainly contribute to drug taking and relapse to addiction. Therefore, there is a great need to provide successful treatment for early amphetamine withdrawal. However, evaluation of full expression of

dysphoric state during amphetamine withdrawal in experimental animals is often confusing and highly depends on the dose and time schedules. The primary goal of the study was to choose a method, which allows the most effective induction of the anxiety-like behavior in amphetamine withdrawal in Wistar rats. Amphetamine (2.5 mg/kg *ip*) was administered once daily for 14 consecutive days. The anxiety-like behavior was estimated 24 hours or 14 days after withdrawal from amphetamine treatment in the elevated plus maze (EPM) test as the percent of time spent in open arms and the percent of entries into the open arms. Our results indicated that rats that have been withdrawn from repeated amphetamine, displayed a significant increase in anxiety-like behavior after 24 hours of withdrawal, while no changes in the locomotor activity was observed. Extending the withdrawal period from 1 to 14 days caused no significant changes in rats' behavior. This symptom of withdrawal was also estimated 24 hours after single injection of amphetamine (2.5 mg/kg) and revealed reduced anxiety-like behavior in the amphetamine-treated rats with no change in locomotor activity. These results indicate that the anxiety-like behavior is time limited only to an early withdrawal after chronic amphetamine administration.

Key words: amphetamine withdrawal, rats, withdrawal schedule, EPM

Abbreviations: EPM – elevated plus maze test

The expression of NMDA(R)(NR2B) subunit in rats selected for low and high anxiety

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In the experiment, we studied differences in the density of NMDA receptor NR2B subunit in the brain structures of low (LR) and high (HR) anxiety rats in basal condition and after exposure to extinction trials and re-learning of a conditioned fear response. Classification of animals as LR or HR was determined by fear-induced freezing responses in the contextual fear test (the duration of a freezing response was used as a discriminating variable). In basal conditions we found increased concentrations of NR2B in the amygdala of HR rats as compared to the unconditioned control group. An exposure of HR animals to fear conditioned context on re-test elevated the expression of NR2B subunits in the basolateral amygdala, prefrontal cortex and dentate gyrus of HR animals. Together, these data suggest that animals that are more anxious have altered patterns of NR2B subunit expression in the frontal cortex and limbic structures, which control emotional behavior. In the HR group the density of NR2B subunits in the cortex correlated negatively with the duration of a freezing response upon re-test of a conditioned fear. The current results suggest that drugs targeting NMDA receptors containing the NR2B subunit may be useful for the treatment of anxiety disorders.

The influence of CRF_2 receptor antagonists on rat conditioned fear responses and c-Fos and CRF expression in the brain limbic structures

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The aim of this study was to examine the influence of intracerebroventricular-administered selective corticotropin-releasing factor receptor 2 (CRF_2) antagonists (antisauvagine-30, astressin-2B), on rat conditioned fear responses, expression levels of c-Fos and CRF, and plasma corticosterone concentration. In fear-conditioned animals, both CRF receptor antagonists enhanced a conditioned freezing fear response and increased the conditioned fear-elevated concentration of serum corticosterone. Exogenously administered antisauvagine-30 increased the aversive context-induced expression of c-Fos in the 1 and 2 areas of the cingulate cortex (Cg1, Cg2), the central amygdala (CeA) and parvocellular neurons of the paraventricular hypothalamic nucleus (pPVN). Immunocytochemistry demonstrated an increase in CRF expression in the Cg1, M2 areas of the cortex, and pPVN, and it revealed the effect of conditioned fear in the CeA 35 min after antisauvagine-30 administration and 10 min after the conditioned fear test. Furthermore, astressin-2B, another CRF_2 receptor antagonist, enhanced expression of c-Fos and CRF in the CeA and pPVN, and revealed the effect of conditioned fear in the Cg1. These data support a model in which an excess in CRF_1 receptor activation, combined with reduced CRF_2 receptor signaling, may contribute to stronger expression of anxiety-like responses.

The effects of D-cycloserine and midazolam on the expression of alpha-2 subunit of GABA-A receptor and gephyrin of high and low anxiety rats

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We investigated how D-cycloserine and midazolam affect the behavior and expression of alpha-2 subunit of GABA-A receptor and gephyrin in brain structures of high and low anxiety rats during extinction session of conditioned fear test. High (HR) and low (LR) anxiety rats, were selected according to their behavior in the contextual fear test (i.e., the duration of a freezing response was used as a discriminating variable). Administration of D-cycloserine (15 mg/kg, *ip*), significantly enhanced the inhibition of an aversive context-induced freezing response observed during the extinction session in HR and LR rats 7 days after contextual fear test. In contrast, midazolam (0.75 mg/kg, *ip*), accelerated the attenuation of fear responses only in HR rats. HR rats pretreated with saline had higher expression of alpha-2 subunits of GABA-A receptor in basolateral amygdala (BLA) compared to LR rats. Administration of D-cycloserine and midazolam in-

creased the expression of alpha-2 subunits in the BLA of HR rats compared to HR rats pretreated with saline, and to drug administered LR rats. Moreover, D-cycloserine enhanced the expression of alpha-2 subunits and gephyrin in the prefrontal cortex of HR rats. Together, these findings suggest that animals that are more vulnerable to stress differ in the expression of alpha-2 subunits of GABA-A receptor in amygdala and prefrontal cortex, which are involved in the control of emotional behavior.

SESSION: **Depression and antidepressants**

A search for the model of "depressive-like" behavior in parkinsonian rats

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Primary motor symptoms of Parkinson's disease (PD) (bradykinesia, muscle rigidity, tremor) result from massive degeneration of dopaminergic neurons of the nigrostriatal pathway and a drammatic decrease in the dopamine (DA) level in the putamen and caudate nucleus. However, it is generally accepted that the clinical phase of PD is preceded by a preclinical period during which some non-motor symptoms: autonomic disturbances, olfactory dysfunctions and sleep disorders may occur. Among those symptoms depression is a frequent comorbid disturbance in (PD). The pathomechanisms underlying PD have also been suggested to be responsible for the PD-related depression.

The aim of this study was to search for the model of the "depressive-like" behavior in rats injected bilaterally with a very low dose of 6-hydroxydopamine (6-OHDA) into the ventrolateral region of the caudate-putamen (CP). The experiment was performed on male Wistar rats weighing 300–360 g. Rats were operated under the pentobarbital anaesthesia. 6-OHDA was injected bilaterally at a dose of 3.75 μg (free base)/2.5 μl per side into the ventrolateral region of the CP (AP: 1.2 mm, L: ± 3.1 mm, V: 7.0 mm according to Paxinos and Watson's, 2007). The "depressive-like behavior" was measured at 2 and 4 weeks after the surgery in the forced swimming test (FS) and three parameters were evaluated (immobility, climbing and swimming). Moreover, the locomotor activity was measured in actometers. The lesion extent was analysed by HPLC with EC detector and immunohistochemically with tyrosine hydroxylase (TH-ir). The verification of the placement of cannula tips was performed using an image analysis system on cresyl violet stained sections of CP.

Two weeks after the operation rats treated with 6-OHDA displayed a prolonged immobility in FS. This effect disappeared after 4 weeks. The locomotor activity was not influenced by 6-OHDA. Levels of DA and its metabolites (DOPAC and HVA) were decreased (ca 65%) in the nucleus accumbens 2 weeks after 6-OHDA but not changed in the CP, frontal cortex and substantia nigra (SN). Moreover, increases in the serotonin level were noted in the SN (2 weeks) and CP (2 and 4 weeks). Two weeks after the injections the level of 5-HIAA was elevated in the CP and lowered in the nucleus accumbens. No significant effect of 6-OHDA on TH-ir in the CP and nucleus accumbens were found.

The present study seems to indicate that a relatively small lesion of dopaminergic terminals in the striatum, which does not produce any motor disturbances, may induce "depressive-like" effects in rats which may be confirmed by the prolonged immobility observed in 6-OHDA lesioned rats in the FS test.

