Summary

ANXIETY DISORDERS AND OESTROGENS – THE STUDY IN OESTROGEN RECEPTOR BETA KNOCK–OUT MICE

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Doctoral thesis was carried out at the Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn under the guidance of associate professor Anna Kozłowska, PhD (supervisor) and Krystyna Bogus-Nowakowska, PhD (ancillary supervisor).

The oestrogen receptor (ERβ) knock-out female mice are characterized by increased anxiety and fear responses. The amygdala regulates all emotional behaviour in animals, which generates anxiety. In this region of the brain, the activity of projection neurons (glutamatergic) is constantly inhibited by GABAergic interneurons, which leads to a balance between excitation and inhibition processes. However, it is known that in ERβ knock-out female mice, GABAergic transmission is reduced in the amygdala. