# ORAL PRESENTATIONS

## IMMUNOENDOCRINE EFFECTS OF NEUROACTIVE STEROIDS: *IN VITRO* STUDY

*Agnieszka Basta-Kaim, Bogusława Budziszewska, Lucylla Jaworska-Feil, Marta Kubera, Magdalena Tetich, Monika Leśkiewicz, Barbara Korzeniak, Władysław Lasoń*

Department of Endocrinology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Neuroactive steroids are precursors or metabolites of steroid hormones, which act mainly through modulation of $GABA_A$ and NMDA receptors. In contrast to numerous data on neuroactive steroid action in the brain, little is known about their interaction with peripheral immune and endocrine systems.

In the present study, we found that the most potent steroid $GABA_A$ receptor agonist – allopregnanolone (0.1–1 μM) enhanced the proliferative and metabolic activity of mouse splenocytes, whereas at high supraphysiological concentrations (30 and 100 μM), it had an opposite effect. On the other hand, 5β-pregnan-3α-ol-20-one (a weak $GABA_A$ agonist and NMDA receptor antagonist) showed only inhibitory effect on the ability of splenocytes to proliferate.

Since glucocorticoids are known immunosuppressants, in the next part of this study we evaluated effects of the neuroactive steroids on the GR receptor function. Allopregnanolone and, to a lesser extent, its 5β-isomer inhibited the GR-mediated gene transcription in LMCAT cells.

These data indicate that, besides their central action, neuroactive steroids may play an important role in regulation of peripheral immune system and glucocorticoid receptor function.

## MECHANISM OF ANXIOLYTIC ACTIVITY OF MELATONIN AND AGOMELATINE IN TWO ANIMAL MODELS

*Ewa Bień, Piotr Gruca, Elżbieta Moryl, Mariusz Papp*

Behavioral Pharmacology Laboratory, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

In previous studies, we found that melatonin as well as agomelatine, a mixed agonist of mt1/MT2 melatonin and antagonist of $5\text{-}HT_{2C}$ receptors, showed potent activity in two animal models of

anxiety (elevated plus-maze and Vogel conflict tests) and in the chronic mild stress paradigm (a well-validated animal model of depression). Both these actions clearly depended on the time of administration. Thus, melatonin was active only when administered in the evening, while agomelatine was effective also after morning administrations, i.e. when the compound is devoid of chronobiotic activity.

In the present study, we investigated the involvement of melatonin and 5-$HT_2$ receptors in the mechanism of anxiolytic action of melatonin and agomelatine. In elevated plus-maze test, melatonin receptor antagonist S22153 (20 mg/kg) blocked the effect of melatonin (50 mg/kg), insignificantly attenuated action of evening agomelatine (50 mg/kg), and was without any effect on morning agomelatine. In Vogel test, S22153 potentiated the effects of the two compounds administered both in the morning and in the evening. SB 206553 (3 mg/kg), a 5-$HT_{2C/2B}$ receptor antagonist, enhanced the effects of morning and evening administration of melatonin as well in elevated plus-maze test as in the Vogel test. In consequence, the magnitude of action of joint administration of melatonin and SB 206553 was comparable to that of agomelatine. These results indicate that both melatonin and 5-$HT_{2C}$ receptors are involved in the mechanism of agomelatine activity in these animal models of anxiety. It is also hypothesized that the existence of serotonergic component deprives agomelatine of chronobiotic dependence, which may have beneficial implication for its putative use in the therapy of anxiety/depression disorders.

# EFFECTS OF CHRONIC PRETREATMENT WITH GINKGO BILOBA EXTRACT (EGb761) ON LEARNING, SPATIAL MEMORY AND MOTOR ACTIVITY IN OLD MALE RATS

*Kamilla Blecharz-Klin, Agnieszka Piechal, Ilona Joniec, Ewa Widy-Tyszkiewicz*

Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Krakowskie Przedmieście 26/28, PL 00-927 Warszawa, Poland

The medicinal use of *Ginkgo biloba* have been known for thousands years. At present, medical professionals use it to aid in the treatment of cognitive deficiency associated with aging and in circulatory disorders.

Effect of administration the rigorously standardized extract of Ginkgo biloba leaves (EGb 761) on learning, memory, exploratory behavior and motor activity was estimated in Water Maze (Exp. 1) and hole board (Exp. 2) tests. Eighteen months old Wistar rats (580–600 g) received for three months solution of EGb 761 in drinking water at three doses: 50, 100 and 150 mg/kg per day.

Exp. 1. Water Maze paradigm was carried out for 4 days by training rats using 4 training trials per day. Memory impairment was assessed by a trial conducted 24 h after the last training. Motor activity and motivation were analyzed in the experiment with visible platform task. In the probe trial on day 5, the EGb150 group showed improvement of memory of the position of the platform compared to the control. The mean escape latency in Water Maze with visible platform for EGb 761-pretreated groups was significantly decreased.

Exp. 2. Exploratory motor activity was significantly increased in the EGb-treated groups in the hole board task.

HPLC study showed significant differences in monoamine and metabolite levels in the prefrontal cortex, hippocampus and striatum between the groups pretreated with EGb and the control group.

These findings suggest improvement of spatial memory, cognitive performance and exploratory behavior in the groups pretreated with EGb.

# REPEATED IMIPRAMINE OR CITALOPRAM DECREASE GLUTAMATERGIC SYNAPTIC TRANSMISSION IN THE RAT FRONTAL CORTEX

*Bartosz Bobula, Krzysztof Tokarski, Grzegorz Hess*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

The effects of repeated administration of a tricyclic antidepressant, imipramine, and a selective serotonin reuptake blocker, citalopram, for 14 days (10 mg/kg *po*, twice daily), were studied *ex vivo* in the rat frontal cortex slices prepared 48 h after the last dose of the drug. Treatment with either of the two antidepressants resulted in a reduction of the amplitude of field potentials evoked in layer II/III by electrical stimuli applied to cortical layer V over a wide range of stimulation intensities. The relationship between amplitudes of pharmacologically isolated NMDA and AMPA/kainate receptor-dependent components of the field potential was decreased. These results indicate that repetitive treatment with imipramine or citalopram results in a weakening of glutamatergic synaptic transmission in the cerebral cortex.

# REDUCTION OF INTERLEUKIN (IL)-2 CONCENTRATION IN PATIENTS WITH HYPERCHOLESTEROLEMIA TREATED WITH SIMVASTATIN

*Józef Cecha[1], Janusz Szkodziński[2], Wojciech Romanowski[1], Małgorzata Muc-Wierzgoń[1], Aleksander Danikiewicz[1], Manfred Żelawski[1], Anna Piętka-Rzycka[1], Barbara Zubelewicz-Szkodzińska[1]*

[1]Department of Internal Medicine, Silesian Medical University Żeromskiego 7, PL 41-902 Bytom, Poland;
[2]Silesian Centre of Cardiology, Szpitalna 2, PL 41-800 Zabrze, Poland

**Background**

Statins, 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitors, are a class of drugs with a potent lipid-lowering effect that have been shown to reduce LDL-cholesterol. The pharmacological effects of statins is far beyond the mere reduction of LDL-cholesterol. They are able to inhibit proliferation of smooth muscle cells, macrophages and T-lymphocytes, to restore the endothelial activity and to inhibit the inflammatory responses to macrophages. These effects have been called "pleiotropic effect" of statins. These metabolic activities of statins play an important role in counteracting the inflammatory elements of the atherosclerotic plaque.

Statins have demonstrated to prevent the inflammatory activity of macrophages. That is why we designed, in our study, to assess the influence of simvastatin on serum concentration of IL-2 in hypercholesterolemic patients.

**Methods**

In 123 asymptomatic men with total cholesterol (TC) > 6.5mmol/l (*group-1*) and in 40 with borderline-high cholesterol level (5.2–6.5 mmol/l) (*group-2*), serum IL-2 was determined before and after first 3 month of diet, then, after a 3-month simvastatin therapy (20 mg daily) in those who did not respond diet.

**Results**

There were no reduction of IL-2 observed in *group 1*, after 3 months of diet. After 3 months of simvastatin treatment IL-2 concentration was decreased ($p < 0.005$).

In conclusion, in men with hypercholesterolemia simvastatin treatment lowers IL-2 concentration presenting anti-inflammatory properties.