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Influence of new high affinity serotonin transporter ligands on cellular cAMP levels

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The 5-HT_{1A} receptors are inhibitory and their activation attenuates both the rate of firing of raphe 5-HT neurons and, consequently, the 5-HT synthesis and release from axon terminals [Blier et al., Ann NY Acad Sci, 1998; Fornal et al., J Pharmacol Exp Ther, 1996]. 5-HT_{1A} receptors are negatively coupled to adenylate cyclase and cellular cAMP level via G_s protein. Their agonists (e.g. 8-OH-DPAT) evoke decrease in rectal body temperature in mice (hypothermia) the effect being supposed to be connected to the activation of presynaptic 5-HT_{1A} receptors. It has also been found that the hypothermia may be caused by some serotonin reuptake inhibitors (e.g. fluoxetine) [Li et al., Eur J Pharmacol, 2009].

In the present paper we examined the influence of new serotonin transporter (SERT) inhibitors on the cellular cAMP levels. For that purpose several new high affinity SERT ligands were synthesized. Simultaneously CHO-K1 cell lines with stable over-expression of the HTRA1 gene were prepared. On those lines the influence of new high affinity SERT ligands on the cAMP level have been examined. Some of the ligands, devoid of substantial 5-HT_{1A} receptor affinity (but active in hypothermia test in mice [Nowak, Chilmonczyk, unpublished results]) diminished cellular cAMP levels in the obtained CHO cells with the over-expression of HTRA1 gene.

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Evidence for antidepressant- and anxiolytic-like properties of ketamine in animal models

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Clinical reports indicate a high degree of comorbidity of depression and anxiety, with symptomatology reflecting affective, somatic and cognitive dysregulation associated with both disorders. The frequent presence of anxiety symptoms in depressive patients suggests that the pathophysiology of anxiety and depression may have a common neurochemical mechanism. A large number of experimental data indicate that the N-methyl-D-aspartate (NMDA) receptors may be involved in the mechanism of action of antidepressant and anxiolytic drugs and, by implication, in the pathogenesis of depression and anxiety. A number of different classes of NMDA receptor antagonists, acting at various sites on the NMDA receptor complex, can mimic clinically effective agents in animal models predictive of antidepressant and anxiolytic action, and therefore, these receptors are suggested to play an important role in the neurobiology and treatment of these mood disorders. Ketamine, a dissociative anesthetic agent, is a non-competitive NMDA antagonist, which shows antidepressant and anxiolytic effects in animal studies and appears to have similar activity in clinically depressed patients.

The present study was design to evaluate an antidepressant-like activity of ketamine in a well-validated animal model of depression, the chronic mild stress

(CMS), and to compare its action with that of the reference drug, imipramine. The CMS procedure consists of a sequential exposure of rats to a variety of mild stressors for a prolonged period of time. This causes, among other behavioral, physiological and biochemical abnormalities, a behavioral hedonic deficit, which can be measured as decreased consumption of a 1% sucrose solution. The CMS-induced decrease in sucrose intakes can be effectively reversed by chronic, but not acute, treatment with various classes of antidepressant drugs.

After completion of the treatment period the level of anxiety in both the control and the stressed animals administered imipramine and ketamine was evaluated in the elevated plus-maze and the open field tests.

Ketamine (5–15 mg/kg) appears to be active in the CMS model of depression; 5 weeks of treatment with the compound did not affect the behavior of control animals and gradually reversed the decrease in sucrose intakes in stressed animals. The magnitude of this effect of ketamine was comparable to that of imipramine (10 mg/kg) but its onset of action appears to be faster than that of imipramine and most of other antidepressant drugs tested in the CMS model; the increase of sucrose consumption in stressed animals reached statistical significance after already the first week of treatment with the most active dose of 10 mg/kg, compared to three weeks required for imipramine.

In the elevated plus maze test the stressed animals administered vehicle showed a significantly lower number of open arm entries and this effect was not observed in animals treated with imipramine or ketamine. Imipramine slightly decreased the activity of stressed, but not control, rats in the open field test and ketamine had no effect on the exploratory activity of both the control and the stressed animals.

In conclusion, these findings provide further support for the hypothesis that the NMDA receptors are involved in the mechanisms of mood-related disorders and appear to confirm recent clinical observations that ketamine may have a potent and rapid antidepressant and anxiolytic potential.

Depressive-like behavior induced in rats by unilateral administration of different doses of 6-OHDA

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Depression is a common psychiatric non-motor factor impacting on quality of life of patients with Parkinson's disease (PD). Clinical studies suggest that at least 40% of all PD patients exhibit depressive symptoms. The proposed neurobiological background of the pathological state includes mesocortico-limbic dopaminergic denervation accompanied by noradrenergic and serotonergic deficiency caused by neurodegenerative changes in the locus coeruleus and the brain stem raphe nucleus. However, available data on the effectiveness of antidepressants therapies in PD are insufficient and there is a controversy about their application in combination with L-dopa.

The aim of the present study was to establish an animal model of PD corresponding to the advanced-stage of the disease with coexisting depressive symptoms. Experiments were performed on 6 groups of male Wistar rats injected with a single dose of 6-OHDA (8 μg/4 μl, 12 μg/4 μl or 16 μg/4 μl) unilaterally into the left medial forebrain bundle (MFB). To protect noradrenergic neurons, 3 groups of rat were pretreated with desipramine (Des) at a dose of 25 mg/kg. One week before surgery, all rats were tested for the level of consumption of 3% sucrose solution. The test was repeated one and three weeks after surgery. Two and four weeks after stereotaxic injection of 6-OHDA, animals were subjected to rotational behavior testing. Rats were injected with apomorphine (0.25 mg/kg, *sc*), and the number of rotations, both ipsilateral and contralateral, was recorded

over a 1 h period using an automatic rotometer. Rats were sacrificed on the following day after the last measurement of rotational behavior. Both limbic and motor brain structures such as, prefrontal cortex (PFC), hippocampus (HIP), striatum (STR) and substantia nigra (SN) were isolated. Noradrenaline (NA), serotonin (5-HT) and its metabolite as well as dopamine (DA) and its metabolites were determined in homogenates prepared from isolated brain structures using an HPLC method.

Unilaterally 6-OHDA-lesioned rats showed a significantly lower water intake rate than intact animals. Preference for sucrose consumption declined gradually with an increasing dose of 6-OHDA injected into the MFB. The loss of preference for sucrose solution was observed in rats treated with 6-OHDA at a dose 16 μg/4 μl both with and without Des. In all groups receiving 6-OHDA without Des, the toxin was found to produce a dramatic, unilateral decline of the level of DA and its metabolites in all examined structures. Similar effect of 6-OHDA on NA levels was also observed. In addition, there was a dose-dependent decrease of 5-HT in the PFC and HIP. However, such alterations were not observed in STR and SN.

The obtained results are discussed in the context of motor and depressive dysfunctions observed in an advanced-stage of Parkinson's disease. Rats treated unilaterally with a high dose of 6-OHDA can be a useful model for testing interactions between L-dopa and antidepressant drugs.

Effects of acute and repeated administration of N-acetyl-L-cysteine in animals model of depression

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Depression is a common mental disorder that exerts great impute on a quality of everyday life, characterized by long term mood lowering, accompanied by attention and cognition impairment and ranked fourth in the leading causes of disease burden [WHO, 2005]. Theories on the mechanisms of the disease have often focused on either its neurobiology or its cognitive and behavioral manifestations, but because of variety of triggers which may initiate the illness, its cause is still poorly understood. Due to the above, the pharmacological treatment of depression is still not satisfying and there is a critical need for developing more efficient treatment. Recently the oxidative stress has been implicated in the pathophysiology of several somatic and/or psychiatric diseases, including depression. Major depression is characterized among others, by significantly lowered antioxidant enzyme activity, e.g. glutathione peroxidase. Among antioxidants, N-acetyl-L-cysteine (NAC) was shown to mimic glutathione peroxidase by stimulating the synthesis of glutathione and its S-transferaze activity, or to scavenge free radicals. NAC was widely used as a mucolytic agent and an antidote in case of paracetamol overdose. According to latest preclinical findings [Ferreira et al., Behav Pharmacol, 2008], NAC may display beneficial effects in treating depression.

The aim of this paper was to examine the potential antidepressive properties of NAC in the bulbectomized rats (BULB; an animal model of depression) exposed to the forced swimming test (FST). To verify the specific action of NAC, locomotor activity measurements were also conducted. Data were analyzed by using one-way ANOVA or test *t*-Student followed by Dunnett test.

