Symposium "An Intriguing Nature of Estrogens" was organized jointly by the Institute of Pharmacology of the Polish Academy of Sciences in Kraków, Regional Branch of the Polish Pharmaceutical Society and Polish Pharmacological Society. The symposium was focused on new aspects of research on estrogens and xenobiotics, particularly in relation to the central nervous system.

The following lectures were delivered:

**10.30 – 11.00** Molecular characteristics of estrogen receptors and their function in the central nervous system
Katarzyna Kalita M.Sc., Department of Molecular and Cellular Neurobiology, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warszawa, Poland

**11.10 – 11.40** New mechanisms of estrogen action in the central nervous system via membrane estrogen receptors
Agnieszka Lachowicz Ph.D., Department of Experimental Endocrinology and Hormonal Diagnostics, Institute of Endocrinology, Medical University, Łódź, Poland

**11.50 – 12.20** Xenobiotics as environmental estrogenic factors
Ewa L. Gregoraszczuk Prof., Laboratory of Physiology and Toxicology of Reproduction, Department of Animal Physiology, Institute of Zoology, Jagiellonian University, Kraków, Poland

**13.00 – 13.30** Estrogens and immune processes in the brain
Agnieszka Ciesielska M.Sc., Second Department of Neurology, Institute of Psychiatry and Neurology, and Department of Experimental and Clinical Pharmacology, Medical University, Warszawa, Poland

**13.40 – 14.10** Estrogen implication in apoptotic processes in the central nervous system
Małgorzata Kajta Ph.D., Department of Endocrinology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Symposium venue: **Institute of Pharmacology**  
**Polish Academy of Sciences**  
Smętna 12, PL 31-343 Kraków, Poland
MOLECULAR CHARACTERISTICS OF ESTROGEN RECEPTORS AND THEIR FUNCTION IN THE CENTRAL NERVOUS SYSTEM

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Estrogens play an important role not only in brain development, but also in promotion of neuronal growth, regeneration, and neuroprotection throughout the whole lifespan. Estrogen mediates changes in the number and density of dendritic spines on CA1 pyramidal neurons in the hippocampus, thereby influencing neuronal plasticity. Two types of estrogen receptors, named ERα and ERβ, operating as transcription factors, have been found to be the major mediators of estrogen biological functions. An interesting feature of the ERβ receptor is the surprising variety of its mRNA isoforms. Protein structures predicted from the pre-mRNAs differ significantly, suggesting different function of particular ERβ isoforms in the regulation of estrogen action in the brain. Additionally, actions of estrogens via membrane or intracellular receptors have been suggested. Autoradiography, immunohistochemistry, and in situ hybridization have been employed to identify estrogen receptors in the brain. The ERα is highly expressed in the areas of the brain that are responsible for reproduction, whereas ERβ is concentrated in the regions, such as hippocampus and amygdala, responsible for cognitive functions. The role played by ERα and ERβ in the brain may be explored by studies on knockout mice. ERα-knockout mice show impaired reproductive behaviors, while ERβ-knockouts remain intact in reproductive capabilities but have morphological abnormalities in the brain. These results suggest that each receptor type is responsible for different biological functions. The pattern of expression of estrogen receptors in the brain is not static but undergoes rapid changes in response to brain injury, neurodegeneration and apoptosis. The wide range of estrogen action in the brain can be explained by their interaction with various growth factors. The synergistic action of this hormone through, both, the estrogen receptors and estrogen receptor-independent pathways cannot be excluded.

NEW MECHANISMS OF ESTROGEN ACTION IN THE CENTRAL NERVOUS SYSTEM VIA MEMBRANE ESTROGEN RECEPTORS

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Classic mode of steroid hormone (SH) action consists in stimulation of specific nuclear receptor, that activates or inhibits gene function. However, results of clinical and molecular studies suggest that hormones with so multidirectional action as SH cannot execute their function through a single, relatively uncomplicated mechanism. Reports of recent years indicate that apart from classic mode of SH action, they can modulate intracellular processes by binding to membrane receptors. Such mechanism of action, called extragenomic, triggers biological effects within several minutes or even
seconds. This pathway is involved, among other things, in activation of protein kinases C, adenylyl cyclase, mitogen- and G-protein-activated protein kinases. Many important cellular processes, such as growth and differentiation, or migration are controlled through this route. In addition to signal transduction from plasma membrane, SH can initiate transcription acting via membrane receptors, independently of or in cooperation with nuclear receptors. Membrane SH receptors are probably located in tiny structures of plasma membrane, called calveole, and such localization enables SH-receptor complexes to interact optimally with target protein. Structure of membrane SH receptors has not been elucidated. It has been suggested that they have the same structure as nuclear receptors and are formed by transcription on the same genes. However, the mechanism of translocation of nuclear receptors to the membrane is unknown. It has also been speculated that SH act via yet another route, viz. they can exert their effects by stimulation of receptors of other ligands (like in the case of GABA_A, oxytocin or EGRF receptors), by changing membrane polarization (altering the function of ion channels) or by interaction with specific membrane receptors structurally dissimilar to nuclear receptors. Biological function of membrane receptors is also not fully understood. It can consist in distinct cooperation between membrane and nuclear receptors. Action of SH on plasma membrane leads to subtle changes in cellular homeostasis in order to prepare the most optimal conditions for functioning of new proteins produced in response to stimulation of nuclear receptors.