# AGONIST AND ANTAGONIST BINDING SITES AT 5-HT$_{1A}$ RECEPTOR

*Zdzisław Chilmonczyk[1,3], Anna A. Strzelczyk[1], Małgorzata Jarończyk[1], Aleksander P. Mazurek[1], Ewa Chojnacka-Wójcik[4], Ingebrigt Sylte[2]*

[1]National Institute of Public Health, Chełmska 30/34 PL 00-725 Warszawa, Poland; [2]Department of Pharmacology, Institute of Medical Biology, University of Tromsø, N-9035 Tromsø, Norway; [3]Institute of Chemistry, Białystok University, Pilsudskiego 11/4, PL 15-443 Białystok, Poland; [4]Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

In the CNS, the 5-HT$_{1A}$ receptors exist in two different populations which exhibit different behavioral and physiological effects. 1) Somatodendritic autoreceptors located presynaptically on 5-HT-containing neurons. 2) Receptors located postsynaptically on 5-HT-containing neurons. Clinical studies have shown that partial agonists of 5-HT$_{1A}$ receptor have anxiolytic properties, while antagonists of presynaptical autoreceptors shorten the onset of action of selective 5-HT re-uptake inhibitors (SSRIs). Pharmacophore identification suggests that agonists and antagonists may occupy different binding sites or bind to different receptor states [2]. In the present study, a three dimensional model of the 5-HT$_{1A}$ receptor was used to study the molecular interactions of buspirone analogous with different intrinsic activity at pre- and postsynaptic 5 HT$_{1A}$ receptors. The predicted ligand-receptor interactions indicated similarities in their receptor binding modes, with no clear relationship between receptor contact residues and intrinsic activity [1]. Comparative molecular dynamics simulations for 650 ps indicated substantial differences in the behavior of pre- and postsynaptic agonists and antagonists upon binding.

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# COCAINE ENHANCES EXPRESSION OF ΔFosB AND FosB-LIKE PROTEINS IN THE RAT PARAVENTRICULAR NUCLEUS OF HYPOTHALAMUS

*Agnieszka Chocyk, Anna Czyrak, Marzena Maćkowiak, Katarzyna Fijał, Krzysztof Wędzony*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

FosB and ΔFosB belong to the Fos family of transcription factors encoded by immediate early genes. They are derived from fosB gene by the process of alternative splicing and can be produced rapidly and transiently in specific brain regions in response to many types of acute treatments and environmental changes. Moreover, some isoforms of ΔFosB are known to accumulate in the brain after many different chronic perturbations, including chronic cocaine treatment. These ΔFosB isoforms are very stable and can persist in the brain for a relatively long time. They are considered as molecular regulators of long-term neural and behavioral plasticity. Recently, there have been many data showing that cocaine regulates the expression of ΔFosB in the rat striatum and nucleus accumbens. Above results and those performed on transgenic mice (lacking or overexpressing fosB) strongly implicate fosB gene products as important determinants of cocaine abuse. Since the effects of cocaine

are not limited to the striatum and nucleus accumbens, we decided to extend above findings to other brain regions, such as paraventricular nucleus of hypothalamus (PVN). It is well known that cocaine can stimulate hypothalamic-pituitary-adrenal (HPA) axis by activation of CRH neurons in PVN and these alterations in HPA axis are involved in many aspects of drug addiction. We investigated FosB and ΔFosB expression in the PVN after acute and chronic cocaine treatments, in order to find out whether this psychostimulant can produce long-lasting changes in the activity of the PVN neurons. In our study, we applied immunohistochemical technique with antibody recognizing both FosB and ΔFosB isoforms. Cocaine (25 mg/kg *ip*) given acutely induced robust expression of FosB/ΔFosB proteins mainly in parvocellular part of the PVN. The effect was the most pronounced after 2 h and persisted, at lower but still significant level, even 72 h after cocaine injection. Chronic cocaine treatment (25 mg/kg, 5 days) induced very strong expression of FosB/ΔFosB immunoreactivity in the PVN, measured 18 h after the final injection. Pretreatment with dopamine D1 receptor antagonist SCH 23390 (1 mg/kg *sc*) attenuated cocaine-induced FosB/ΔFosB expression. Further experiments using Western blot analysis will help to identify the isoforms of ΔFosB present in the pool of FosB-like proteins obtained in our study. The results of the present study indicate that cocaine treatment can produce long-term changes in activity of the PVN neurons, and dopamine D1 receptors are involved in above effects. Finally, long-lasting expression of ΔFosB in the PVN may underlie some changes in activity of HPA axis present in cocaine abusers.

# REDUCTION OF ALCOHOL DRINKING BY ORAL NALTREXONE IN ETHANOL-PREFERRING WHP RATS

*Wanda Dyr, Wojciech Kostowski*

Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Sobieskiego 9, PL 02-957 Warszawa, Poland

The opioid system has been implicated in the reinforcing effect of ethanol (EtOH) self-administration. Morphine, an opioid agonist, is able to increase the EtOH consumption, while opioid antagonists, such as naloxone and naltrexone, decrease the EtOH intake in both animals and humans.

Rats selected for the phenotype of high EtOH preference obtained in our laboratory (WHP Warsaw High Preferring) were treated orally with 1.0, 2.5 and 5.0 mg/kg of naltrexone (NX) or water before 15 min of access to 10% ETOH daily for 4 days. In this study, the test of limited access to ETOH (4 h daily of ETOH intake) was used. This test measures the 10% ETOH intake at 30, 60, 120, 180 and 240 min of drinking period. Compounds were administered 15 min before the 4 h ETOH access period.

Ours results have shown that NX produced dose-dependent suppression of EtOH intake with maximal effect of 5.0 mg/kg dose at 240 min of drinking period on the third and fourth day of treatment. These results are with agreement with literature data showing that NX suppressed alcohol intake [1, 2, 3]. Ours results support the hypothesis that opioid receptors mediate reinforcing effect of alcohol and show that selected WHP line of Wistar rats may serve to investigate the effect of drugs on excessive EtOH intake.

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# CLINICAL EFFECTS OF JOINT ADMINISTRATION OF IMIPRAMINE AND AMANTADINE IN PATIENTS WITH DRUG-RESISTANT UNIPOLAR DEPRESSION

*Marta Dziedzicka-Wasylewska[1], Zofia Rogóż[1], Joanna Solich[1], Władysława Daniel[2], Jacek Wójcikowski[2], Dominika Dudek[3], Andrzej Wróbel[3], Andrzej Zięba[3]*

[1]Department of Pharmacology, [2]Department of Pharmacokinetics and Drug Metabolism, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland; [3]Department of Psychiatry, Collegium Medicum, Jagiellonian University, Kopernika 21a, PL 31-501 Kraków, Poland

Usually ca. 30% of patients diagnosed as suffering from unipolar depression do not respond to conventional therapy, therefore, a wide variety of combined treatments have been applied, yet with no apparent therapeutic success.

In the present study, we approached this problem by treating the carefully selected drug-resistant group of patients with imipramine (IMI), an antidepressant drug widely used in clinical practice with amantadine (AMA), an uncompetitive NMDA receptor antagonist, already admitted to clinic practice for other reasons (e.g. treatment of Parkinson's disease). Patients were recruited on the basis of history of their illness and therapy. The mean duration of the illness was 15.6 ± 2.5 years, with a number of depressive episodes averaging 8.8 ± 1.0. At the beginning of the present experiment, after 2 weeks of washout period, the patients (3 men and 9 women, aged 33–52 years) were treated with IMI (Imipramin, Polfa Stargard Szczeciński, Poland, twice daily, 100–150 mg/day) for 2–3 and 4–6 weeks, and then AMA (Amantix, Merz Pharmaceuticals, Germany, twice daily, 150 mg/day) was introduced and administered for further 4 and 6 weeks. Thereafter, AMA was withdrawn, and the patients were treated with IMI alone for additional 2 weeks. The plasma level of IMI was 150–300 ng/ml, and did not change during joint treatment with AMA, indicating the lack of pharmacokinetic interaction. At each (six) time points, the patients were rated with the various depression rating scales in order to estimate their clinical improvement, which has been significantly positive and long-lasting. The obtained results are as follow: according to Hamilton Depression Rating Scale (HDRS) the drop was from 32.2 ± 1.2 to 12.2 ± 1.3, and using Beck Depression Inventary (BECK), the drop was from 48.3 ± 2.3 to 18.6 ± 1.6 points has been observed; Automatic Thoughts Quastionaire (ATQ) has shown a decrease from 132.3 ± 3.5 to 86.3 ± 5.5; Hopelessness Scale (HS) – 18.2 ± 0.4 to 9.5 ± 0.9 points. On the other hand, the Rosenberg Self Esteem Scale (RS) has shown an increase from 15.1 ± 1.1 to 45.0 ± 4.9 points.

Additionally we simultaneously measured the specific binding of [$^3$H]7-OH-DPAT to dopamine $D_3$ receptors on peripheral blood lymphocytes of these patients. Four to six weeks of treatment with IMI has increased the binding of [$^3$H]7-OH-DPAT, however, with no statistical significance. Joint treatment with IMI and AMA for additional six weeks increased the apparent density of dopamine $D_3$ receptors on the lymphocytes of depressed patients, which correlated well with their clinical improvement.

In the light of the above data, it seems justified to postulate that joint administration of IMI with AMA may be successful in the treatment-resistant unipolar depression. However, the question remains whether the binding of [$^3$H]7-OH-DPAT to the peripheral blood lymphocytes might be of diagnostic value in checking the clinical improvement of the patients with unipolar depression.

# ANTIDEPRESSANT EFFECT ON ADENOSINE RELEASE IN THE RAT PREFRONTAL CORTEX

*Anna Dziubina, Krystyna Gołembiowska*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Purine nucleoside adenosine released from cells in response to various stimuli modulates action of other neurotransmitters in the CNS. Our previous data indicate adenosine involvement in the effect of antidepressant drugs on excitatory neurotransmission.

The aim of this study was to investigate the effect of some antidepressant drugs on adenosine release in the rat prefrontal cortex using *in vivo* microdialysis and HPLC with fluorescence detection. Application of $Na^+$ channel activator veratridine (10 μM) or activation of ionotropic amino acid receptors with NMDA (15–50 μM) or kainate (10 μM) into perfusing medium produced significant increase in adenosine release. Amitriptyline (100 μM) perfused through microdialysis probe did not affect spontaneous extracellular adenosine level. However, amitriptyline completely inhibited veratridine- and NMDA-evoked adenosine release and did not change kainate-induced adenosine release.

The obtained results suggest that effect of antidepressants on adenosine release seems to be exerted *via* blockade of ionic channels and is not likely to be receptor mediated. It should be taken into consideration that adenosine level may be affected differently by antidepressant drugs depending on changes in ionic gradient in the synaptic cleft.

# EFFECT OF ADENOSINE $A_{2A}$ RECEPTOR ANTAGONIST ON L-DOPA-DERIVED DOPAMINE RELEASE IN RAT STRIATUM

*Krystyna Gołembiowska, Anna Dziubina*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Adenosine $A_{2A}$ receptor antagonists have attracted attention as potential antiparkinsonian drugs. Selective $A_{2A}$ receptor antagonists can reduce the activity in the striatopallidal pathway and counteract motor inhibition. However, it is not known, whether blockade of adenosine $A_{2A}$ receptors influences the activity of nigrostriatal neurons and has an effect on the ability of these neurons to convert L-DOPA into dopamine (DA).

In the present study, we have examined effect of new selective adenosine $A_{2A}$ receptor antagonist 8-(3-chlorostyryl)caffeine (CSC) on L-DOPA-induced DA release in the rat striatum using *in vivo* microdialysis and HPLC with electrochemical detection. Administration of L-DOPA (100 mg/kg *ip*) together with peripheral DOPA-decarboxylase inhibitor benserazide (50 mg/kg *ip*) produced a 6 h increase in extracellular DA level. CSC (5 mg/kg *ip*) given 30 min before L-DOPA administration potently enhanced L-DOPA-derived DA release as well as the concentration of L-DOPA in extracellular space, but did not affect DOPAC and HVA level.

The obtained results indicate that blockade of striatal adenosine $A_{2A}$ receptors increases the L-DOPA-derived DA release. In addition, CSC seems to increase accessibility of L-DOPA in the CNS, and by MAO inhibition, it may disturb utilization of DA. Selective adenosine $A_{2A}$ receptor antagonists may enhance the therapeutic efficacy of L-DOPA applied exogenously.

# EFFECTS OF CHRONIC STRESS ON THE DEVELOPMENT OF DRUG DEPENDENCE IN RATS

*Piotr Gruca, Ewa Bień, Sławomir Mrowiec, Mariusz Papp*

Behavioral Pharmacology Laboratory, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

A growing number of data suggest that stress is a major factor, which increases vulnerability to drug abuse, dependence and relapse. Therefore, the present study was designed to evaluate effects of chronic mild stress (CMS) procedure on the development of motivational and physical aspects of dependence in rats.

The animals were subjected to a variety of mild stressors (e.g. cage tilting, group housing, food or water deprivation, cage wetting) for a period of five weeks. A second group of animals, the non-stressed controls, were housed in separate rooms and had no contact with the stressed animals. After initial four weeks of stress, both groups (i.e. stressed and control animals) were administered twice daily with morphine (10 mg/kg), nicotine (2.5 mg/kg) and diazepam (10 mg/kg) for 5 days and 24 h later the motivational symptoms of withdrawal precipitated by low doses of naloxone (0.1 mg/kg), mecamylamine (1 mg/kg) and flumazenil (7 mg/kg), respectively, were studied using conditioned place aversion (CPA) paradigm. After completion of this assay, the drug administration was continued for further 5 days and physical signs of withdrawal precipitated by naloxone (1 mg/kg), mecamylamine (2 mg/kg) and flumazenil (15 mg/kg) in stressed and non-stressed rats were assessed. The aversive effects of withdrawal from morphine and nicotine precipitated by respective antagonists were observed in stressed but not in control animals. Furthermore, in stressed animals physical signs of withdrawal from morphine, nicotine and diazepam were more severe and robust than in the non-stressed controls. Taken together, these findings suggest that the CMS procedure can be successfully used in further studies on the relationship between stress and various aspects of drug addiction.

# REPEATED TREATMENT WITH IMIPRAMINE INDUCES SUBSENSITIVITY TO THE EFFECT OF 5-HT$_7$ RECEPTOR ACTIVATION IN THE RAT HIPPOCAMPUS

*Małgorzata Grzegorzewska, Bartosz Bobula, Krzysztof Tokarski, Grzegorz Hess*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Previous work, using recording of stimulation-evoked field potentials, has demonstrated that repetitive administration of imipramine, a tricyclic antidepressant, results in an enhancement of the inhibitory effects of postsynaptic 5-HT$_{1A}$ receptor activation and in a subsensitivity to the activation of postsynaptic 5-HT$_4$ receptor in the CA1 area of rat hippocampus. We have now investigated the influence of treatment with imipramine (14 days, 10 mg/kg *po*, twice daily) on the effects of the activation of 5-HT$_7$ receptor in rat hippocampal slices *in vitro*. Spontaneous epileptiform activity, which occurs under low $Mg^{2+}$ incubation conditions was recorded. The application of 5-carboxytryptamine (5-CT, 0.01–1 μM) in the presence of WAY 100635 (1 μM) resulted in a dose-dependent increase in bursting frequency and 0.025 μM 5-CT increased the frequency of discharges in control preparations by 46 ± 5% (n = 23). The effect of 5-CT was significantly weaker in slices prepared from imipramine-treated animals (increase by 28 ± 4%, $p < 0.01$, *t*-test, n = 21). This result indicates that adaptive changes induced by imipramine involve a reduction of the function of 5-HT$_7$ receptor. The treatment with imipramine results in a stronger inhibitory action of 5-HT on the excitability of hippocampal neurons.

# EFFECT OF ANTIDEPRESSANT DRUGS ON CYTOCHROME P-450 2D (CYP2D) AND 3A (CYP3A) IN RATS

*Anna Haduch, Jacek Wójcikowski, Władysława Daniel*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland

The influence of tricyclic antidepressant drugs (TADs), selective serotonine reuptake inhibitors (SSRIs) and novel drugs (mirtazapine, nefazodone) on the activity of cytochrome P-450 was studied *in vitro* in the following models: 1) estimation of cytochrome P-450 activity in control liver microsomes in the absence and presence of antidepressants added *in vitro*; 2) estimation of cytochrome P-450 activity in liver microsomes of rats treated for 1 day with antidepressants; 3) estimation of the cytochrome P-450 activity in liver microsomes of rats treated with antidepressants for two weeks.

The activity of CYP3A was assesed by measuring the rate of testosterone hydroxylation in two positions: 2β and 6β. The activity of CYP2D was estimated by measuring the rate of ethylmorphine O-deethylation. The amount of the metabolites formed *in vitro* was assayed using the HPLC method. The hepatic level of cytochrome P-450 proteins was determined by Western blotting using specific polyclonal antibodies.

The investigated antidepressants added to liver microsomes *in vitro* showed a moderate or weak inhibitory effect on the activity of CYP2D or CYP3A. One-day exposure to TADs usually decreased the activities of these isoenzymes, while SSRIs reduced only the activity of CYP2D. After chronic treatment with TADs, the decreased activities of CYP2D and CYP3A were still observed; moreover, a reduction in CYP2D activity by SSRIs was also still visible. Additionally, chronic mirtazapine elevated and nefazodone lowered the activity of CYP2D, while sertraline enhanced that of CYP3A. The level of CYP3A protein was increased by chronic treatment with TADs and sertraline. The obtained results indicate complex interactions of antidepressants with cytochrome P-450 proceeding *via* different mechanisms.