Male Wistar rats were divided into two groups that were either bulbectomized (removal of the olfactory bulbs) or exposed to the SHAM surgery (olfactory bulbs were left undestroyed). After 14-day recovery, half of each group was further given acute (1×) or chronic (10×) injections of NAC (50–100 mg/kg; *ip*; –120 min) or its solvent (saline; *ip*; –120 min). On the

day 9, all rats were placed into a cylinder (30 cm diameter and 45 cm deep) filled with 22–24°C water for a 15-min pre-test. On day 10, locomotor activity measurements (expressed as distance traveled during a 30-min recording) followed by the FST were conducted on the all groups of animals.

The results show that BULB rats displayed longer immobility time and distance traveled than SHAM controls. Acute NAC (50 or 100 mg/kg) administration had no effect on the time of immobility nor changed the animals' locomotor activity. Chronic administration of NAC (50–100 mg/kg) in a dose-dependent manner resulted in a significant reduce ($p < 0.05$ and $p < 0.001$ respectively) of the immobility time in BULB rats (but not in SHAM group), remaining without influence on the locomotor activity in both groups of animals.

Our findings extend the previous observation by Ferreira [Ferreira et al., Behav Pharmacol, 2008] that NAC mimics the action of antidepressant drugs in naive and BULB animals in the FST and it action is specific (not related to influence on locomotor activity). Based on the well-known antioxidant properties of NAC, the present results confirm the hypothesis that the oxidative stress is a key component of depression.

1,2,3,4-Tetrahydroisoquinoline and its neuroprotective methyl derivative, 1MeTIQ as potential antidepressant substances in the forced swimming test in mice

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1,2,3,4-Tetrahydroisoquinoline (TIQ) and its methyl derivative, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) are amines present in the mammalian brain. They may be natural regulators of monoaminergic systems with a visible neuroprotective potency how it was recently demonstrated in the rodent brain [Antkiewicz-Michaluk et al., J Neurochem, 2006]. In the present study we investigated a potential antidepressant properties of TIQ and 1MeTIQ and compare their effects with a classic antidepressant drug, imipramine by using the forced swimming test (FST) in mice. Additionally, the biochemical *ex vivo* study was carried out to determine the concentration of monoamines: dopamine (DA), noradrenaline (NA), serotonin (5-HT), and their metabolites in different mice brain structures. Moreover, the rate of monoamines metabolism and the inhibition of their re-uptake was calculated. The neurochemical data was established by HPLC methodology with electrochemical detection. All experiments were performed on male Albino Swiss mice weighing 25–35 g.

Results: FST has shown that TIQ and 1MeTIQ administrated in doses 10, 25 and 50 mg/kg, *ip* possess antidepressant-like activity, and significantly decreased immobility time (by about 30 to 40%, $p < 0.05$) similarly to imipramine in dose 30 mg/kg, *ip*. The biochemical analysis demonstrated that both investigated substances increased the levels of DA, NA and 5-HT in mice brain structures. Moreover, TIQ and 1MeTIQ caused a significant inhibition of DA and NA re-uptake in the dose dependent manner, expressed as the ratio [3-MT]/[DOPAC] and [NMN]/[NA], respectively. The rate of 5-HT metabolism calculated as the ratio [5-HIAA]/[5-HT], was strongly decreased in a dose dependent manner (by about 50%, $p < 0.01$) after administration of TIQ and 1MeTIQ.

Conclusions: The obtained data suggest that TIQ and its close methyl derivative, 1MeTIQ possess marked antidepressant-like effect in FST with potency comparable to imipramine. Thus, in that light and additionally taken into account their neuroprotective potential of action in the brain TIQ and 1MeTIQ may be useful in clinical practice as antidepressant drugs in patients.

1-Methyl-1,2,3,4-tetrahydroisoquinoline evoked antidepressant-like effects and intensified imipramine activity in the forced swimming test in rat

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It is generally known that monoaminergic transmission in the brain play an important role in the etiopathology of depression. In treatment of depression are used drugs which selectively inhibit the neuronal reuptake of noradrenaline and serotonin. Unfortunately, medicines are till now have shown poor efficiency. Thus there is an urgent medical need for a better, more effective pharmacotherapy. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) is an endogenous substance which exhibits neuroprotective, antiaddictive and MAO-inhibiting properties [Antkiewicz-Michaluk et al., J Neurochem, 2006; Wąsik et al., J Physiol Pharmacol, 2007] and it was proposed as an endogenous regulator of dopaminergic and serotoninergic activity. Imipramine is tricyclic antidepressant and inhibits the serotonin and norepinephrine transporters. In the present study we tested antidepressant-like effects of 1MeTIQ, imipramine and co-administration of these drugs in the forced swimming test in rat. Additionally, using *in vivo* microdialysis methodology we analyzed the effects of 1MeTIQ on release of monoamines: dopamine, serotonin and noradrenaline in rat striatum.

Results: 1MeTIQ in dose related manner has shown antidepressant-like effects, and significantly reduced the time of immobility with potency similar to imipramine. Moreover, 1MeTIQ intensified the effects of a low, not working doses of imipramine in this swimming test. The locomotor activity test has shown that 1MeTIQ (25 mg/kg, *ip*) given alone did not change the locomotor activity of rats but co-administered with imipramine (30 mg/kg, *ip*) partially antagonized the depression of locomotor activity induced by imipramine. *In vivo* microdialysis study demonstrated that 1MeTIQ (25 mg/kg) given concomitantly with imipramine (15 mg/kg) produced a long-lasting and significant increase of dopamine (up to 300%; $p < 0.01$), noradrenaline (about 900%; $p < 0.01$), and serotonin (approx. 400%; $p < 0.01$) concentrations in the extracellular area.

Conclusions: 1MeTIQ, an endogenous neuroprotective substance has shown also antidepressant-like properties in behavioral and neurochemical studies. Additionally, it strongly intensified antidepressant-like effects induced by a low doses of imipramine in the forced swimming test. The data strongly suggest that 1MeTIQ may be useful in clinical practice as an antidepressant drug in patients.

SESSION: **Neurodegenerations**

The ceramide levels in the brain of rats with streptozotocin-induced diabetes

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Ceramide, a second messenger in the so-called sphingomyelin signalling pathway has been implicated in a variety of physiological functions including apoptosis, cell growth arrest, differentiation and calcium homeostasis. Roles for ceramide and its downstream metabolites have also been suggested in a number of pathological states including diabetes. Ceramide is likely to play a role in the induction of insulin resistance. Diabetes mellitus (DM) increases the risk of dementia while the defects in lipid metabolism have been linked with the Alzheimer's disease (AD) and other neurodegenerative conditions. Additionally, a number of studies have consistently demonstrated that ceramide levels are increased in the AD brain tissue. It is suggested, that elevations in the ceramide levels can be toxic to neurons. There is no information available on the ceramide content in the brain of subjects with diabetes. The aim of the present study was to examine the ceramide level in the brain of rats with streptozotocin-induced diabetes exposed to myriocin, the inhibitor of the key enzymes of ceramide metabolism. The content and composition of ceramide fatty acids were determined by gas-liquid chromatography.

We found, that the brain ceramide content level nearly doubled in the subjects with diabetes and this level is significantly decreased by myriocin. The present results demonstrate for the first time that streptozotocin-induced diabetes elevates the ceramide content in the brain. Thus, inhibitor of de novo ceramide synthesis may provide an important new agent for this disease. The above results suggest, that sphingomyelin signalling pathway in the brain is an important mechanism by which functions of the brain of a patient with diabetes can be improved.

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Time course of changes in rotational behavior and DA metabolism in rats lesioned unilaterally with the selective proteasome inhibitor lactacystin

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A growing body of evidence suggests that proteasomal dysfunction may play an important role in the pathogenesis of PD. The aim of our study was to examine the effect of unilateral administration of lactacystin, a selective proteasome inhibitor, on rotational behavior and striatal and nigral metabolism of dopamine (DA).

Male Wistar rats were used for the study. The animals were injected unilaterally with a single dose of lactacystin (2.5 μg/2 μl) into the substantia nigra (SN) pars compacta. After injection, the rats were examined for spontaneous and apomorphine (0.25 mg/kg)-induced rotational behavior in rotameter bowls throughout 60 min. All the testing was done on days 1, 4, 7, 14 and 21 after lactacystin administration. The animals were killed by decapitation on post-lesional days 1, 4, 7 and 21. The levels of DA and its metabolites were assayed in striatal and nigral homogenates using an HPLC method. Behavioral studies showed that the lactacystin-lesioned rats, but not the sham-operated animals, displayed a strong spontaneous circling behavior contralateral to the lesioned side on the 1st and 4th day after lactacystin treatment. This effect disappeared 1 week after the lesion. After apomorfine treatment on day 7, 14 and 21 days after surgery, no contralateral turning was observed in the lesioned rats. Biochemical studies demonstrated that lactacystin evoked a progressive loss in DA and its metabolites in the ipsilateral striatum and SN compared to the ipsilateral striatum of the sham-operated rats. The decreases started on the 7th day after lesion except for the drop in the nigral DA level which began already on the 4th day after surgery. After 21 days, the decline in DA level exceed 90% both in the striatum and SN. As regards DA catabolism, lactacystin evoked a progressive acceleration of MAO-dependent oxidative DA catabolism (DOPAC/DA), COMT-dependent O-methylation (3-MT/DA) and total DA catabolism (HVA/DA), both in the ipsilateral striatum and SN.

The present study shows that lactacystin produces biochemical changes characteristic of degeneration of the DA system, namely a robust progressive decrease in DA level and an increase in DA catabolism. However, the presence of spontaneous contralateral rotations on the first few days after lesion indicates that other, probably non-DA mechanisms may play a role in this phenomenon. On the other hand, the lack of contralateral rotations after apomorphine up to three weeks after surgery suggests that the lactacystin-induced dramatic loss of striatal DA does not lead to supersensitivity of the striatal postsynaptic DA receptors.

Metformin increases phagocytosis and acidifies lysosomal/endosomal compartments in AMPK-dependent manner in rat primary microglia

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Recent evidence suggests that metformin shows beneficial effects in experimental models of neuroinflammatory diseases. The aim of the present study was to determine the effect of metformin on phagocytosis and acidification of lysosomal/endosomal compartments in rat primary microglia in the presence of LPS and/or beta peptide (25–35), (1–40) and (1–42).

Metformin increased the phagocytosis of fluorescent microspheres in the presence or absence of the beta-peptide (1–40). However, the drug had no effect on the phagocytosis in LPS-stimulated microglia regardless of the presence of the beta-peptide (1–40). Metformin acidified the lysosomal/endosomal compartments in the presence or absence of the beta-peptide 1–40 in both resting and activated microglia. To elucidate the mechanism of metformin action we used 5-aminoimidazole-4-carboxamide-1- -D-ribofuranoside (AICAR) as an activator of AMP-activated protein kinase (AMPK) and compound C as an confirmed pharmacological inhibitor of AMPK. We have showed that metformin increased AMPK activity in microglial cells, and that all observed effects are AMPK-dependent because the pretreatment of microglia with compound C reversed the effects of the drug. Since degradation of proteins in lysosomal/endosomal compartments depend largely on their phagocytosis and acidification, metformin may be beneficial in proteinopathies affecting the brain.

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Effects of ethylene glycol ethers on neuronal cell viability and total antioxidant capacity

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Ethylene glycol ethers (EGEs) are a class of chemicals used extensively in the manufacture of a wide range of domestic and industrial products which may result in human exposure and toxicity. The most well-known EGEs include 2-methoxyethanol, 2-ethoxy-ethanol, 2-butoxyethanol, 2-propoxyethanol, 2-isopropoxyethanol and 2-phenoxyethanol. 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. Contrary to peripheral action the effects of EGEs on the central nervous system (CNS) are expected, but has not been studied so far. Therefore, the aim of the present study was to find out if EGEs exert cytotoxic effects in human neuronal cell lines or disturbed lipid peroxidation in some brain structures.

In the *in vitro* study the effects of some EGEs on cell viability and on the hydrogen peroxide-induced damage in the human neuroblastoma (SH-SY5Y) cells were determined. It has been found that 2-phenoxyethanol in a concentration-dependent manner (5–25 mM, 24h) increased the basal and H_2O_2-induced lactate dehydrogenase (LDH) release and 3-[4,5-dimethylthiazol-2-yl]2,5-di-phenyl tetrazolium bromide (MTT) reduction. 2-Isopropoxy-ethanol significantly increased basal LDH release and MTT reduction but did not affect H_2O_2-induced cytotoxicity. 2-Butoxyethanol enhanced only cytotoxic effect of H_2O_2 but had no effect on MTT reduction and LDH release in basal condition.

Since data from peripheral organs indicated that EGEs attenuate antioxidant defense system and increase lipid peroxidation the aim of second part of the present study was to find out if similar changes are also present in rat hippocampus. Male Wistar rats were administered for 30 days (*sc*, 5 days a week) with 2-methoxyethanol (ME), 2-ethoxyethanol (EE) alone or in combination with 2-butoxyetanol (BE) or 2-isopropoxyethanol (IPE). Control rats were administered with physiological saline. The rats were decapitated 24 hr 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 co-administration of ME with BE, ME with IPE and EE with BE in statistically significant manner decreased antioxidant capacity in hippocampus. There were no significant changes in hippocampal antioxidant capacity in animals treated with 2-methoxyethanol, 2-ethoxyethanol or combination ME + EE.

The obtained results indicated that 2-phenoxyethanol, 2-butoxyethanol and 2-isopropoxyethanol, but not 2-methoxyethanol, induced damage or potentiated hydrogen peroxide-induced cytotoxicity in the human neuroblastoma (SH-SY5Y) cells. Also in *in vivo* condition 2-butoxyethanol and 2-isopropoxyethanol, but not 2-methoxyethanol and 2-ethoxyethanol decreased hippocampal antioxidant capacity. Comparison of the intensity of action of the investigated monoalkyl- and monoaryl-EGEs suggests that lipophilicity may play a key role in their neurotoxic action and that these compounds may be responsible for initiation or exacerbation of the harmful action of oxidative stress or aging on neuronal damage.

The involvement of kynurenic acid in the processes of epileptogenesis

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Kynurenic acid (KYNA), an antagonist of α7 nACh and ionotropic glutamate receptors is thought to be important endogenous inhibitory agent. Considering the role of KYNA in regulation of neuronal activity we investigated the involvement of KYNA in the processes associated with development of seizures.

We found that the electrically-induced kindling of seizures was associated with significant increase in the concentration of KYNA and its precursor, tryptophan (TRP) and the KYNA/TRP ratio in the amygdala and the hippocampus. Moreover these changes were accompanied by decrease in GABA levels, and an increase of the Glu/GABA ratio.

Further we investigated the effects of group III metabotropic glutamate receptor (mGluR)-selective ligands (L-AP4 and CPPG) on the KYNA level in the hippocampus of PTZ-kindled rats (*in vivo*). ICV administration of CPPG increased the concentrations of KYNA in the hippocampus of non-kindled rats, however this effect disappeared in the fully kindled rats. Administration of L-AP4 did not affect the hippocampal KYNA concentration. These data suggest that in the fully kin-

dled rats, there appears tendency to attenuate endogenous inhibitory processes, and that process is may be mediated by group III mGluR receptors.

Finally we attempted to determine whether KYNA plays a role in the central effects of valproic acid (VPA). We found a remarkably increase (more than 1600%) of the kynurenic acid level in the hippocampus (*in vivo*), following VPA administration (400 mg/kg; *ip*) to the PTZ-kindled rats. Furthermore, this increase in KYNA was accompanied by, and correlated with a significant rise in local GABA level.

Our study suggests that allow to suspect that KYNA play an important role in the process of epileptogenesis as well as in the central effects of VPA, an important antiepileptic drug.

Effects of hypoxia on cyclic AMP signaling and VEGF/bFGF generation in different types of cultured cells

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Introduction: Hypoxia is a pathological condition leading to multiple changes in the brain tissue, including neuronal and glial cell degeneration. Simultaneously, hypoxia-affected cells/tissue mobilizes their/its defense mechanisms, whose role is to protect against cell/tissue damage and death. Among such protective mechanisms is hypoxia-accompanied expression of endogenous substances endowed with (neuro)protective potential, such as pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP), an issue that was studied in detail by the present authors. In addition, hypoxia can affect the activation of different cells by coordinated expression of a number of genes, including vascular endothelial growth factor (VEGF), glycolytic enzymes, glucose transporter (Glut), inducible NO synthase, and others.

Aim of work: The present work focuses on the effects of hypoxia on several cellular mechanisms related to cell survival, cyclic AMP (cAMP) signaling, and expression of VEGF or bFGF. The study was performed on different cultured cells, such as: the rat primary glial cells (astrocytes) and neuronal cells, as well as human endothelial cells lining both micro- and macrovesells, i.e. HMEC-1 and HUVEC, respectively.

Results: Using MTT method, we have found that the all cell systems subjected to "chemical" hypoxia (100–800 μM $CoCl_2$) for 24 h responded similarly – there was a concentration-dependent suppression of cell survival, independent on cell type. Concerning the cAMP signaling, the effect of hypoxia was studied in a model system where cAMP generation was stimulated by adrenergic agonists, i.e. adrenaline and isoprenaline. In cultured rat astrocytes and neuronal cells both compounds stimulated cAMP generation acting preferentially *via* β_1-subtype adrenoceptors. However, in microvascular endothelial HMEC-1 cells, adrenaline strongly stimulated cyclic nucleotide response *via* all three subtypes of β-adrenoceptors, acting preferentially *via* β_2-subtype [Wiktorowska-Owczarek et al., Pharmacol Rep, 2008]. In addition to decreasing survival of these three cell types, hypoxia produced suppression of the cAMP response to adrenaline and isoprenaline. In contrast, hypoxia showed only slight effect on the adrenaline-driven cAMP response in human umbilical vein endothelial cells (HUVEC), representing a macrovascular system [Wiktorowska-Owczarek et al., Pharmacol Rep, 2007]. In additional studies, hypoxia ($CoCl_2$) stimulated VEGF production, but did not affected the expression of bFGF in HMEC-1 cells. These findings are consistent with previous results coming from other laboratory showing that hypoxia decreased endothelial cells proliferation and suppressed the ability of human microvascular endothelial cells (HMEC-1) to express bFGF, whereas it increased bFGF receptors [Kuwabara et al., Proc Natl Acad Sci USA, 1995].

Lipopolysaccharide (LPS) derived from bacterial cell walls is responsible for inducing an inflammatory process. In our hands, LPS in a concentration-dependent manner (0.1–100 μg/mL) increased the level of VEGF and bFGF in HMEC-1 cells, with statistically significant effects seen at 10 and 100 μg/mL. Summarizing, our studies have proved that hypoxia stimulated the production and secretion of VEGF, but not bFGF, whereas LPS stimulated both. We observed important differences in production and secretion of VEGF and bFGF by the human microvascular endothelial cells (HMEC-1).

Conclusions: Based on the presented results we conclude that hypoxia: 1. decreased survival of cultured rat glial (astrocytes) and neuronal cells, as well as human endothelial cells (representing both micro- and macrovessels), 2. largely suppressed the β-type adrenoceptor-driven cAMP effect in the all studied cell systems, 3. contributes to expression of VEGF in human microvascular endothelial cells (HMEC-1).

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SESSION: **ModALL**

Characterization of compound C46: a novel 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, $mGluR_4$ is primarily found presynaptically in several brain areas and they function as an autoreceptors or heteroreceptors: mGlu4 receptor activation inhibits GABA and glutamate neurotransmission. Intervention in glutamatergic neurotransmission through mGlu4 receptor has been pursued intensively for the treatment of vast number of neurological and psychiatric disorders such as anxiety, schizophrenia, epilepsy, and addiction.

$mGluR_4$, a G protein-coupled receptor (GPCR), couples to adenyl cyclace (AC) through Gi signaling and decreases cytosolic cyclic AMP upon receptor activation.

Specific for Class C GPCRs, to which $mGlu_4$ receptor belongs, is their large N-terminal extracellular domain, where the endogenous agonist binds. Ligands can bind the orthosteric, endogenous agonist, binding site or an allosteric domain which is located in the transmembrane region.

A lack of success in developing highly subtype-selective agonists and antagonists that act at the orthosteric glutamate binding site has led to the development of a novel approach to the activation of $mGluR_4$ using highly selective positive allosteric modulators (PAMs) of this receptor. As opposed to direct activation of $mGluR_4$, PAMs dramatically potentiate the response of the receptor to its endogenous ligand glutamate by interaction with transmembrane region of $mGlu_4$ receptor.

Aim: Identification novel chemical scaffold possessing $mGluR_4$ positive allosteric modulation activity.

Methods: The screening study and activity of potential PAM was determined using forskolin-induced cAMP accumulation, in a HEK-293 T-Rex cell line stably expressing $mGluR_4$ or mock transfected HEK-293 T-Rex cell line. Cells were labeled [^{3}H] adenine. [^{3}H] cAMP was then purified by sequential chromatography over Dowex resin and aluminum oxide columns.

Results: Endogenous $mGluR_4$ orthosteric agonists L-Glutamic acid (L-Glu) decreased the forskolin-induced cAMP production in cells, with EC_{50} value of 10.2 M.

Non selective $mGluR_4$ orthosteric agonists L-AP4, L-SOP, ACPT-I and DCPG decreased the forskolin-induced cAMP production in cells, with EC_{50} values of 1.3 μM, 1.2 μM, 3.3 μM and 4.1 μM, respectively.

Compound C46 (10 uM and 30 uM) alone and in presence of L-Glu decreased the forskolin-induced cAMP production in HEK-293 T-Rex mGluR4 cell line.

The orthosteric antagonist CPPG did not fully block the agonism of the L-Glutamic acid in the presence of compound C46.

Compound C46 (10 uM and 30 uM) alone and in presence of L-Glu decreased the forskolin-induced cAMP production in mock transfected HEK-293 T-Rex cell line.

Conclusions: Compound C46 is not a positive allosteric modulator of $mGluR_4$.

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Synthesis and characterization of novel metabotropic glutamate receptor 2/3 ($mGluR_{2/3}$) positive allosteric modulator (PAM)

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Background: Glutamate is the major excitatory neurotransmitter in the central nervous system. Its effects are modulated by both ionotropic and metabotropic glutamate receptors (mGluRs). The Group II mGluRs are highly expressed in the cerebral cortex, hippocampus, striatum, amygdala, frontal cortex and nucleus accumbens. $MGluR_2$ and $mGluR_3$ are found predominantly presynaptically but outside of the active zone of glutamate release, where they largely function to regulate neurotransmitter release *via* coupling to $G_{\alpha i/o}$-associated signal transduction cascades and through ion channel regulation *via* $G_{\beta\gamma}$ subunit activity. Specific for Class C GPCRs, to which $mGlu_{2/3}$ receptors belong, is their large N-terminal extracellular domain, where the endogenous agonist binds. Ligands can bind the orthosteric, endogenous agonist, binding site or an allosteric domain which is located in the transmembrane region.

A lack of success in developing highly subtype-selective agonists and antagonists that act at the orthosteric glutamate binding site has led to the development of a novel approach to the activation of $mGlu_{2/3}$ using highly selective positive allosteric modulators (PAMs) of this receptor. As opposed to direct activation of $mGlu_{2/3}$ PAMs dramatically potentiate the response of the receptor to its endogenous ligand glutamate by interaction with transmembrane region of $mGlu_{2/3}$ receptors.

Group II metabotropic glutamate receptors have shown activity in a range of preclinical animal models of anxiety schizophrenia and addiction.

Aim: Identification novel chemical scaffold possessing $mGluR_{2/3}$ positive allosteric modulation activity.

Methods: The screening study and activity of potential PAM was determined using [^{35}S]-GTPγS assay.

Results: *Via* screening of in-house compound collection we have identified novel chemical scaffold possessing $mGluR_{2/3}$ PAM activity.

Series of 28 compounds have been designed, synthesized, and characterized.

The SKS-11d compound induced a leftward-shift of the glutamate concentration-response curve.

None of compounds did interact with $mGluR_4$ and $mGluR_8$ receptors up to 30 μM.

Compound SKS-11d was active in SIH and four plate test in mice.

Conclusions: Compound SKS-11d is a novel positive allosteric modulator of mGlu2/3 receptors.

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Validation of HEK-293 cell line as a molecular tool for pharmacological study of $mGluR_7$ receptor

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Background: $mGluR_7$ is a very promising target receptor for psychopharmacological research and pharmacological industry. It is suggested potential role the $mGluR_7$ in the pathophysiology of schizophrenia,

Alzheimer's disease, anxiety, depression, addiction, epilepsy and pain.

