In search of new orexins functions: effects of orexins on survival of rat astrocytes cultures

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Orexins (orexin A and B) are recently discovered hypothalamic neuropeptides that have been implicated in a variety of behaviors, e.g. arousal and sleep, food-seeking and feeding, reaction to stress. They also control functions of miscellaneous peripheral organs. In addition, proapoptotic activity of orexins has been demonstrated in several cancer cell lines. Orexins exert their numerous actions by interacting with two G protein-coupled receptors: OX1R and OX2R. We have recently demonstrated that orexin A (a nonselective agonist of OX1R and OX2R) stimulates cAMP synthesis in primary cultures of rat astrocytes. The aim of this work was to evaluate expression of orexins receptors in primary astrocyte cultures from rat cerebral cortex and to analyze effects of orexins on their survival. Quantitative RT-PCR analysis revealed that both subtypes of orexin receptors are expressed in rat astrocytes at similar levels, 15236 and 18378 specific RNA copies numbers per ng total RNA for OX1R and OX2R, respectively. Results of the MTT assay revealed that 48 h incubation of astrocytes with orexin A, orexin B and [Ala¹¹,D-Leu¹⁵]orexin B (selective agonists of OX2R) increased their viability. In addition, orexins markedly stimulated proliferation of astrocytes as measured by incorporation of [³H]thymidine. We concluded that orexins, acting at their receptors, increase survival of cultured primary astrocytes from rat cerebral cortex.

Acknowledgment:
Supported by the grant No. PO1.0101/36 from the Ministry of Science and Higher Education, Wroclaw, Poland.

Imipramine and fluoxetine influence phenotype of microglial cells in the rat primary mixed glial cell cultures

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Experimental studies indicate that glial cells in the CNS represent new targets of action of antidepressants. Moreover, it has been reported that these drugs have positive influence on neurogenesis and neurodifferentiation in the rodent hippocampus [Maes et al., Metab Brain Dis, 2009]. Recently, it has been observed for the first time that imipramine induced human astrocytes to differentiate into cells with neuronal phenotype [Cabras et al., Int J Neuropsychopharmacol, 2010]. The aim of our study was to
determine if imipramine and fluoxetine are able to modulate phenotype of lipopolysaccharide (LPS)-stimulated and unstimulated microglial cells.

Experiments were performed on rat primary mixed glial cell cultures prepared according to the method described previously [Bielecka et al., Naunyn Schmiedeberg’s Archiv Pharmacol, 2010]. On day 13, staining of cells with *Ricinus communis* agglutinin-1 – marker of microglia and the antibodies against GFAP – marker of astrocytes, evidenced that approximately 60–65% of cells were microglial cells and 30–35% were astrocytes. On day 13, culture medium was replaced with a medium containing additionally: 1) antidepressant drug (imipramine or fluoxetine at not cytotoxic concentration 10 µM) or 2) LPS (2 µg/ml) or 3) LPS and antidepressant. After 24-h incubation in the standard condition (37°C, 95% air, 5% CO₂) imipramine induced transformation of unstimulated microglial cells into cells with a neuronal phenotype characterized by the smaller cell bodies and thinner and longer processes. Fluoxetine did not modulate cell morphology as imipramine but it changed cell arrangement. Both drugs modified phenotype and arrangement of cells in LPS-stimulated cultures. The round-shaped activated microglial cells returned to resting ramified forms which were not conglomerated in a “grape structure”. These data suggest that imipramine stronger than fluoxetine influence on phenotype of microglial cells and that both drugs may attenuate activation of microglia.

**Histamine content in rat brains with rodent model of Parkinson’s disease exposed to manganese during pre- and postnatal development**

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Rats lesioned shortly after birth with 6-hydroxydopamine (6-OHDA; 134 µg icv⁴) represent a near-ideal model of severe Parkinson’s disease because of the near-total destruction of nigrostriatal dopaminergic fibres. There are scarce data that in Parkinson’s disease, activity of the central histaminergic system is increased. The element manganese, an essential cofactor for many enzymatic reactions, itself in toxic amount, replicates some clinical features similar to those of Parkinson’s disease. Therefore, the aim of this study was to examine the effect of neonatal manganese exposure on 6-OHDA modeling of Parkinson’s disease in rats, and effect on histamine content in the brain of adult rats.

Manganese (MnCl₂ • 4H₂O), 10,000 ppm was included in the drinking water of pregnant Wistar rats from the time of conception until the 21st day after delivery, the age when neonatal rats were weaned. Control rats consumed tap water. Other groups of neonatal rat pups, on the 3rd day after birth, were pretreated with desipramine (20 mg/kg, ip 1 h) prior to bilateral icv administration of 6-OHDA (60 or 137 µg) or its vehicle saline-ascorbic (0,1%) (control). At 2-months after birth, in rats lesioned with 60 or 134 µg 6-OHDA, endogenous dopamine (DA) content in the frontal cortex was reduced (HPLC/ED), and co-exposure of these groups to perinatal manganese magnify the DA depletion. There was prominent enhancement of histamine content in the frontal cortex, hippocampus, hypothalamus and medulla oblongata of adult rats’ brains after 6-OHDA 60 and 134 µg injection on the day 3rd of life and exposure to manganese.

These findings indicate that histamine and the central histaminergic system was altered in the brain of rats lesioned to model Parkinson’s disease, and that manganese enhanced effect of 6-OHDA on the histamine content in the brain of mammalians.
Concentrations of glucose metabolites in the brain in an animal model of depression

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Recent data have indicated that reduced action of insulin and disturbed glucose metabolism in the brain may play an important role in pathogenesis of depression. Glucose is an essential energy source for the brain and most of energy in the brain is needed for restoration of the resting membrane potential following neuronal depolarization and for neurotransmitter recycling and transport. Distorted glucose metabolism in some brain regions, mainly in the prefrontal cortex, especially in humans, results from hyperactivity of hypothalamic-pituitary-adrenal (HPA) axis. Interaction between glucocorticoids and insulin and their effect on metabolic processes are well established in peripheral tissues, however, their action in the brain is largely unknown. Previously, we found that in prenatally stressed rats (an animal model of depression), HPA axis activity was increased and glucose and glycogen concentration in the frontal cortex were elevated. The aim of the present study was to find out whether there are also changes in pyruvate and lactate concentrations (an index of glycolytic glucose metabolism) in this model of depression.

Pregnant Sprague-Dawley rats were subjected to stress sessions (three per day) from 14th to 21st day of pregnancy, while control pregnant females were left undisturbed in their home cages. After weaning, male rats from control and stress group were housed for 3 months. In 3 months old male rats, the immobility time in the forced swim test (Porsolt test) was determined in order to verify the animal model of depression used in this study. Two days after Porsolt test, the animals were killed by rapid decapitation and the brain structures were rapidly dissected out and stored at –80°C. The frontal cortex was homogenized and centrifuged to obtain the cytosol fractions. Pyruvate and lactate concentrations were determined by colorimetric methods using pyruvate assay kit and lactate assay kit (BioVision).

It was found that prenatally stressed rats had statistically significantly higher levels of immobility behavior in the forced swimming test than control animals, i.e. they showed depression-like behavior. Pyruvate concentration in the frontal cortex was by about 50% higher in prenatally stressed rats than in control animals. Also the level of lactate was higher in prenatally stressed rats. In contrast, there were no changes in pyruvate and lactate concentration in serum.

The obtained data indicated that depression-like behavior induced by prenatal stress evoked long-term changes in brain glycolytic glucose metabolism. The elevated levels of glucose, pyruvate and lactate in the frontal cortex indicated that not only glucose uptake to this structure was enhanced but also glycolytic metabolism was more intense. So, the excessive brain glucocorticoid concentration in the last gestation period may lead to the disturbance in glucose concentration and metabolism. However, only examination of glucose transporters and oxidative metabolism of glucose in this model of depression will permit to draw a final conclusion about prenatal exaggerated glucocorticoid action on glucose utilization.