# CHANGES IN REACTIVITY OF NOREPINEPHRINE RECEPTORS ON VISUAL TRACT IN RATS AFTER PRENATAL EXPOSURE TO HEAVY METALS

*Ewa Herba[1], Agata R. Plech[1], Katarzyna Makowiecka-Obidzińska[1], Dorota Pojda-Wilczek[1], Stefan M. Pojda[1], Andrzej Plech[2]*

[1]Department of Ophthalmology, Medical University of Silesia, Żeromskiego 7, PL 41-902 Bytom, Poland;
[2]Department of Pharmacology, Medical University of Silesia, H. Jordana 38, PL 41-808 Zabrze, Poland

**Purpose**

Some metals are known to be neurotoxic, because of their deleterious effects on the nervous system. We examined influence of some neurotoxic metals, such manganese (Mn), mercury (Hg), lead (Pb), cadmium (Cd), cooper (Cu) on visual system's function by measuring disturbance of visual transmission (Flash Visual Evoked Potentials FVEP). In this study, we measured changes in FVEP after intracerebroventricular (*icv*) injections of norepinephrine (NE) in rats after prenatal heavy metals intoxication.

**Method**

The experiments were performed on 30 female adult Wistar rats. The rats were stereotaxically implanted with polyethylene cannulas into the lateral

ventricle (*icv*) and electrodes: active under the skull on dura mater in occipital region of the brain and reference one on the skull in the interorbital space. After next 5–7 days, the FVEP was recorded by the1000 LKC (USA) electrophysiological system, with standard stimulation of both mydriatic eyes (by 1% tropicamide and 1% atropine). The animals were divided into 6 groups (5 rats in each): control, Cd-, Pb-, Hg-, Cu- and Mn-treated groups. The amplitudes and the latencies of negative peak N1 and following it positive $P_1$ were analyzed before and after *icv* injections of NE.

**Results**

Intoxication with heavy metals caused prolonged latencies and diminished amplitudes of FVEP. Reactivity of visual system after NE *icv* injection was greater in all metal treated groups than in control one.

**Conclusion**

Prenatal exposure to heavy metals enhanced susceptibility of FVEP to NE in rats.

# LACK OF EFFECTS OF ANGIOTENSIN IV ON THE ACQUISITION OF A WATER MAZE TASK AND ON THE FUNCTION OF RYANODINE CHANNEL IN THE RAT HIPPOCAMPAL ENDOPLASMIC RETICULUM

*Adam Holownia, Jan J. Braszko*

Department of Clinical Pharmacology, Medical University of Białystok, Waszyngtona 15A, PL 15-274 Białystok, Poland

We investigated the effect of angiotensin IV on the acquisition of spatial task by rats and on $Ca^{2+}$ transport in microsomal membranes isolated from the rat hippocampus, the brain structure essential for spatial memory.

Wistar rats injected intracerebroventricularly with 1 nmol of angiotensin IV or saline were subjected to the water maze training using hidden (learning) or visible (nonlearning) escape platform. Rats showed overall good acquisition of the task and mean escape latency decreased from 55 s to less than 10 s during 5-days training. Learning significantly increased [$^3$H]ryanodine binding to microsomal ryanodine receptors and markedly increased receptor affinity for the ligand and decreased microsomal $Ca^{2+}$ uptake.

Angiotensin IV was without effect on the rate of acquisition of the spatial task but increased (by 47%) maximal ryanodine binding in the hippocampal microsomes of the learning rats. The peptide, however, did not affect decreased net $Ca^{2+}$ uptake in rats subject to learning procedure. Since microsomal $Ca^{2+}$-ATPase activity was similar in all tested groups, the lower net $Ca^{2+}$ uptake in learning rats could be attributed to elevated expression of ryanodine receptors and resulting increased $Ca^{2+}$ release.

Our results do not support the view about predominant role of angiotensin IV in acquisition of spatial memory, although they cannot exclude the possible modulatory effects of the peptide.

# ANXIOLYTIC-LIKE ACTIVITY OF AIDA (1-AMINOINDAN-1,5-DICARBOXYLIC ACID), AN mGLu1 RECEPTOR ANTAGONIST

*Aleksandra Kłodzińska, Ewa Tatarczyńska, Ewa Chojnacka-Wójcik*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Antagonists of group I metabotropic glutamate receptors (mGluRs) were shown to induce anti-anxiety-like effects in different animal models. In the present study, we examined the effects of 1-aminoindan-1,5-dicarboxylic acid (AIDA), regarded as a selective and competitive mGluR1 antagonist, in animal models of anxiety. Diazepam was used as a reference drug. After intraperitoneal administration, AIDA (0.5–2 mg/kg) produced anxiolytic-like effects in the conflict drinking test and the elevated plus-maze test in rats; however, at doses up to 8 mg/kg, it was inactive in the four-plate test in mice. AIDA (at a dose of 4 mg/kg, but not lower) increased the exploratory locomotor activity of rats measured in the open field test, but it did not disturb rat motor coordination in the rotarod test. The above results indicate that selective mGlu1 receptor antagonists may be useful in the therapy of anxiety at a low risk of side effects characteristic of benzodiazepines.

# EFFECT OF NEW ANTIEPILEPTIC DRUGS ON KYNURENIC ACID SYNTHESIS *IN VITRO*

*Tomasz Kocki, Tomasz Tomczyk, Ewa M. Urbańska, Marian Wielosz, Waldemer A. Turski*

Department of Pharmacology, Medical University of Lublin, Jaczewskiego 8, PL 20-090 Lublin, Poland

Kynurenic acid (KYNA) is an endogenous brain constituent that inhibits the activity of all three ionotropic excitatory amino acid (EAA) receptors. Cerebral synthesis of KYNA from its bioprecursor L-kynurenine is catalyzed by aminotransferases localized preferentially within astrocytes. The possible role of altered KYNA-mediated modulation of EAA receptors in the human neuropathology has been postulated. In particular, the disturbances of KYNA production have been linked to the occurrence of epilepsy, Huntington's disease, Alzheimer's disease, schizophrenia, AIDS-related dementia and others.

In the present study, the influence of new antiepileptic drugs (AEDs): felbamate, vigabatrin and gabapentin on KYNA synthesis in the rat brain cortex was investigated. Kynurenic acid was subjected to HPLC and detected fluorometrically. Felbamate at the concentration of 0.5 and 1 mM significantly increased KYNA production up to 140 and 150% of control values, respectively. Vigabatrin and gabapentin at the concentration of 0.01, 0.1, 0.5, 1 and 3 mM did not influence KYNA production *in vitro*. Our data suggested that some AEDs may modulate KYNA production in the brain tissue.

*Acknowledgment.* Supported by grant No 6 P05A 053 21 from State Committee for Scientific Research, Warszawa, Poland.

# ROLE OF GROUP I AND II METABOTROPIC GLUTAMATE RECEPTORS IN ANTIPARKINSONIAN-LIKE EFFECTS IN RATS

*Jolanta Konieczny, Krystyna Gołembiowska, Stanisław Wolfarth, Krystyna Ossowska*

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

It has been postulated that the dopaminergic deficiency observed in Parkinson's disease (PD) leads to secondary overactivity of the glutamatergic neurotransmission. The abundance of metabotropic glutamate receptors (mGluRs) in the basal ganglia suggests that they may play a role in modulatig the dopaminergic function in the brain. The aim of the present study was to find out whether inhibition of glutamatergic transmission at a level of mGluRs may alleviate parkinsonian-like symptoms in rats. MPEP, a non-competitive antagonist of mGluR5, and LY354740, a selective agonist of group II mGluRs, decreased both the haloperidol-induced muscle rigidity and catalepsy. Intrastriatal injection of AIDA, a competitive antagonist of mGluR1, also reduced the haloperidol effects, while intrastriatal injection of APDC, a selective agonist of group II mGluRs, was ineffective. Methamphetamine (MTH) is a potent neurotoxin. This compound produces a massive release of dopamine (DA) and glutamate (GLU), which finally leads to degeneration of striatal DA terminals. Hence, the antagonistic effect of mGluR ligands on excitotoxicity may be a potential neuroprotective therapy in PD. We found that MPEP injection diminished the MTH-stimulated DA release in the striatum and reversed the MTH-induced degeneration of striatal DA terminals. The obtained results suggest that blockade of group I mGluRs, or stimulation of group II mGluRs may be effective in reducing parkinsonian-like symptoms in rats. However, in the striatum, only group I mGluRs seem to be involved in this effect. Moreover, the blockade of mGluR5 receptors may protect DA neurons against the MTH-induced toxicity.