Aim: In most cases as a host cells for glutamate receptors study serves HEK-293 cell line, but does it work also for $mGluR_7$? In this study we compeered pharmacological features of HEK-293 cell with or without expression $mGluR_7$.

Methods: GRM7 was cloned into genome of HEK-293 cells contained T-Rex expression system (Invitrogen). Expression of the receptor was analyzed by means of qRT-PCR and Western blot in both: cells before and after stable transfection with GRM7. For the functional characterization of $mGluR_7$ was used cAMP assay with use of radiolabeled [^{3}H]-adenine and forskolin as an adenylate cyclase stimulatory agent. During experimental procedures cell with expression of $mGluR_7$ were incubated with various concentration of L-AP4, L-Glu with or without presence modulators: AMN-082 or MMPIP respectively. Percent of conversion radiolabeled adenine to [^{3}H]-cAMP was plotted against concentration of compound (L-AP4, L-Glu). This same experimental procedures were performed for HEK-293 T-REx cell without expression $mGluR_7$.

Results: Inhibitory effects of increased concentration of L-AP4 on [^{3}H]-adenine conversion to [^{3}H]-cAMP was noted only in cell with expression of $mGluR_7$ protein but in high doses. Contrary to L-AP4 biological effects of increased concentration of L-Glu was observed in both HEK-293 after stable transfection with GRM7 and what is more interesting in cell without expression of mGluR7. EC_{50} calculated for both group of cells in presence of L-Glu were 1.66 mM and 8.0 mM, respectively. These data are in kipping with available information concerning the $mGluR_7$ EC_{50} that was estimated for 2.3 mM.

Conclusions: The present data demonstrated that expression system based on HEK-293 cell line might be not adequate for mGluR7 study especially L-Glu effects.

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Adjustment of adenylate cyclase [EC 4.6.1.1] activity in HEK293 cells transiently expressing mGluRs group III

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Background: Expression of metabotropic glutamate receptors in heterologous mammalian cells is a method of choice to study ligand binding and activation. We optimize the method of transient overexpression of mGluRs group III in HEK293 cells, which should be an effort- and cost-balanced tool to study allosteric modulation of this receptors. Proper downstream signaling is essential for the indirect functional evaluation of mGluRs activity. Group III mGluR ligand binding triggers G protein dissociation and activation, with $\alpha 1$ subunit inhibiting adenylate cyclase (AC) activity.

Aim: Our goal is to improve the settings for transient transfection in order to get a highly efficient and reliable method for functional screening of the effects of amino acid substitutions in receptor protein, in response to potential allosteric modulators.

Results: Transient transfection enables high level of protein produced in short time, but this way of delivering GRM4, 7, 8 genes to HEK293 seems to affect adenylate cyclase activity: the rate of forskolin stimulated [^{3}H]-adenine conversion to [^{3}H]-cAMP conversion is significantly reduced. It could be an effect of membrane fluctuations caused by the uptake of transfectant-DNA complexes or lowered number of adenylate cyclase associated with the cell membrane-due to endocytosis. Irrespective of the cause, low [^{3}H]-adenine conversion to [^{3}H]-cAMP conversion rate precludes reliable evaluation of glutamate receptor activity.

As the exact cellular origin of HEK293 is not resolved yet and AC is a family of at least dozen of genes, we analyzed which exactly are expressed in this cell line and estimated the level of expression (qRT-PCR). AC isoforms differ in the regulation of enzyme regulation in response to G protein or other stimuli. Therefore this provided us the information whether the particular isoform is the most convenient for our application, or it should be delivered in trans by means of stable overexpression.

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Different approaches in homology modeling of transmembrane domain of metabotropic glutamate receptor type 4 (mGluR4)

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Class C GPCRs are well known of being conceivable drug targets for many Central Nervous System (CNS) diseases. Among the class, the group III (mGluR4, -6, -7, -8) of metaboropic glutamate receptors (mGluRs) lie in field of our interest due to their involvement in depression and anxiety treatment [Lavreysen et al., Curr Med Chem, 2008]. Metabotropic glutamate receptors are composed of two domains: an extracellular Venus Flytrap – binding glutamate or other orthosteric ligands, and heptahelical transmembrane 7TM – bearing allosteric site. Due to allosteric modulation being in scope of our interest, computational models of investigated receptor would be used in our virtual screening protocol to help discriminate active/inactive compounds. However lack of crystallographic structures of transmembrane domain of mGluRs forces us to use Rhodopsin and β2AR (both class A GPCRs) as templates for the models.

Despite several crystal structures of class A GPCRs published so far, untrivial evolutionary relationships between class A and classes B & C GPCR make homology modeling of mGluRs a highly challenging task. In this study we present two different approaches to creating homology models 7TM region of mGluR4 that were attempted in our lab, along with methods and tools created to validate the models.

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How to find a needle in a haystack? The application of *in silico* methods in searching of potentially new PAM of mGluR III

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In recent years the *in silico* technology have become increasingly popular in the pharmaceutical and academic researches, especially in hit discovery and lead optimization [Shoichet, Nature, 2004; Vyas, Sci

Pharm, 2008]. In general, these methods, which support the various stages of drug design, include molecular modeling techniques, cheminformatics and bioinfomatics. They offer the advantage of delivering new drug candidates more quickly and at a lower cost. Major roles of *in silico* methods in drug discovery are; (1) Virtual Screening, (2) *de novo* design, (3) *in silico* ADME/T prediction and (4) advanced methods for determining protein-ligand binding.

Here, we show the implementation of *in silico* technology to the searching of potentially new Positive Allosteric Modulators (PAM) of mGlu receptors family III. For this purpose a broad range of computational techniques (e.g. 2D fingerprints, 1D molecular descriptors, docking and scoring, pharmacophore similarity search, clustering), machine learning (support vector machines, SVM) and statistical (e.g. PCA, ROC) methods were applied. Additionally, the largest vendors databases, such as Enamine, ChemBridge and ChemDiv have been analyzed, adopted, and used as a molecular screening space (approx. 3M compounds).

In order to increase the overall efficiency, great efforts are being made to develop and validate new tools, methodology and infrastructure linking work and results of different research teams.

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Anxiolytic-like action of LY487379 after central administration in rats

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The present study was carried out to investigate which brain structure was involved in the anxiolytic-like activity of LY487379. A Vogel test in rats was used to assess the anxiolytic-like effect. LY487379 [N-(4-(2-methoxyphenoxy)phenyl)-N-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine] is a selective positive allosteric modulator (PAM) of mGlu2 receptor, with behavioral effects similar to mGlu2/3 receptor agonists. Brain regions involved in the anxiolysis, i.e. hippocampus and amygdala were chosen as sites for drug administration (*ihp* and *ia,* respectively). LY487379 given into the CA1 region of dorsal hippocampus showed anxiolytic-like action in the Vogel test at the dose of 3 nmol/µl (but not in doses of 1, 6 and 10 nmol/µl). After *ia* administration only the highest dose (10 nmol) of LY487379 produced an anxiolytic-like action. Effect of LY487379 was blocked by peripheral injection of group II mGlu receptor antagonist, LY341495, confirming the specificity of action of PAM. The results obtained suggest that positive modulator of group II mGlu receptor exerts anxiolytic action by stimulation of receptors placed in both investigated brain areas.

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On the mechanisms of anxiolytic-like effects of mGlu2/3 receptor agonist LY 379268 and positive modulator LY487379 in stress-induced hyperthermia model in mice

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The present study was carried out to investigate the potential anxiolytic-like activity of LY379268, selective mGlu2/3 receptor agonist and LY487379, a positive allosteric modulator of mGlu2 receptors, in the modified stress-induced hyperthermia (SIH) test. LY379268, administered at the doses of 0.5, 2.5 and 5 mg/kg, *ip*, produced a dose-dependent anxiolytic-like effect, which was statistically significant after 2.5 and 5 mg/kg. LY487379 was administered at doses of 0.1, 0.5, 1 and 5 mg/kg; the anxiolytic-like effect of the compound was observed at the doses of 0.5 and 1 mg/kg. $GABA_A$ receptor antagonist, flumazenil (10 mg/kg, *ip*), did not abolished the effects of drugs.