Acknowledgments:
This work was supported by the Operating Program of Innovative Economy 2007-2013, grant No. POIG.01.01.02-12-004/09.
Influence of chemical convulsants on kynurenic acid production in the rat brain
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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 (KAT I and KAT II) localized preferentially within astrocytes. The possible role of altered KYNA-mediated modulation of EAA receptors in the human neuropathology has been postulated. The disturbances of KYNA production have been linked to the occurrence of epilepsy. The anticonvulsant and neuroprotective role of KYNA in vivo and in vitro is well documented.

In this study the influence of chemical convulsants: bicuculine and pentylenetetrazole on the brain production of KYNA was investigated.

The experiments were performed in vitro in rat cortical slices. Bicuculine at the concentration of 0.5, 1 and 5 mM increased KYNA production in the brain cortical slices to 163% (p < 0.01), 170% (p < 0.01) and 173% (p < 0.01) of control, respectively. Pentylenetetrazole at the concentration of 0.5, 1, and 5 mM enhanced KYNA synthesis to 120% (p < 0.05), 124% (p < 0.01), and 130% (p < 0.01) of control, respectively.

The activity of KAT I was enhanced by bicuculine at the concentration of 0.1; 0.5; and 5.0 mM to 128% (p < 0.01); 145% (p < 0.001); 156% (p < 0.001) of control, respectively. Pentylenetetrazole at the concentration of 0.01–5 mM did not alter the activity of KAT I.

Both, bicuculine and pentylenetetrazole used at the concentration of 0.01–5 mM did not affect of KAT II activity.

Our data suggest that chemical convulsants can modulate the brain production of KYNA.

Interaction between central noradrenergic system and serotoninergic 5-HT₃ receptor mediated analgesia in rats
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The goal of the present study was to examine the effect of the central noradrenergic system on the serotoninergic 5-HT₃ receptor mediated analgesia in rats. Noradrenergic system was lesioned in male rats shortly after birth by subcutaneous (sc) injections of the neurotoxin DSP-4 [(N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] (50 mg/kg × 2) given on postnatal days 1 and 3. Rats continued to be housed until they were 10 weeks old, for further experimentation. The anti-nociceptive effects of the central serotoninergic 5-HT₃ receptor agonist (1-phenylbiguanide; 7.5 mg/kg), antagonist (ondansetron; 1.0 mg/kg) and both drugs administration (intraperitoneal; ip) were examined in models of exteroceptive sensation using thermal (tail immersion and hot plate tests) and mechanical stimuli (paw pressure test). Furthermore accumulation of 5-hydroxytryptamine (5-HTP) in some parts of the brain were determined using high pressure chromatography with electrochemical detection method (HPLC/ED). In the tail immersion test we did not observe differences between control and DSP-4 treated rats as far as the anti-nociceptive effect evoked by the central serotonergic 5-HT₃ receptor agonist (1-phenylbiguanide; 7.5 mg/kg, ip) is concerned. Conversely in the hot plate test 1-phenylbiguanide (7.5 mg/kg, ip) produced significantly diminished analgesic reaction in DSP-4.
lesioned rats in comparison to control (in all tested intervals (20, 40, 60 and 80 min; \( p < 0.05 \)); this effect was abolished by 5-HT\(_3\) receptor antagonist (ondansetron; 1.0 mg/kg, \( ip \)) pretreatment. Similar effects were observed in paw pressure test; in this case significant changes were noticed in 20 and 40 min of testing (\( p < 0.05 \)). In biochemical assay we found that 1-phenylbiguanide significantly increased 5-HTP level in the prefrontal cortex of control rats being without effect in DSP-4 group in this regard. Ondansetron did not affect 5-HTP content when given alone but injected before 1-phenylbiguanide abolished its effect in control group. In the thalamus with hypothalamus (control) as well as in the brain stem (control and DSP-4) 1-phenylbiguanide only non-significantly elevated 5-HTP level. Ondansetron alone did not affect examined parameters but in the brain stem administered before 1-phenylbiguanide statistically lowered 5-HTP (in both tested groups) in comparison to respective controls (1-phenylbiguanide). The results of the present study indicate that the noradrenergic system participates in the analgesic properties of 5-HT\(_3\) acting drugs integrated in the higher brain structures (e.g. thalamus, cortex) being without effect on spinal analgesia. Additionally, obtained data pointed out on the possibility of nociception disturbances (mediated by 5-HT\(_3\) receptor) in patients with noradrenergic system dysfunction (e.g., depression and/or anxiety disorders).