# SOME BIOCHEMICAL EFFECTS OF IMIPRAMINE ARE TIME-, RATHER THAN DOSAGE-, DEPENDENT

*Maciej Kuśmider, Zofia Rogóż, Joanna Siwanowicz, Marta Dziedzicka-Wasylewska*

Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Imipramine (IMI) is an antidepressant drug widely used in clinical practice. Since it is therapeutically active after a few weeks of administration, therefore, the animal studies designed to define the changes, which might be important for the mechanism of its action entail drug administration for at least two weeks. In the present studies, we treated rats with IMI (10 mg/kg *po*) repeatedly (twice daily for 14 days), and also acutely (single dose of IMI). After the treatment, the rat brains were quickly removed and frozen, until receptor autoradiography and *in situ* hybridization have been performed. The animals receiving acute dose of IMI were sacrificed 2, 72 h, or 14 days after the treatment.

The repeated treatment with IMI resulted in the increase in the binding of [$^3$H]7-OH-DPAT in the shell part of nucleus accumbens septi and in the islands of Calleja. In the latter brain region, the increase in the apparent density of dopamine $D_3$ receptors was observed also 72 h or 14 days after single administration of IMI.

No statistically significant effects were observed in the binding of [$^3$H]ketanserin to serotonin 5-$HT_{2A}$ receptors in the rat brain cortex, no matter which

lamina, or what kind of IMI treatment (acute or repeated) was examined.

We also measured the amount of mRNA coding for dopamine $D_2$ autoreceptors ($D_2$ mRNA) in the rat mesencephalon, following acute or repeated administration of IMI. No significant changes in the amount of mRNA were observed in the substantia nigra. In the ventral tegmental area repeated treatment with IMI increased the amount of $D_2$ mRNA in the lateral part of this brain region. The increase in the amount of $D_2$ mRNA started to be significant at 72 h after acute IMI administration. Moreover, this increase was also observed after 14 drug-free days following acute administration of the drug.

An interesting finding of this study is the observation that acute treatment with IMI seems to be sufficient to trigger at least some changes as a function of time regardless of whether it was administered again, providing a possible explanation for the delayed therapeutic effect of the drug.

# EFFECT OF NEONATAL DSP-4 ADMINISTRATION ON BIOGENIC AMINE LEVELS IN THE BRAIN OF ADULT RATS

*Łukasz Labus, Przemysław Nowak, Aleksandra Bortel, Joanna Dąbrowska, Ryszard Brus*

Department of Pharmacology, Medical University of Silesia, H. Jordana 38, PL 41-808 Zabrze, Poland

DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] is a neurotoxin which induces acute and relatively selective degeneration of both central and peripheral noradrenergic nerve terminals in mammals [2]. In adult rats, DSP-4 can, in contrast to 6-hydroxydopamine, pass the blood-brain barrier and produce long-lasting degenerative action on the central noradrenergic neurons [1].

The present study was undertaken to examine and compare effect of DSP-4 administration to adult and newborn rats on the noradrenaline (NA) and other biogenic amine levels in the brain. Two-month-old male Wistar rats were injected with DSP-4 (50 mg/kg *ip*) and seven days later they were decapitated, the brains were immediately excised from the skull and placed on ice. The corpus striatum and hippocampus were separated, placed on dry ice and stored at 70°C till biogenic amine assay.

Three groups of newborn Wistar rats were injected with DSP-4 at 50 mg/kg *ip* once (on the 1st day of life), twice (on the day 1st and 3rd of life) and three times (on the day 1st , 3rd and 5th of life). Control adult and newborn rats were injected with saline. When rats attained age of 8 weeks, they were decapitated and procedure was performed as above. In the corpus striatum and hippocampus NA, 4-hydroxyl-3-methoxyphenyl-glycol (MOPEG), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were assayed by means of HPLC/ED technique [3].

The decreased brain level of NA in DSP-4-treated adult rats was confirmed [1]. The level of NA in the striatum and hippocampus decreased to 78.5 and 5.8% of the control values (127.8 to 100.3 and 533.3 to 31.1 ng/g of wet tissue, respectively). In the rats treated neonataly twice with DSP-4, the significant decrease in NA level in the hippocampus to 1.4% of the control group was noticed (5.6 and 392.1 ng/g of wet tissue, respectively). After single and triple injections of DSP-4 to newborn rats, the level of NA in the hippocampus dropped to 2.8 and 1.9% of the value in the control group (11.0 and 7.3 ng/g of wet tissue, respectively). Two injections of DSP-4 to neonatal rats decreased the MOPEG and 5-HT level in the hippocampus of adult rats to 84.2 and 75.6% of the control value. No changes in DA and other amine levels in the hippocampus and corpus striatum were observed.

The results showed that DSP-4 injected to newborn rats induced long-term depletion of NA content in limbic structures of the adult rat brain. The obtained animal model seems to be useful for study of interaction between other neurotransmitter systems in the brain.

*Acknowledgment.* Supported by Medical University of Silesia (NN-1-002/03).

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# MORPHINE MODULATES HIPPOCAMPAL THETA RHYTHM EVOKED BY CHOLINERGIC STIMULATION OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS

*Emilia Leszkowicz, Anita Nowacka*

Department of Animal Physiology, University of Gdańsk, Kładki 24, PL 80-822 Gdańsk, Poland

Cholinergic neurons of the pedunculopontine tegmental nucleus (PPN) are involved in generation of hippocampal theta rhythm. In the present experiment, we tested whether opioid system of the PPN exerts neuromodulatory influence on theta-relevant cholinergic transmission.

In urethane-anesthetized rats, direct microinjection of a cholinergic agonist, carbachol (5 μg), into the PPN generated theta oscillations in the hippocampal EEG. Simultaneous intra PPN administration of an opioid agonist, morphine (5 μg), prolonged latency and shortened duration of carbachol-elicited theta episodes. Morphine itself did not influence sensory-induced synchronous hippocampal EEG in a range of theta band.

These results suggest that PPN opioids can modulate cholinergically mediated generation of hippocampal theta rhythm.

# INFLUENCE OF ADENOSINE RECEPTOR AGONISTS AND ANTAGONISTS ON BENZODIAZEPINE WITHDRAWAL SYDROME IN MICE

*Joanna Listos, Danuta Malec, Sylwia Fidecka*

Department of Pharmacodynamics, Medical University School, Staszica 4, PL 20-081 Lublin, Poland

Benzodiazepines (BDZ) are used clinically, primarily as sedative-antianxiety drugs. However, chronic treatment with BDZ may produce dependence. There are data suggesting that adenosinergic system participates in morphine and ethanol abstinence signs. The present study aimed to determine

the role of adenosinergic system in temazepam (TZ) withdrawal syndrome, characterized by the seizure susceptibility and reactivity. The influence of adenosine receptor agonists (CPA, CGS 21680 and NECA) and antagonists (DPCPX, DMPX and caffeine) on the intensification of pentetrazole (PTZ)-induced seizures resulting from the chronic TZ administration was studied in mice.

TZ dependence was evoked by *sc* implantation of one pellet (75 mg) per mouse. Additionally, mice were injected *sc* (two times a day) with TZ during 2 weeks. Following this time, pellet was removed, and 26 h later the experiments were conducted (preceded by *ip* injection of 10 or 5 mg/kg of flumazenil, BDZ receptor antagonist). CPA (0.5 and 1 mg/kg) – selective $A_1$, CGS 21680 (1 and 2 mg/kg) – selective $A_2$, and NECA (0.1 and 0.2 mg/kg) $A_1/A_2$ receptor agonist significantly and dose-dependently decreased the number of mice responding with clonic seizures and protected TZ-dependent mice against tonic seizures and death. On the other hand, the observed withdrawal syndrome was significantly intensified by selective adenosine receptor antagonists: $A_1$ – DPCPX and $A_2$ – DMPX (both at doses of 3 and 6 mg/kg) and also by caffeine (10 and 20 mg/kg) – $A_1/A_2$ receptor antagonist.

The obtained results have shown that adenosine mechanisms may play some role in the TZ withdrawal signs.

# INVOLVEMENT OF HIPPOCAMPAL mGlu1 RECEPTORS IN CONSOLIDATION OF CONTEXTUAL FEAR CONDITIONING: BEHAVIORAL AND IMMUNO-CYTOCHEMICAL STUDY

*Piotr Maciejak[1], Ewa Taracha[1], Małgorzata Lehner[1], Aleksandra Wisłowska[2], Małgorzata Zienowicz[2], Anna Skórzewska[1], Andrzej Bidziński[1], Janusz Szyndler[2], Adam Płaźnik[1,2]*

[1]Department of Neurochemistry, Institute of Psychiatry and Neurology, Sobieskiego 1/9, PL 02-957 Warszawa, Poland;
[2]Department of Pharmacology, Medical University, Krakowskie Przedmieście 26/28, PL 00-927 Warszawa, Poland

The effects of post-training intra-hippocampal injections of group I mGluR agonists and antagonists were examined in the contextual fear test, in rats. It was found that DHPG (a mGluR1-5 agonist) decreased, and AIDA (a mGluR1 antagonist) increased fear conditioning (a freezing reaction), examined 24 h after conditioning session. CHPG (a mGluR5 agonist), and MPEP (a mGluR5 antagonist) did not cause any effect. In the immunocytochemical study, the post-conditioning administration of AIDA decreased the c-FOS induction in the dentate gyrus and CA-1 layer of the hippocampus proper, 2 h after exposure of animals to the aversive context, and 24 h after conditioning session. It is suggested that overactivation of glutamatergic transmission in the structure, critical for memory trace formation and period of time, may result in an attenuation of memory consolidation. The immunocytochemical study and factor analysis of experimental data revealed that hippocampal mGlu1 receptors significantly contribute to memory consolidation in a way dependent on the phase of memory trace processing. Furthermore, they indicate that changes in glutamatergic activity within the brain limbic structures can modify the threshold for the induction of the long-term neuronal plastic alterations, involved in some forms of learning and memory processes.

# INFLUENCE OF ADENOSINE RECEPTOR AGONISTS AND ANTAGONISTS ON KETAMINE-INDUCED MOTOR ACTIVITY IN MICE

*Marcin Mandryk, Sylwia Fidecka, Danuta Malec*

Department of Pharmacodynamics, Medical University School, Staszica 4, PL 20-081 Lublin, Poland

Ketamine, a short-acting dissociative anesthetic agent, produces psychomimetic effects and analgesia. In animals, ketamine evokes hypermotility, rotation and stereotypy, which may be connected with its stimulatory influence on dopamine neurotransmission. Adenosine, neuromodulator in the CNS, seems to play an opposite role to dopaminenergic system in the brain. The aim of our study was to define the influence of adenosine receptor ligands on ketamine-induced locomotor activity in mice. Locomotor activity was measured in actometer cages for 30 min. Ketamine-induced hyperactivity (10 mg/kg) was significantly and dose-dependently attenuated by CGS 21680 and NECA, selective $A_2$ and $A_1/A_2$ adenosine receptor agonists, respectively, but not by CPA, a selective $A_1$ adenosine receptor agonist. Motor activity produced by threshold dose (2.5 mg/kg) of ketamine was significantly increased by DMPX and caffeine, selective $A_2$ and $A_1/A_2$ adenosine receptor antagonists, respectively, but not by DPCPX, a selective $A_1$ adenosine receptor antagonist.

The present results suggest that adenosine system is involved in ketamine-induced motor activity and seem to indicate a predominant role of $A_2$ adenosine receptor in this effect.

# AMPHETAMINE SENSITIZATION OF THE CENTRAL DOPAMINERGIC SYSTEM CONTRIBUTES TO INCREASE IN ANTIOXIDANT DEFENSE: *IN VIVO* STRIATAL MICRODIALYSIS STUDY

*Przemysław Nowak[1], Aleksandra Bortel[1], Joanna Dąbrowska[1], Ryszard Brus[1], Richard M. Kostrzewa[2]*

[1]Department of Pharmacology, Medical University of Silesia, H. Jordana 38, PL 41-808 Zabrze, Poland;
[2]Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA

Alternation in the normal metabolism of dopamine (DA) might lead to elevated concentrations of this neurotransmitter, thereby accelerating its oxidation in the cellular compartments in which its concentration has increased. The oxidative metabolism of DA proceeds *via* both enzymatic and nonenzimatic pathways. The latter is auto-oxidation, which produces DA orto-quinone, superoxide radicals, hydroxyl radicals ($HO^{\bullet}$) and other reactive oxygen species (ROS). DA is also easily metabolized *via* monoamine oxidase to 3,4-dihydroxyphenylacetic acid (DOPAC) and also to $H_2O_2$. Repeated exposure to psychostimulants, such as amphetamine and cocaine, induces behavioral sensitization, which is characterized by an augmented locomotor response to a subsequent psychostymulant challenge in rats. Alteration in the central DA receptors sensitivity, DA synthesis and its release or metabolism may contribute to the augmentation of DA neurotransmission in the brain. Therefore, the major objective of this study was to test the hypothesis that the behavioral sensitization is accompanied by altered basal $HO^{\bullet}$ production and/or different susceptibility to 6-OHDA-induced $HO^{\bullet}$ generation. In our study, we used salicylate-trap methods as the indirect measurements of hydroxyl radical, described in details by Giovanni et al. [1] and Obata and Yamanaka [2].

Adult male Wistar rats were treated for 10 days with amphetamine at 5 mg/kg *ip* followed by 30 days of abstinence. On the test day, the rats were challenged with salicylic acid (100 mg/kg *ip*), and 30 min later they were decapitated. The level of 2,3- and 2,5-dihydroxybenzoic acids (2,3- and 2,5-DHBA) in the striatum was measured by HPLC method. In the second part of experiment, *in vivo* microdialysis of the striatum was performed, and ROS level was estimated in the microdialysate. After taking three baseline samples, the neurotoxin, 6-hydroxydopamine (6-OHDA), was added to the perfusate at a concentration of 1 mM to generate excess of $HO^{\bullet}$ and the 2,3- and 2,5-DHBA were estimated in microdialysates.

The changes in ROS formation under basal (steady-sate) and dynamic conditions (microdialysis) may be caused by altered DA metabolism in amphetamine-sensitized animals.

*Acknowledgment.* Supported by Medical University of Silesia (NN-2-007/03).

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# AMPHETAMINE vs. QUINPIROLE SENSITIZATION OF THE CENTRAL DOPAMINE RECEPTORS: BIOCHEMICAL STUDIES

*Przemysław Nowak, Aleksandra Bortel, Joanna Dąbrowska, Łukasz Labus, Ryszard Brus*

Department of Pharmacology, Medical University of Silesia, H. Jordana 38, PL 41-808 Zabrze, Poland

Behavioral manifestation of sensitization to many indirectly (amphetamine, cocaine) or directly (quinpirole, bromocriptine) acting substances is a progressive and enduring enhancement in the motor stimulant effects elicited by repeated administration of these agents [2]. The present study was undertaken to compare biochemical profile of amphetamine-, and quinpirole-induced sensitization.

Most attempts to identify a neural correlate of behavioral sensitization have focused on the nigrostriatal dopaminergic system. Therefore, the aim of this study was to evaluate striatal levels of dopamine and its metabolites as well as dopamine synthesis rate. Behavioral sensitization was developed by intermittent amphetamine (1 mg/kg *ip*) injections to rats from 22nd to 33rd day of postnatal life, and quinpirole (0.05 mg/kg *ip*) from 1st to 11th day. The measurement of dopamine synthesis rate (turnover) was obtained under either steady-state (resting) condition, or following challenge with amphetamine and quinpirole. Additionally, presynaptic dopamine receptor functions were evaluated using autoreceptor GBL model described by Walters and Roth [3].

The obtained results indicate that, in spite of one common mechanism shared by both types of sensitization, dopamine release to extrasynaptic space is augmented after amphetamine challenge [1], while the dopamine synthesis and utilization differ between both types of sensitization.

*Acknowledgment.* Supported by Medical University of Silesia (NN-1-001/03).

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# *IN VITRO* AND *IN VIVO* ACTION OF SALSOLINOL ON DOPAMINE METABOLISM AND HYDROXYL RADICAL PRODUCTION

*Antoni Patsenka[1,2], Sergej Zimatkin[2], Lucyna Antkiewicz-Michaluk[1]*

[1]Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland;
[2]Grodno State Medical University, Gorkogo 80, 230-015 Grodno, Belarus

1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol, SAL), is an tetrahydroisoquinoline alkaloid, one of the endogenous substance of the mammalian brain. SAL is produced both by the nonenzymatic Pictet-Spengler type condensation of dopamine with acetaldehyde or enzymatically with pyruvic acid followed by decarboxylation and reduction. SAL has been suggested to play a crucial role in progressive neurodegeneration of the nigrostriatal/mesolimbic dopaminergic neurons as well as in the pathogenesis of alcoholism and Parkinson's disease. The aim of this study was to investigate the effects of SAL on the type A and B monoamine oxidase (MAO) with radioactive 5-hydroxytryptamine and β-phenylethylamine as substrates, in isolated mitochondria from different brain structures (striatum, frontal cortex, brain stem), and to examine promotion/scavenging properties of SAL in production of hydroxyl radicals *in vitro* Fenton reaction HPLC with electrochemical detection was used to estimate the hydroxylated products of salicylic acid. In *in vivo* study, we investigated in microdialysis experiments the effect of SAL on dopaminergic system after local infusion into ventral tegmental area (VTA). The concentration of dopamine and its metabolites was investigated in VTA and nucleus accumbens by HPLC. The results obtained from these experiments indicate that SAL inhibits MAO A, but not MAO B activity, possess hydroxyl radical scavenging activity in *in vitro* study, and stimulates somato-dendric dopamine release. Our study may suggest for the first time that SAL may play important physiological role in CNS.