The anxiolytic-like activity of drugs was not serotonin dependent, as both compounds were effective in serotonin-depleted animals. 5-HT2A/2C receptor antagonist ritanserin (0.5 mg/kg, *ip*) did not abolished the effect of drugs, either. In contrast, WAY100635 (0.1 mg/kg, *sc*) did not have any influence on the LY487379-induced effect, but antagonized the effect exerted by LY379268. The obtained results suggest that group II mGlu receptor agonist evokes its anxiolytic-like action which is not dependent on GABAergic and partially (after stimulation of mGlu3 receptors) dependent on serotonergic system.

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SESSION: **Addiction. Varia**

Effects of cannabinoid CB_1, CB_2 and vanilloid TRPV1 receptor antagonists on cocaine reward and seeking behavior in rats

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Cocaine addiction is a chronic illness attached mainly with loss control of substance use and with episodes of reinstatements which are critical in persist this lingering disease. Nowadays, despite well-understood neurobiological action of cocaine in the central nervous system, no effective therapy exists, and further examination should concentrate on anti-relapse treatment. Recent preclinical reports indicate, that drugs affected endocannabinoid (eCB) system may play key role in cocaine addiction, especially in reinstatement of cocaine seeking behavior [Adamczyk et al., J Physiol Pharmacol, 2009].

The aim of the present study was to examine the effect of the selective CB_1 receptor antagonist AM251, the selective CB_2 receptor antagonist SR144528 and the selective TRPV1 receptor antagonist SB366791 in the cocaine- or food-maintained self-administration and in the cocaine-, cue- or food-induced reinstatement in rats.

Male Wistar rats were implanted with a catheter (*iv*) and trained to self-administer cocaine (0.5 mg/kg/infusion) in experimental operant chambers under a fixed ratio (FR) 5 schedule of reinforcement, during 2-h daily sessions performed 6 days/week. After the stabilized self administration, the extinction reinstatement procedures were carried out. During extinction active lever presses resulted in neither the delivery of cocaine (saline was substituted for cocaine) nor the presentation of the conditioned stimulus. Then, the rats were tested for response reinstatement induced by cocaine (10 mg/kg, *ip*) or by the cue (light + tone). Food self-administration paralleled closely cocaine self-administration; instead of cocaine access was given to sweetened milk. After acquisition of food self-administration, the extinction/reinstatement procedures were carried out. During the reinstatement tests, active lever presses on the FR 5 schedule resulted in a delivery of sweetened milk.

AM251 (1–3 mg/kg), SR144528 (0.1–1 mg/kg) and SB366791 (0.1–1 mg/kg) did not alter the cocaine-maintenance responding. Cocaine-induced cocaine seeking behavior was altered dose-dependently by AM251 (0.1–1 mg/kg), SR144528 (0.1–1 mg/kg) and SB366791 (0.1–1 mg/kg). In cue-induced reinstatement of cocaine seeking behavior only CB_1 receptor antagonist AM251 (0.3–1 mg/kg) significantly reduced active lever presses, while nor SR144528 (0.1–1 mg/kg) nor SB366791 (0.1–1 mg/kg) affected animals operant responding.

In food self-administration AM251 in the highest dose (3 mg/kg) significantly reduced active lever presses and food intake, while SR144528 (0.1–1 mg/kg) and SB366791 (0.1–1 mg/kg) attenuated only operant respond (active lever presses). Reinstatement of food-induced taking behavior expressed as active lever presses and food intake was attenuated by AM251 (1 mg/kg). Administration of SR144528 (0.1–1 mg/kg) dose-dependently reduced active lever presses, while highest dose (1 mg/kg) significantly also decreased food reinforcement.

Our results indicate that inhibitory effects of CB_1, CB_2 or TRPV1 receptor antagonists on drug primed cocaine seeking behavior were related to decrease in motivation for appetitive stimuli or other aspects of cocaine

addiction. Howsoever its interesting that only CB_1 receptor antagonist attenuated cue induced reinstatement of cocaine seeking behavior, what may suggests a crucial role of this receptor in drug associates cues.

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Frequency modulated 50-kHz ultrasonic appetitive vocalization as a model of drug abuse

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50-kilohertz (kHz) range frequency-modulated ultrasonic vocalization (USVs) emitted by rats is modulated by enhanced reactivity on dopaminergic neurons that project from ventral tegmental area to the nucleus accumbens and prefrontal cortex [Wintink and Brudzynski, Pharmacol Biochem Behav, 2001]. Intra-NAcc amphetamine (AMPH) injections selectively evoke 50-kHz USVs in rats, supporting the notion that dopamine elevations in NAcc may unconditionally elicit a state of reward anticipation. The range of 50-kHz reflects a positive emotional state. It is well known that amphetamine increase appetitive vocalization. We mentioned in our study that another drug – morphine after acute administration in a dose 2.5; 5.0; 10 mg/kg, reduce 50-kHz USVs in the low reward model. In the model of chronic (14 days) administration of morphine we found that anticipation of a drug (morphine) enhanced frequency modulated 50-kHz USVs on the challenge day (28 day), and injections of morphine in a dose 5.0 mg/kg inhibited the emission of appetitive related vocalization. These results indicate an important role of the VTA/NAcc μ-opioid and κ-opioid receptors in neurochemical regulation of the mesolimbic dopamine system. In another paper [Hamed et al., Physiol Behav, 2009] it was found that long-term social isolation of adult rats enhanced the emission of high frequency calls in the appetitive band (50-kHz), during rats encounter. Diazepam (1.0 mg/kg) significantly increased isolation induced appetitive USVs. We found that morphine in a dose 5.0 and 10.0 mg/kg, but not 2.5 mg/kg, inhibited ultrasonic vocalization in a range of 50-kHz induced by social interaction after long-term isolation. All together, these data indicate that emission of appetitive USVs could be a very useful tool to study the role of neurochemical interactions in the brain reward systems.

Acute cocaine administration enhances the polysialylation of the NCAM molecule in the rat medial prefrontal cortex

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The present study investigated the impact of acute cocaine treatment on changes in the expression of the neural cell adhesion molecule (NCAM) and its polysialylated form (PSA-NCAM). Results are given on the level of mRNA and proteins expression in the medial prefrontal cortex (mPFC) of rats. NCAM/PSA-NCAM is required for the structural-activity dependent-remodelling of neuronal shape and synaptic contacts and is constitutively expressed in the mPFC. The rats were treated with a single dose of cocaine (15 mg/kg, *ip*), and the mRNA levels of NCAM, the polysialyltransferases ST8SiaII and ST8SiaIV, the enzymes involved in the polysialylation of NCAM, were measured at 3, 6, and 24 h after cocaine treatment. At the same time points, the level of expression of the NCAM and PSA-NCAM proteins was measured via western blotting. An acute cocaine injection did not affect the mRNA levels of NCAM and ST8SiaIV, but it increased the mRNA level of ST8SiaII 3 h after the injection. At the same time point, an increase in the PSA-NCAM protein level but not in the NCAM protein level was observed. Cocaine administration did not change mRNA and proteins level of NCAM and polysialylation of NCAM molecule when measured 6 and 24 h after cocaine injection. Morphological studies of the PSA-NCAM protein expression patterns using immunohistochemistry method and unbiased stereological analysis were performed 3 h after cocaine administration. It was found an enhancement of the PSA-NCAM immunostaining in the perisomatic-like sites and in the length density of PSA-NCAM-positive neuropil. It was also observed that a single injection of raclopride (0.4 mg/kg) or SCH 23390 (0.5 mg/kg), D2/D3 and D1 dopamine receptors antagonists that are ineffective when given alone, abolished the effects of cocaine administration, including those on the level of mRNA expression and the total content of the proteins. The data in the present study indicate that constitutive synaptic plasticity in the mPFC may be modified by cocaine administration. The observed increase in the polysialylation of NCAM in the perisomatic innervations of pyramidal neurons engaging dopamine D1 and D2/D3 receptors may influence activity of cortical neurons and formation memory traces.