Prenatal stress modifies the insulin-like growth factor-1 levels in the hippocampus and prefrontal cortex of adult rats

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Numerous epidemiological studies have evidenced that early adverse experiences are closely connected with an increased risk of stress-related psychiatric disorders, such as depression, in adulthood. As yet, the mechanism for the precipitation of the onset of stress-related disorders remains to be determined, but it has been postulated that an early-life adversity produces definite morphological and functional changes in the central nervous system. Recent data have suggested that the modulation of neurotrophic factors and their receptors may be associated with an altered hippocampal and front cortical dysfunction. It has been postulated that the weaker activity of growth factors, such as brain-derived growth factor (BDNF) and insulin-like growth factor (IGF) plays a key role in pathogenesis of depression. IGF family members exert multidirectional effects: intensify cell proliferation and differentiation, enhance neurogenesis in the hippocampus in adult animals and exert antidepressant activity.

The aim of the present study was to find out whether the prenatal stress, which is an animal model of depression, can influence the IGF-1 levels in the hippocampus as well as in the frontal cortex, i.e. the regions in which synaptic plasticity is particularly disturbed in depression.

Pregnant Sprague-Dawley rats were subjected daily to three stress sessions from day 14 of pregnancy until delivery. Control pregnant females were left undisturbed in their home cages. After weaning, male rats from each experimental group were housed collectively. At 3 months of age, the control and prenatally stressed male rats were tested for behavioral changes in the Porsolt test and in the elevated plus-maze test. The animals were killed by rapid decapitation two days after a behavioral test and the hippocampus and the prefrontal cortex were rapidly dissected out and stored at –80°C. The level of IGF-1 was determined by a Western blot method, using specific primary antibody from Santa Cruz Biotechnology and ELISA tests.

Prenatally stressed rats showed depression-like behaviors, i.e. they displayed high levels of immobility.
behavior in the forced swimming test, an enhanced exploratory activity and changes in the elevated plus-maze test (less time spent in the open arm). In our study, Western blot and ELISA analysis showed that the level of IGF-I in the hippocampus and frontal cortex of 3 months old prenatally stressed animals was significantly lowered in comparison to adult control rats. On the other hand, the peripheral serum IGF-1 level in stressed animals was non-significantly different from its concentration in control animals.

In conclusion, we report that prenatal stress evoked a long-term depression-like behavior what confirms the usefulness of this model for examination of the mechanisms involved in pathogenesis of depression. IGF-1 down regulation in the hippocampus and frontal cortex can be one of possible mechanisms by which early stress precipitates the onset of depression in adulthood. This hypothesis will be verified in our next study.

Acknowledgments: This work was supported by the Operating Program of Innovative Economy 2007–2013, grant No. POIG.01.01.02-13-004/09.