*Acknowledgment.* This study was supported by grant from the Kasa im. J. Mianowskiego and Foundation for Polish Science.

# INFLUENCE OF INTRACEREBROVENTRICULAR INJECTIONS OF NOREPINEPHRINE ON FLASH VISUAL EVOKED POTENTIALS IN RATS AFTER PRENATAL EXPOSURE TO ZINC

*Dorota Pojda-Wilczek[1], Agata R. Plech[1], Ewa Herba[1], Katarzyna Makowiecka-Obidzińska[1], Stefan M. Pojda[1], Ryszard Brus[2]*

[1]Department of Ophthalmology, Medical University of Silesia, Żeromskiego 7, PL 41-902 Bytom, Poland;
[2]Department of Pharmacology, Medical University of Silesia, H. Jordana 38, PL 41-808 Zabrze, Poland

## Purpose

In previous studies, we observed changes in reactivity to various neurotransmitters or neuromodulators in rats prenatally exposed to heavy metals. In this paper, we want to show the effect of prenatal exposure to zinc on flash visual evoked potentials (FVEP) after norepinephrine (NE) injection into the lateral brain ventricle (*icv*).

## Method

Pregnant Wistar rats were allowed to drink water with zinc for entire pregnancies until the parturition. Six rats of their offspring and six control rats (the offspring of rats which drank only tap water during pregnancy) were examined when they became four-month-old. The rats were implanted with active and reference electrodes (fixed on dura ma-

ter and on the skull, respectively) and the caniula was introduced into the right lateral brain ventricle under chloral hydrate anesthesia. FVEP were obtained before and after injections into *icv* of NE at doses of 25 and 50 nmol.

**Results**

There was no difference in mean amplitude and latency of $N_1$ and $P_1$ waves between the groups. After NE injections, latencies became about 10% longer in control group but they were shorter by about 5% in zinc-treated group after both NE doses. The amplitude of $N_1P_1$ increased by about 5% in control group, and by about 10% in the examined group after 50 nmol of NE only.

**Conclusion**

NE injection into the lateral brain ventricle intensifies visual transmission in the rat brain much more in animals prenataly exposured to zinc.

# EFFECT OF COMBINED ADMINISTRATION OF IMIPRAMINE WITH AMANTADINE ON THE CENTRAL $\alpha_1$-ADRENERGIC SYSTEM: BEHAVIORAL AND BIOCHEMICAL STUDIES

*Zofia Rogóż, Agata Faron-Górecka, Joanna Solich, Marta Dziedzicka-Wasylewska*

Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

The problem of treatment-resistant depression has been the subject of extensive studies, yet with no apparent therapeutic success. Our previous studies demonstrated that joint administration of tricyclic antidepressant drug, imipramine (IMI) with the uncompetitive antagonist of NMDA receptors, amantadine (AMA), produced stronger "antidepressive" effects in the forced swimming test than the treatment with either drug given alone. Since it has also been shown that antidepressant drugs administered repeatedly increase the responsiveness of $\alpha_1$-adrenergic receptors, we have designed the present experiments to determine whether combined treatment of IMI (5 and 10 mg/kg) with AMA (10 mg/kg), given separately or jointly, at a single dose or repeatedly (twice daily for 14 days) evokes similar effects.

In the behavioral studies, the responsiveness of $\alpha_1$-adrenergic receptors was estimated in the clonidine-evoked aggression test. IMI (10 but not 5 mg/kg) given repeatedly, significantly enhanced the effect of clonidine, so did AMA (10 mg/kg). Combined administration of these two drugs produced even stronger effect. Enhancement of clonidine-evoked aggressive behavior was also observed when lower dose of IMI (5 mg/kg) was administered jointly with AMA (10 mg/kg).

Binding of [$^3$H]prazosin to $\alpha_1$-adrenergic receptors in the rat cerebral cortex was not altered by the repeated administration of IMI or AMA, as far as the values of $B_{max}$ or $K_d$ are concerned. However, the ability of the $\alpha_1$-adrenergic receptor agonist, phenylephrine, to compete for these sites was significantly increased upon repeated administration of IMI together with AMA, which indicated the enhancement of their affinity for an agonist.

The above results show that repeated administration of IMI together with AMA induces the adaptive changes in the $\alpha_1$-adrenergic receptors, especially it enhances their functional responsiveness. However, the question whether this functional responsiveness is important for the clinical antidepressant efficacy, remains to be elucidated.

# SIDE EFFECTS OF ANTIDEPRESSANT AND NORMOTHYMIC TREATMENT: ASSOCIATIONS WITH THE MOOD, GENDER AND AGE

*Aleksandra Suwalska, Maria Chłopocka-Woźniak, Agnieszka Heymann, Filip Rybakowski, Dorota Łojko, Janusz Rybakowski*

Department of Psychiatry, University of Medical Sciences in Poznań, Szpitalna 27/33, PL 60-572 Poznań, Poland

The aim of study was to evaluate severity of side effects of antidepressant treatment in patients with affective disorders.

**Methods**

Fifty-two patients (14 males, 38 females) with affective disorders (22 bipolar affective disorder, 30 major depressive disorder) aged 50.8 ± 13.4 years were included into the study. Twenty-nine patients were hospitalized, 23 were outpatients. Side effects were assessed using UKU side effects rating scale. Severity of depression [Beck Depression Inventory (BDI)] and quality of life (WHOQOL Bref) were also evaluated.

**Results**

Female patients had more pronounced increased dream activity, akathisia, constipation and reduced sexual desire comparing to male subjects. Male patients had significantly higher level of sleepiness. In depressed patients, significantly higher levels of psychiatric side effects (concentration difficulties, memory problems, depression, inner tension, emotional indifference), neurological side effects (hypokinesia/akinesia, paraesthesias), disturbances of accomodation, palpitations/tachycardia, photosensitization, weight loss and reduced sexual desire were observed. Non-depressed patients had only higher scores in weight gain. Younger patients (aged under 50 years) had significantly worse concentration difficulties, sleepiness/sedation, emotional indifference, hypokinesia/akinesia, orthostatic dizziness, and weight loss than the remaining group. Patients treated with lithium reported less concentration difficulties, emotional indifference, more tremor and less increased salivation than the remaining subjects. Severity of psychiatric side effect was lower in the lithium-treated group. Monotherapy (in 19 patients) was associated only with less tremor than treatment with drug combinations. Quality of life of the patients was influenced by psychiatric, neurological side effects and several other side effects including sexual dysfunctions.

**Conclusions**

Results of our study suggest the importance of age- and gender differences in the side effects of treatment of affective disorders.

# EFFECT OF PENTETRAZOLE-INDUCED KINDLING OF SEIZURES ON *IN VIVO* STRIATAL METABOLISM OF DOPAMINE

*Janusz Szyndler[2], Piotr Maciejak[1], Jerzy Walkowiak[1], Małgorzata Lehner[1], Aleksandra Wisłowska[2], Małgorzata Zienowicz[2], Anna Skórzewska[1], Andrzej Bidziński[1], Adam Płaźnik[1,2]*

[1]Department of Neurochemistry, Institute of Psychiatry and Neurology, Sobieskiego 1/9, PL 02-957 Warszawa, Poland;
[2]Department of Pharmacology, Medical University, Krakowskie Przedmieście 26/28, PL 00-927 Warszawa, Poland

The influence of pentetrazole (PTZ)-induced kindling of seizures on the turnover rate of monoamines in the striatum, was measured *in vivo* in rats. The repeated administration of PTZ (35 mg/kg, *ip*) evoked kindled seizures in rats (stage 4 or 5 of clonic-tonic convulsions – maximum). Simultane-

ously, in these rats there appeared an enhancement of dopamine metabolism (an increase in the HVA/DA ratio). The concentration of serotonin metabolite, 5-HIAA, also increased, indicating stimulation of local serotonin neuron activity, however, the differences in 5-HIAA/5-HT ratio did not reach the significance level. The changes in the metabolism of monoamines occurred independently of a direct seizure activity, i.e. several days after the last episode of chemically induced convulsions. The presented data indicate an important role of changes in the striatal dopaminergic activity in the phenomenon of kindling of seizures.