ESTROGENS AND IMMUNE PROCESSES IN THE BRAIN

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Both epidemiological and clinical reports indicate that the prevalence of various neurological diseases changes with gender. This phenomenon is observed in many different types of diseases, classified as abnormalities in neurotransmitter systems (depression), disorders caused by trauma (stroke, head injury), or by defects in immune (multiple sclerosis) or cardiovascular (stroke) systems and those of unknown etiology (neurodegenerative diseases). Although, the reason for the gender-related differences in the course of these diseases is still unknown, the concentration of the estrogens or progesterone or expression of their receptors may be considered. Several lines of evidence support the idea that immune mechanisms contribute to the etiopathogenesis of the neurodegenerative disease, such as Parkinson’s or Alzheimer’s diseases; psychiatric disorders, such as schizophrenia or depression; multiple sclerosis or stroke. The neuroinflammatory reaction observed in neurodegenerative disorders leads to the glial activation regulated by numerous inflammatory mediators, including cytokines and infiltration of circulating immune cells into the CNS. The increased levels of inflammatory cytokines were reported in psychiatric patients, revealing a potential role of these compounds also in schizophrenia and depression. Estrogens have been shown to play a major role in inflammatory processes. This hormone may regulate the glia morphology and gene expression in these cells. Furthermore, estrogens may be involved in the regulation of the formation of reactive gliosis. Estrogens may also influence the production of pro-inflammatory cytokines. Thus, an intriguing hypothesis can be proposed to explain beneficial effects of estrogen in so many human neurological pathologies, namely that there could be a link between hormone action and the machinery sustaining the inflammatory response.
AN INTRIGUING NATURE OF ESTROGENS

ESTROGEN IMPLICATION IN APOPTOTIC PROCESSES IN THE CENTRAL NERVOUS SYSTEM

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Naturally occurring neuronal cell death, which is essential for normal development and tissue homeostasis, has been viewed traditionally as a fate reserved for differentiating neurons that are in the process of making synaptic connections. However, recent studies on the embryonic cerebral cortex have shown that many neuroblasts in the proliferative ventricular zone die also via apoptosis. Furthermore, apoptosis in the central nervous system has been considered a defense mechanism against pathogens. At the molecular level, apoptosis is regulated by the activation of the aspartate-specific cysteine protease (caspase) cascade, which depends either upon the participation of mitochondria and the activation of procaspase-9 or on the interaction of the death receptor with its ligand and subsequent activation of procaspase-8. Pro- (bax, bid, bad, bak) and anti-apoptotic (bcl-2, bcl-xl) members of the bcl-2 family control the mitochondrial apoptotic pathway triggering or preventing, respectively, cytochrome C release from mitochondria and, thus, regulating caspase-3. Caspase-3, the main executioner protease of the apoptotic cascade, activates a specific DNase (DFF40 or CAD), which induces the internucleosomal fragmentation of DNA and, finally, cell death. The apoptotic cell death may be counteracted by cellular mechanisms which limit the activation of the caspase cascade, suppress oxygen radicals, and stabilize calcium homeostasis and mitochondrial function. There is a line of evidence suggesting a direct interference of estrogens with apoptotic processes triggered by mitochondrial pathway. Recently, estradiol has been found to prevent caspase-6-mediated neuronal cell death, possibly by inducing a caspase inhibitory factor (CIF). Another example is the stimulation of the expression of anti-apoptotic proteins, like bcl-2 or bcl-xl. An anti-apoptotic action of estrogens may also depend on suppression of pro-apoptotic gene transcription, such as Nip-2 or Bad, possibly through the AP-1 site down-stream of JNK and caspase-3 activation. As indicated in nigral dopaminergic neurons in rat primary cultures. Apart from mitochondrial pathway, estrogens appear also to be involved in death receptor-mediated apoptosis affecting cytokine signaling components, such as nitric oxide synthesis and activation of transcription factor NFκB.