Effect of ontogenetic manganese exposure on dopaminergic system in 6-hydroxydopamine lesioned rats

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The present study investigated the effect of neonatal manganese (Mn) exposure in a 6-hydroxydopamine (6-OHDA) rat model of Parkinson’s disease. Pregnant Wistar rats were given drinking water with 10,000 ppm of manganese (MnCl₂·4H₂O) from the time of conception until weaning on the 21st day after delivery. Control rats consumed tap water. Three days after birth, other groups of neonatal rat pups were pretreated with desipramine (20 mg/kg, ip 1 h) prior to bilateral icv administration of 6-OHDA (30, 60, or 137 µg) or its vehicle, saline-ascorbic (0.1%) (control). Two months after birth, striatal dopamine and homovanillic acid efflux was measured using microdialysis. Both dopamine and homovanillic acid were reduced in rats lesioned with 30, 60, or 134 µg 6-OHDA. Co-exposure to perinatal Mn did not intensify neurotransmission disturbances; however, there were prominent abnormalities in behavioral testing (locomotor activity, exploratory activity, and irritability) in rats perinatally exposed to Mn and treated neonatally with 6-OHDA. These findings demonstrate that although Mn did not further damage neurotransmitter activity in the neostriatum, ontogenetic exposure to Mn enhances the behavioral toxicity to 6-OHDA.

Key words: manganese, ontogenetic, exposure, behavior, brain, microdialysis
Effect of β-adrenoceptor antagonists and antiepileptic drugs against aminophylline-induced convulsions and lethality in mice

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The convulsive activity of methylxanthines has been known for many years. Conventional antiepileptic drugs very poorly control this type of convulsions and are practically ineffective on aminophylline-induced mortality. Protective activity of phenobarbital, and valproate combined with propranolol, atenolol, labetalol and pindolol against aminophylline-induced seizures and mortality was evaluated. The experiments were carried out on male Swiss mice weighing 20–25 g. Chemical seizures were induced by intraperitoneal (ip) injections of aminophylline and defined as clonus of the whole body with an accompanying loss of righting reflex. Phenobarbital (up to 75 mg/kg) and valproate (up to 300 mg/kg) markedly reduced the incidence of convulsions, but were not effective in preventing mortality. β-Adrenoceptor antagonists at the maximal doses used did not reveal protective action against seizures and lethality. Phenobarbital and valproate co-applied with atenolol and propranolol led to a significant protection against aminophylline-induced convulsions and mortality. On the contrary, antiepileptics co-administered with labetalol and pindolol did not protect against aminophylline-induced seizures and mortality. The obtained results point to a novel method sufficiently reducing convulsion and lethal effects of aminophylline overdose.

Effect of propranolol on the anticonvulsant action of diazepam and phenobarbital in caffeine-induced seizures and lethality in mice

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Caffeine is a natural methylxantine which stimulates the secretion of neurotransmitters in central nervous system. Moreover, caffeine as well as methylxantines such as theophylline or aminophylline, may exhibit many side effects like nervousness, anxiety, cardiac arrhythmias and even convulsions in high doses. On the other hand, experimental data shown that caffeine decreased anticonvulsant properties of some antiepileptic drugs against maximal electroshock-induced seizures in mice.

We evaluated the protective activity of phenobarbital and diazepam combined with propranolol against caffeine-induced convulsions and mortality. The experiments were performed on male Swiss mice weighing 20–25 g, kept under standard laboratory conditions. Chemical seizures were induced by intraperitoneal (ip) injections of caffeine and defined as clonus of the whole body with an accompanying loss of righting reflex lasting for over 3 s. All experimental procedures were approved by Ethical Committee (Medical University of Lublin).

Phenobarbital, diazepam and propranolol were administered intraperitoneally 60 min before the test. The mean times to performed seizures after injection of the convulsant agent were 15 min. Mice were observed in transparent cages for 60 min after the test and 24 hours later the lethality was recorded. Propranolol (up to 5 mg/kg) did not affect the convulsive action of caffeine. Phenobarbital (up to 50 mg/kg) and diazepam (up to 5 mg/kg) were effective in this re-
spect but had no influence on mortality of mice. Diazepam and phenobarbital combined with propranolol did not protect against caffeine-induced seizures otherwise led to a significantly reduction of mortality.

The results indicate that the combined treatment of some antiepileptic drugs with propranolol may provide a good protection against methylxantine-induced mortality. It seems importance to notice that this β-adrenoceptor antagonist at the same time exacerbate onset of seizures.