## EFFECT OF AN ANALOGUE OF 1, 25α-DIHYDROXYVITAMIN $D_3$ (PRI-2191) ON SEIZURE-INDUCED CHANGES IN BDNF GENE EXPRESSION IN THE RAT BRAIN

*Magdalena Tetich[1], Andrzej Kutner[3], Mariusz Skowroński[4], Marta Dziedzicka-Wasylewska[2], Maciej Kuśmider[2], Bogusława Budziszewska[1], Lucylla Jaworska-Feil[1], Agnieszka Basta-Kaim[1], Marta Kubera[1], Władysław Lasoń[1]*

[1]Department of Endocrinology and [2]Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland; [3]Institute of Pharmacy, Rydygiera 8, PL 01-793 Warszawa, Poland; [4]Department of Animal Physiology, University of Warmia and Mazury, Pl. Łódzki 3, PL 10-727 Olsztyn, Poland

Active forms of vitamin $D_3$ are thought to exert neuroprotective effects, at least partly *via* stimulation of NGF, GDNF and NT-3 synthesis, whereas their interaction with the brain-derived neurotrophic factor (BDNF) is largely unknown. We studied effects of a new low-calcemic analog of 1, 25α-dihydroxyvitamin $D_3$ (PRI-2191) on seizure-induced changes in BDNF mRNA levels in the rat brain. *In situ* hybridization study showed that pilocarpine-induced seizures elevated the BDNF mRNA level in the cortex and hippocampus, the most potent effect being observed at 3 h after pilocarpine injection. Treatment of rats with PRI-2191 for 8 days, once a day (0.05 μg/kg) had no effect on BDNF mRNA level in control animals, but attenuated the seizures-induced increase in this parameter. These data suggest that PRI-2191 can interfere with seizure-induced changes in BDNF mRNA levels, however, a possible involvement of those effects in the neuroprotective action of the vitamin $D_3$ analog remains to be elucidated.

## EFFECT OF METABOTROPIC AND IONOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS ON NEUROPEPTIDE Y AND CORTICOLIBERIN SYNTHESIS AND EXPRESSION IN THE AMYGDALA

*Joanna M. Wierońska, Piotr Brański, Agnieszka Pałucha, Bernadetta Szewczyk, Maria Śmiałowska*

Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Neuropeptide Y (NPY), a 36-amino acid peptide, is widely distributed in neurons of many brain structures. In amygdala, NPY is present mainly in interneurons and is engaged in antianxiety effects.

Corticoliberin (CRF) is a 41-amino acid peptide, which is present in a number of nerve terminals and in cell bodies which form a cluster in the central nucleus of the amygdala. Previous studies have indicated that NPY and CRF neurons may be regulated by classical neurotransmitters. The most abundant excitatory neurotransmitter in the brain is glutamate, acting through both iono- and metabotropic receptors (i- and mGluRs). The prominent role of glutamate in the regulation of NPY and CRF synthesis was found in the amygdala. Therefore, in the present study, the effect of NMDA, an anxiogenic compound or MPEP, group 5 metabotropic glutamate receptor antagonist with anxiolytic activity, on NPY and CRF level and synthesis was investigated in the amygdala.

MPEP (10 mg/kg) or NMDA (50 mg/kg) *ip*, 3× every 8 h, were injected into male Wistar rats. Brains were taken 30 min after the last dose, then amygdalas were analyzed for expression of NPY and CRF (using immunohistochemical method) and of NPYmRNA and CRFmRNA (in situ hybridization).

It was found that MPEP very strongly diminished the NPYmRNA expression in the amygdala, to 8% of control level. Peptide level was only slightly decreased (to 81% of the control). At the same time, a slight, but statistically signifficant increase in CRFmRNA was observed. NMDA caused a slight decrease in both NPY and CRFmRNA in the amygdala.

The obtained results indicate that glutamatergic receptors may regulate NPY and in CRF synthesis in neurons of the amygdala.

# DISTRIBUTION OF DRUGS IN WHOLE BLOOD *IN VITRO*: COMPARISON OF CARBAMAZEPINE WITH CLONAZEPAM

*Marek Zieliński, Krystyna Kmieciak-Kołada, Halina Klimas, Zbigniew S. Herman*

Department of Clinical Pharmacology, Medical University of Silesia, Medyków 18, PL 40-752 Katowice, Poland

Among the cellular constituents of blood, the red blood cells (RBCs) represent the largest populations both in number and cell size. Various enzymes in the RBCs can metabolize many drugs contained in whole blood.

It was shown that the RBC concentration of some drugs correlated better with therapeutic effects or dose than plasma concentrations, particularly if ratio of blood-to-plasma concentration Kb/p is larger than 2. Therefore, measurement of RBC concentrations for therapeutic monitoring is recommended for some drugs. The goal of this study was determination of rate and extent of RBC partitioning in whole blood or in the RBCs suspended in plasma or buffer. In *in vitro* procedure: the drug was added to human whole blood and after mixing the samples were incubated at a temperature of 37°C. After 0.5, 1, 2 and 3 h of incubation of the whole blood, we estimated the following parameters.

*Table 1.* Influence of carbamazepine (CBZ) on the concentration of clonazepam after 30 min *in vitro*

| Study | Clonazepam 50 ng/ml | Clonazepam 50 ng/ml + CBZ 10 ug/ml |
|---|---|---|
| Drug concentration in plasma (**Cp**) | 6.64 ± 1.44 | 5.27 ± 1.31 |
| Drug concentration in red blood cells (**Ce**) | 44.47 ± 2.98 | 39.09 ± 6.12 |
| Drug concentration in red blood cells suspended in buffer (**Cebuf**) | 27.01 ± 1.40 | **9.51 ± 2.21** ** $p < 0.01$ |
| Unbound drug concentration in buffer (**Cp,u**) | 27.93 ± 1.56 | 24.24 ± 8.60 |

**Pharmacokinetics calculations:**

**Ke/p,u** – absolute affinity of drugs to the binding sites in the RBCs, **Ke/p** – relative drug affinity to binding sites in the RBCs, **Kb/p** – the whole blood to plasma concentration, **fu –** the fraction of drug unbound in plasma.

Carbamazepine decrease all estimated parameters particularly (**Cebuf**) for clonazepam.

**Conclusion**

Carbamazepine increases metabolism of some drugs not only *in vivo*, but also *in vitro* in whole blood.

# DOES SIMVASTATIN PLAY A ROLE IN INHIBITION OF TUMOR NECROSIS FACTOR (TNF) α SECRETION IN PATIENTS WITH HYPERLIPIDEMIA?

*Barbara Zubelewicz-Szkodzińska[1], Janusz Szkodziński[2], Adam Błażelonis[1], Wojciech Romanowski[1], Małgorzata Muc-Wierzgoń[1], Zuzanna Muryn[1], Manfred Żelawski[1], Grzegorz Drabek[1]*

[1]Department of Internal Medicine, Medical University of Silesia, Żeromskiego 7, PL 41-902 Bytom, Poland;
[2]Silesian Centre of Cardiology, Szpitalna 2, PL 41-800 Zabrze, Poland

**Background**

Beneficial effects of statins 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitors in prevention of cardiovascular events may depend, at least in part, on their anti-inflammatory action, as statins appear to have several biological effects beyond those of lipid metabolism, they influence endothelial function, plaque stability and trombus formation. Moreover, we hypothesise that immunomodulating effects of statins are important for the regulation of proinflammatory cytokines such as: IL-1β, IL-2 (our own, unpublished observation) and IL-6.

**Aim of the study**

We designed our study to check the effect of simvastatin on TNF α secretion.

**Methods**

The study groups were composed of 60 asymptomatic men with total cholesterol (TC) ≥ 6.5 mmol/l, LDL-cholesterol ≥ 3.5 mmol/l (*group-1*) undergoing simvastatin therapy (20 mg daily) and 20 patients who well respond on a 3-month diet (*group-2*). Serum TNF α level was determined at the onset of study, after 3-month diet and (in those who did not respond to diet) after 3-months of simvastatin therapy. TNF α concentration was estimated with ELISA method, using commercially available kits (R&D System).

**Results**

A significant reduction of TNF α concentration was found in *group 1* ($p < 0.05$). Moreover, we have observed significant reduction of TNF α concentration after 3-months of diet ($p < 0.005$).