Symposium 1

dedicated to Prof. dr hab., dr h.c. EDMUND PRZEGALIŃSKI

New aspects of the mechanism of action of antidepressant drugs

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The present study has addressed the question of what is more important for occurrence of the adaptive changes observed in the organism treated with antidepressant drugs (ADs) – is that a daily dosing of the drug or the period of time necessary for the plastic events to develop, as has been suggested by Antelman et al. [Mol Psychiatry, 2000].

ADs are effective in the clinical practice following at least a few weeks of administration, therefore, it has been widely accepted that adaptive changes in the central nervous system take place. However, as we have shown in the previous behavioral studies, down-regulation of presynaptic dopamine D_2 and D_3 receptors was observed not only after repeated administration of ADs, but also after a single dose, followed by two drug-free weeks. Similar effects were observed at the level of α_2-adrenergic receptors [Dziedzicka-Wasylewska et al., Prog Neuropsychopharmacol Biol Psychiatry, 2000]. Among behavioral tests used to screen the antidepressant efficacy, the forced swimming test (FST) is one of the most widely used. Porsolt et al., [Eur J Pharmacol, 1978] showed that a single injection of ADs reduced immobility time in a dose-dependent manner, but a more pronounced and consistent effects were observed following three administrations in rats. Repeated treatment with most ADs induces similar reduction in the immobility time as three doses of the drug, however, desipramine is more effective following chronic treatment. Therefore, we have chosen desipramine to study its effect in the FST after repeated (14 days) administration, in comparison to an acute dosing of desipramine followed by 13 drug-free days. We have shown that three doses of the drug induced a ca. 30% reduction of immobility time as compared with drug-naive rats. Effect of chronic treatment with desipramine was significantly more pronounced (ca. 70% reduction *vs*. drug-naive animals), but in a group treated with a single dose of desipramine, followed by 13 drug-free days, and then subjected to FST, we also observed a reduction in the immobility time and the effect was almost identical with the effect induced by chronic treatment with desipramine [Kuśmider et al., Behav Pharmacol, 2006].

Additionally, using the procedure of the repeated FST (6 times over 21 days), we have shown that the shortening of immobility time induced by a single dose of imipramine persisted throughout the whole experimental period and was similar as in a group of animals treated repeatedly with that drug. As for citalopram (its effect was measured with the modified FST), its influence on immobility and climbing after acute treatment and delayed testing was similar to the repeated drug exposure [Kuśmider et al., Behav Pharmacol, 2006].

The next step in those studies was to examine the "pulse-therapy" with imipramine in the Chronic Mild Stress (CMS) test, a well-validated animal model of

depression [Papp et al., Psychopharmacology, 1991]. Wistar rats trained to consume 1% sucrose solution in a one-hour test performed once a week, were subjected to the procedure of sequential exposures to a variety of mild stressors for 8 weeks. A decrease in the consumption of the solution was a measure of anhedonia developing in stressed animals. After two weeks of stress exposure, when the sucrose intake between stressed and control animals started to differ significantly, animals were divided into three groups treated with imipramine (10 mg/kg *ip*) in a following way: (i) chronically (i.e. once daily), (ii) once a week, (iii) on the day before the sucrose test and once a week, (iv) on the day after the sucrose test. These two "pulse therapy" groups were used in order to check whether the presence of imipramine in the animals will influence the sucrose intake. As expected, imipramine had no effect on sucrose intake in control (unstressed) animals but in "regular" and both "pulse therapy" groups of stressed rats, it gradually reversed hedonic deficit. The effect reached statistical significance in 6th week of the experiment.

We find the obtained results intriguing. Since some effects of ADs regarded as adaptations to chronic treatment can be also observed after a single administration followed by 2–3 weeks of drug-free period, it may well be that those effects are not so important to therapeutic action of the drugs as we have thought so far. Otherwise, those effects are indeed important for the action of ADs and, therefore, the idea of "pulse therapy" deserves attention. Especially, our CMS study provided the strong argument for reconsideration of the "pulse therapy" with ADs in the clinical trial. Besides the economic aspect, it also could lead to the reduction of side-effects often encountered with the pharmacological therapy of depression.