Amnesic effect of flunitrazepam in mice in the modified elevated plus-maze is prevented by the inhibition of L-arginine:NO:cGMP pathway

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Benzodiazepines (BZs) are useful for treating a variety of specific conditions, like anxiety, sleep disorders or epilepsy. It is also known that BZs induce memory deficits both in humans and in animals. They act by enhancing the γ-aminobutyric acid$_A$ (GABA$_A$) receptor function in the central nervous system. Nitric oxide (NO) is an important bioregulatory molecule that has been suggested to play a critical role in learning

and memory processes. Literature data point to the relationship between L-arginine:NO:cGMP pathway and GABA-mediated transmission in the central nervous system. The aim of this study was to assess the role of L-arginine:NO:cGMP pathway in the amnesic effects of flunitrazepam in the modified elevated plus-maze task in mice. Our experiments indicated that flunitrazepam (0.05 mg/kg, *sc*) impaired elevated plus-maze memory performance in mice. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME; NO synthase nonselective inhibitor; 50, 100 and 200 mg/kg, *ip*); 7-nitroindazole (NO synthase selective inhibitor; 10, 20 and 40 mg/kg, *ip*) and methylene blue (soluble guanylate cyclase inhibitor; 2.5, 5 and 10 mg/kg, *ip*) prevented the amnesic properties of flunitrazepam. It is important to note that flunitrazepam (0.05 mg/kg) does not impair locomotor activity of mice. Moreover, L-NAME, 7-nitroindazole and methylene blue given alone, have no impact on mice's behavior in the modified elevated plus-maze task. In conclusion, our data indicate that an inhibition of L-arginine:NO: cGMP pathway may be involved in the amnesic effect of BZs.

Regulatory effect of some neuropeptides on plasma interferon level

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Interferons, proteins with antiviral antitumoral activity, are also produced by neurons and glial cells in central nervous system. They regulate the hypothalamic-pituitary-adrenocortical axis. Several neuropeptides: tetrapeptide tuftsin, pentapeptide metenkephalin, nociceptin or β-endorphin were reported to increase interferon level. There were demonstrated opioid-induced alterations of human and animal immune status. Morphine suppresses intracellular interferon-α expression in neurons of the central nervous system.

Octapeptide leucopyrokinin, an insect neuropeptide exerts some biological effects, especially antinociceptive effect in rats. Synthetic leucopyrokinin, as well as its active analog [2–8]-leucopyrokinin, injected directly into the lateral ventricle induced significant increase of blood serum γ-interferon level in rats. This effect was blocked by either Naloxone, an opioid antagonist or by synthetic leucopyrokinin analog [D-Ala^5]-[2-8]-leucopyrokinin, an antagonist of leucopyrokinin and of μ-opioid receptors. Obtained results indicate that leucopyrokinin, insect neuropeptide also in mammals modulates interferon release. At present we regard that central opioid system mediates leucopyrokinin effect.

Superoxide dismutase activity changes in the brain and peripheral organs of rats underwent cocaine self-administration and extinction training procedures

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Cocaine is one of the illegal abused psychostimulants in the world and its chronic consumption evokes damage in a range of organs. Numerous different mechanisms have been suggested for cocaine-evoked toxicity, and one of them was indicated as intensification of oxidative stress (OS) in such organs like brain, heart, liver and kidney. OS gives rise to lipid peroxidation, protein oxidation, DNA damage and many enzyme dysfunction, all of them can provide to alterations in neuronal function and injury in the above body organs [Bashkatova et al., Neuroreport, 2005; Numa et al., Neuroscience, 2008; Fan et al., J Pharmacol Exp Ther, 2009]. Interestingly, some results suggest also that chronic cocaine administration can affect cellular enzyme and non-enzyme antioxidant defense systems. Superoxide dismutase (SOD) family play a crucial role in intra- and extracellular antioxidant system [Portugal-Cohen et al., J Dermatol Sci, 2010; Fineschi et al., Int J Legal Med, 2001].

The aim of this study was to examine the SOD activity in some brain structures (the frontal cortex and hippocampus) and peripheral organs (the liver, heart and kidney) during maintenance of cocaine self-administration and after its withdrawal during extinction training.

The experiment was carried out on male Wistar rats. Animals were implanted with catheter (*iv*) and trained to cocaine self-administration (0.5 mg/kg/infusion) in experimental operant chambers under a fixed ratio 5 schedule of reinforcement. Subjects were given access to cocaine during 2-h daily sessions performed 6 days/week. Following stabilization of responding, the extinction procedure was carried out. During extinctions sessions animals had 2-h daily training sessions with no delivery of cocaine (saline was administrated). In this experiment we involved yoked procedure in order to distinguish pharmacological and motivational effects of the psychostimulant. The SOD activity was assessed using Misra and Fridovich method based on the ability of the enzyme to inhibit the autoxidation of epinephrine [Misra et al., J Biol Chem, 1972].

Our findings showed that only active cocaine administration significantly ($p < 0.05$) increases SOD activity in the hippocampus and frontal cortex. During maintenance of self-administration we found an intensification of SOD activity ($p < 0.001$) following either active and yoked cocaine administration in the liver while the enzyme activity was left unchanged in other studied peripheral organs. In a group of rats underwent cocaine self-administration, extinction training induced a significant increase in the SOD activity in the rat hippocampus ($p < 0.001$) and in the kidney ($p < 0.01$).

Our results suggest that repeated active cocaine administration provoked elevation of SOD activity in brain and peripheral organs. They also point a significance for oxidative stress in motivational processes related to voluntary cocaine intake.

Neuromodulation of memory by veratridine in rats

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Veratridine (Ver) a natural alkaloid from *Liliaceae*, preferentially binds to activated Na channels, causing a long lasting open state and their persistent activation. It prevents inactivation and shifts activation to more negative potentials. Drugs which block voltage-gated sodium channels, are applied in therapy of epilepsy, cardiac arrhythmias and as a local anesthetics. There are only a few data on the role of sodium channels in the memorizing action. I suppose that Ver-induced neuronal activation may influence on neuronal plasticity and in this way may modulate memory consolidation in rats. Therefore present study was undertaken in order to verify this hypothesis.

The experiments were performed on Wistar female rats. A week before the beginning of experiment rats were implanted with polyethylene cannulas to the right lateral brain ventricle under ketamine and xylazine anesthesia. On the day of experiment veratridine was injected into the lateral brain ventricle (*icv*) through implanted cannulas at doses of 0.05, 0.2, 1, 5, 20 and 65 nmols. Every applied dose of veratridine was dissolved in a constant volume of 5 µl of 0.9% NaCl. There were used the following three tests: of water maze, of active avoidance and the test of passive avoidance to evaluate the effect of Ver on rats memory.

It was found a biphasic effect Ver on rats memory. Lower doses Ver improved rats memory, while higher doses deteriorated memory processes in rats.

Effect of alloferon 1 on plasma levels of VEGF, IL-2, TNF-alpha and IFN-gamma in rats

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Tridecapeptide Alloferon 1 (Al 1), isolated from blood of experimentally infected larvae of the blow Callifora vicina, displays antitumoral and antiviral activity. It stimulates the activity of NK cells and the synthesis of IFN in humans and experimental animals. Recently it was indicated that Al 1 do not exert any toxic effect on central nervous system in rats after its intracerebroventricularly injection (*icv*) in some behavioral tests: the open field test, the hole test, the water maze test and the score of irritability. However it was demonstrated, that Al 1 indicates antinociceptive activity and this effect is mediated by opioid receptors.

The aim of present study was to determine effect of Al 1 on releasing some cytokines: IL-2, TNF-alpha and IFN-gamma, and releasing VEGF in rats.

7 days before the beginning of the experiment female Wistar rats were anaesthetized with ketamine and xylazine and polyethylene cannulas were implanted into the right lateral brain ventricle (*icv*). On the day of the experiment Al 1 at doses: 5, 25, 50 and 100 nmols were dissolved in a saline in the constant volume of 5 µl and it was injected directly *icv* through implanted cannulas. Rats were divided into 2 groups. Rats from the first group were anaesthetized with

ketamine after 120 min from the beginning of the experiment and the blood was taken by heart puncture. Rats from the second group were subjected to the same procedure after 24 h from Al 1 injection. Plasma levels of IL-2, TNF-alpha, IFN-gamma and VEGF were determined in blood plasma by ELISA method.

Al 1 significantly decreased level of TNF alpha and VEGF in rats' plasma and insignificantly decreased IFN gamma level. Al 1 increased level of IL-2 in rats' plasma.

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