Plenary lectures

Dynamic behavioral, neurochemical and molecular changes induced by antidepressant drugs

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Depression is a serious social problem worldwide, particularly in highly developed countries. Concerning the pathogenesis, number of hypotheses are taken into account. They are usually based on the postulated mechanism of acute action of antidepressants, which, however, is not yet well understood. There are several, pharmacologically well characterized, groups of antidepressants. Among them are drugs inhibiting reuptake of monoamines, compounds affecting monoamines metabolism and release, but also substances acting directly on receptors. Sometimes, despite clinical efficacy, their pharmacological action is mutually opposite. This suggests that their efficacy depends not on direct interaction between drug and target molecule, but rather on the results of this interaction, or even on the secondary effects. A common feature of action of antidepressants is that the therapeutic effect appears after at least several weeks of treatment. The time required for the formation of adaptive changes in the nervous system exposed to the antidepressants has become a subject of some of our studies. Experiments were carried out on Wistar rats, treated with the following antidepressants: desipramine (a selective norepinephrine reuptake inhibitor), imipramine (classical tricyclic antidepressant with a broad pharmacological activity) and citalopram (selective serotonin reuptake inhibitor). A series of behavioral experiments and determinations of biochemical parameters, reported previously to be altered with repeated treatment with antidepressants, has been conducted. The experiments compared the effects of a single injections at various time intervals (1 to 21 days) prior to testing, to the effects of repeated drug applications and to appropriate groups of control animals – not receiving the drug. Behavioral experiments have shown that a single exposure of animals to the antidepressant is not neutral to the behavior of animals in the test, even if this test was carried out after a few days of a single exposure to drug. The response in some cases was identical to that observed after repeated drug injections (e.g.: behavioral response in the Porsolt's test) The biochemical studies also demonstrated changes, that manifest themselves at different time points after single dosing. Most of these changes were observed early (3–7days after drug application), but in the case of dopamine receptors, very similar changes were observed in animals treated with imipramine or desipramine chronically, or once, 21 days before the end of the experiment. The results obtained allowed to propose a pulse therapy regimen, which has been tested in well-validated model of depression – a model of chronic mild stress. Animals treated with imipramine at pulse regimen, with a frequency of once a week, gave the same behavioral outcome as in the case of daily administration. Then, on the tissue collected from these animals, determination of gene expression was performed, by the means of low density microarray technology. Surprisingly, only four transcripts differentiated stressed and the control animals (similarly, rather small number of proteins differentiated stressed and the control animals, as revealed by proteomic approach). Differences in the expression of two of them were turned over by the pulse therapy regimen, one – by chronic imipramine administration. In the next series of studies we employed RT-PCR array to evaluate gene expression changes during a time-course (1, 3, 7,

14 and 21 days) of treatments with desipramine and citalopram. In addition to repeated treatment, we also conducted acute treatment (a single dose of drug followed by the same time intervals as the repeated doses). Appropriate genes (95) were not chosen randomly but the choice was based on the data already published, indicating their either proved or alleged importance for the mechanism of action of antidepressants. We decided to measure alterations in their expression in the same experiment in order to avoid any variables. Time-dependent and structure-specific changes in gene expression patterns allowed us to identify spatiotemporal differences in the molecular action of two ADs. Singular value decomposition analysis revealed differences in the global gene expression profiles between treatment types. The numbers of characteristic modes were generally smaller after citalopram than after desipramine treatment. Analysis of dynamics of gene expression revealed that the most significant changes concerned immediate early genes, whose expression was also visualized by *in situ* hybridization. Transcription factors binding sites analysis revealed an over-representation of SRF binding sites in the promoters of genes that changed upon treatment with both ADs.

The main finding of our work is that gene expression pattern during ADs treatment is highly dynamic, with oscillations and peaks occurring at different time points of treatment. Additionally, our study revealed a number of novel potential targets for antidepressant therapy, such as the *Dbp* and *Id1* genes, that were not previously directly linked to antidepressant action. We are strongly convinced time has come to look at the mechanisms of action of antidepressant drugs through broader window instead of sticking to any particular measure, as e.g. one gene or one protein, and building next clear hypothesis which will lead us to the next dead end. Similar conclusion has been put forward recently by Kessler et al. [Psychosoc Med, 2011], who have analyzed the results of neuroimaging studies of depressed patients. Despite the great effort the outcome of these studies has been "a surprising heterogeneity of results with the eventual lack of practical conclusions". The authors suggest novel approaches using longitudinal designs of neuroimaging studies, i.e. to "take various pictures over the course of treatment". Our studies, although pre-clinical, strongly support that view.

The reactivity of microglia – the role of AMP-dependent kinase and its possible pharmacological modulation

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Microglia is one of the most mysterious and the least-known of the neuroglial cells. Despite the increasing number of research, its diverse structure and roles still remain unclear. It is known that microglial cells present antigens, produce cytokines and chemokines, and act as local 'cleaners'. They have receptors for many neurotransmitters and neurotrophins, and are covered with numerous ion channels, enabling them to contact with 'neighbours'. Since then, microglial cells become important players in the local microenvironment, acting as specific sensors, transmitters and mobile executors. Thus, incorrect microglial reactivity and disturbances in the functioning of intercellular information systems can be crucial for the pathogenesis of the CNS diseases such as Alzheimer's, Parkinson's, Huntington's, multiple sclerosis, dementia associated with HIV infection, ischemia and reperfusion syndrome, and metabolic encephalopathies. This impaired reactivity may result from the cell number imbalance between the 'classic-inflammatory-bad' and 'alternative-anti-inflammatory-good' phenotypes. Looking at this issue from the perspective of pharmacological activation or inhibition of AMP-dependent kinase (AMPK) makes the described

relations really interesting in terms of modern neuropharmacology development. AMPK acts as a sensor of energy level and triggers stress pathways, coordinates cellular metabolism, regulates cell division, mitochondrial biogenesis, autophagy and apoptosis. Therefore, it is not surprising that the search for substances that selectively modulate AMPK functions is tempting. Certain pharmacological tools can promote the alternative, anti-inflammatory and partial phagocytic phenotype, not impairing the secretory activity. In summary, it appears that chronic anti-inflammatory therapy should be based not only on the suppression of the pro-inflammatory phenotype of microglia, but should also enhance the anti-inflammatory response of these cells. Perhaps, AMPK will soon become a therapeutic target in the treatment of selected diseases of the CNS.