Preclinical potential of $GABA_B$ receptor ligands as a pharmacotherapy for cocaine addiction

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Cocaine addiction is characterized by an increase in drug-directed behavior and a simultaneous weakening of other motivated behaviors. A number of recent studies suggest that gamma-aminobutyric acid B-type ($GABA_B$) receptor agonists may be useful in the treatment of cocaine addiction. We found that pharmacological stimulation of $GABA_B$ receptors by agonists or positive allosteric modulators reduced cocaine reinforcement while this property of cocaine was not related to tonic activation of $GABA_B$ receptors. Furthermore, a separation in effects of positive allosteric modulators and direct agonists of the $GABA_B$ receptors on cocaine- and food-maintained responding and on the locomotor activity was demonstrated. On the other hand, tonic activation of $GABA_B$ receptors is required for the cocaine seeking behavior since a $GABA_B$ receptor antagonist selectively altered motivated drug-seeking behavior at doses that failed to alter reinstatement of food-seeking behavior, cocaine and food self-administration or basal locomotor activity. Agonists of $GABA_B$ receptors inhibited cocaine seeking but also caused decreases in cocaine or food self-administration, indicating their nonspecific effects on relapse. In conclusion, a $GABA_B$ receptor antagonist seems to be a good therapeutic choice for reducing craving and preventing relapse in cocaine addicts, while a positive allosteric modulator of the $GABA_B$ receptors attenuates cue-evoked relapses to cocaine as well as the direct rewarding properties of cocaine.

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The function of neuroglia in normal and pathological conditions

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The neuroglia (Greek for "glue") is the most abundant component of the central nervous system, since it constitutes about a half of the cells in the brain. It comprises astrocytes which guide adaptive function in the mammalian central nervous system (CNS), and microglia which is composed of specialized macrophages capable of phagocytosis that protect neurons of the CNS. These two types of glial cells will be the topic of this lecture.

Moreover, neuroglia comprises: oligodendrocytes, which coat axons in the CNS with their cell membrane called myelin, producing so-called myelin sheets, which provides insulation to the axon that allows electrical signals to propagate more efficiently.

Other glial cells include: ependymal cells, radial glia, Schwann cells, satellite cells.

Therefore, it seems an extraordinary phenomenon that this component of the brain for decades had not been the subject of interest for investigations. But perhaps it may be explained by the burst of experiments concerning the role of neurons in the very young field of psychopharmacology born in the middle of the 20th century.

The second element of the greatest importance was an impulse to find, unknown at that time, remedies for psychic illnesses.

Then, till the last decades of the past century neugroglia was considered to be rather biochemically passive component needed as a structural and nutritional entity.

Investigation of the last 15 years brought an evidence that neuroglial cells are very dynamic in order to secure normal function of neurons or to defend them against noxious stimuli.

Astrocytes

Biochemistry

These cells secrete the following neurotrophic factors: nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF).

In neuroglia, several receptors for the transmitters are present, and if stimulated by environmental factors they increase the calcium efflux.

There are several evidences that glutamate and ATP may be transmitters between astrocytes and neurons. Actually, the similar role for D-serine is discussed. On the other hand, a reverse influence is also possible. There are some data indicating that noradrenaline, acetylcholine and GABA released from neurons act on astrocytes.

The other pathway of communication between astrocytes and neurons are gap-junction channels a connection proteins. The other substances present at very low concentrations are: tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), which are the class of inflammatory compounds.

Function

Astrocytes are fundamental cells for normal development of the CNS in fetus, newborn and during maturation of the brain.

Not long ago, it was an axiom that neurogenesis in adulthood did not exist. Actually, it was evident that after puberty, neurogenesis occurred in the subventricular zone and hipocampal subgranular zone. This phenomenon was not observed in the spinal cord. The other important role of astrocytes is regulation of production of synapses.

Function in brain injury

If noxious stimuli act on the brain, the astrocytes as well as microglia are transformed to defend the CNS. If the CNS injury is beyond of astrocyte functional defending capabilities the injures of CNS appear.

Actual studies of the role of astrocytes in brain diseases: cerebral ischemia and inflammation, and degenerative brain disorders: Parkinson's disease, Huntington's chorea, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease; aging are in progress.

Microglia

Microglia is composed of specific macrophages capable of phagocytosis that protect neurons of the CNS. They are derived from a hemopoietic precursor, rather than ectodermal tissues, and have supportive role to neurons.

They constitute 15% of all cells of the CNS.

They synthesize inflammatory cytokines: IL-1β and IL-6 as well as transforming growth factor-β (TGF-β).

It is hypothesized that there is a real relationship between microglia and astrocytes, which this is still under discussion.

At the end of this review, the original data concerning the influence of anti-ischemic drugs in the neuroglia as well as results of the experiments concerning the influence of antidepressants on the release of cytokines from neuroglia will be presented.

Effects of chronic mild stress (CMS) procedure and chronic administration of citalopram on motivational and physical aspects of morphine dependence in rats

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Clinical and animal evidences indicate an important role of stress in development and maintenance of drug abuse and addiction. These observations are confirmed by our studies showing that the chronic mild stress (CMS) paradigm, a procedure which employs a long-term (10 weeks) presentation of a variety of mild stressors, causes a substantial enhancement of the motivational (naloxone- or cues-induced conditioned place aversion) and physical (abstinence syndrome precipitated by naloxone or cues) aspects of morphine dependence in rats. These effects are substantially attenuated by chronic (5 weeks) administration of citalopram (10 mg/kg). Citalopram also inhibits the aversive and physical symptoms of morphine withdrawal precipitated by presentation of conditioned stimuli (vanilla and tone previously associated with injections of naloxone), which, again, are much more severe in animals subjected to the CMS procedure.

These results confirm that the CMS paradigm provides an adequate basis for studying relationship between stress and various aspects of drug addiction in animals. Moreover, our findings suggest a possible use of antidepressant drugs in the therapy of drug abuse and dependence in humans.

The bright sides of old age

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The man is one of a few animal species whose longevity extends over the sexual reproductive period, after which death ensues in prevalent number of animals. The death evolved as a means to optimize the reproductive success of a given species. While the death is unavoidable, the ageing process in the sense of the loss of the functional efficiency (senescence) evolved in mammalians, while lower vertebrates – fish, amphibians, reptilians and birds – age without symptoms of the senilism. Theories of the senescence, whose number exceeds 300, may be divided into two large groups. One of them assumes that the death is genetically planned and the life-span is controlled hereditarily, determined by programmed apoptotic processes or by the rate of shortening of telomeres. Other theories assume that our starting vital potential is prone to dwindling due to external damages, such as the impairment of the mitochondrial machinery by free radicals, the distortion of the hormonal regulation by the stress-induced release of corticosteroids that damage hypothalamic neurons, by the loss of fluidity of cell membranes etc.

The progress of medicine has prolonged human life span and pushed further the onset of age-related malfunction in healthy persons, but instead unmasked the symptoms of slowly developing neurodegenerative diseases, such as Alzheimer's type dementia, which without the present medicine would not have the time to come to light. The constant efforts to combat neurodegenerative diseases met with some, but still limited success.

It is very purposeful to keep an aging brain in good shape. This may be achieved by active seeking of enriched environment (what increases neuroplasticity), a healthy life style, including calorie restriction (what activates the product of the longevity gene, sirtuin), the regular physical exercises (activating BDNF), and the intellectual effort, beneficial for the development of neuronal plasticity and stimulating the release of acetylcholine, which exerts neuroprotective activity. An important aspect of the good senescence is the maintaining of the sexual activity to the late age.

In spite of growing physical inconveniences, the ripe age has its own advantages, such as improvement of emotionality and getting experience permitting better verbal knowledge and appraisal of other people. The older humans, particularly women, played an important role in human evolution, taking care of the youngest members of the society and promoting higher reproduction rate of their offspring. Due to their experience, the old individuals, not only in the human society, but also and in other social animals, like elephants, perform important, often leading social functions.

Developmental animal model of schizophrenia – postnatal administration of a competitive NMDA receptor antagonist

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The malfunction of glutamatergic neurotransmission in the neonatal or postnatal periods may be a risk factor for the appearance of neuroanatomical, neurochemical or functional changes that are characteristic of schizophrenia. The present report summarizes experiments undertaken to investigate whether NMDA

receptor blockade in the postnatal period influences rat behavior in tests characterizing schizophrenia-like deficits, such as psychomotor agitation, impairments of sensorimotor gating, working memory, and intensity of social interactions. Furthermore, we focus our attention on the morphology of the prefrontal cortex, like alterations of dopaminergic innervation of medial prefrontal cortex and changes in density of spines on pyramidal neurons, that are anatomical features of schizophrenia observed *post mortem* in brains of schizophrenics. CGP 40116, a competitive antagonist of NMDA receptors, was given postnatally (1.25 mg/kg on days 1, 3, 6, 9; 2.5 mg/kg on days 12, 15, 18; and finally 5 mg/kg on day 21, all injections *sc*), and rats were tested at 60 days of age. At the behavioral level, we found that NMDA receptor blockade in the postnatal period led to an enhancement of exploration, mimicking psychomotor agitation, to impairments in sensorimotor gating as measured by a prepulse-evoked inhibition of acoustic startle response, and to an impaired working memory, as measured by an increase in the latency to achieve accurate rate of response in the delayed alternation task. Decreases in non-aggressive social interactions and increases in aggressive interactions were also observed. At the anatomical level, it has been found that CGP 40116, given in postnatal period decreased the density of tyrosine hydroxylase immunoreactive axonal arbors in the medial prefrontal cortex of adult animals. The decrease was observed in superficial (II/III) and deep (V/VI) layers of the medial prefrontal cortex, while the average length of tyrosine hydroxylase immunoreactive axonal arbors was increased in both superficial and deep cortical layers. The analysis of the morphology of pyramidal neurons visualized by the Golgi-Cox technique revealed that the exposure to an antagonist of NMDA receptors in the postnatal period diminished the length of basilar dendrites, while that of apical dendrites remained unchanged. It is concluded that the NMDA receptor blockade in the postnatal period may model deficits that are characteristic of schizophrenia. It will be important to investigate in further studies whether such behavioral and anatomical deficits are influenced by neuroleptic drugs and to examine the possible involvement of candidate genes linked to schizophrenia.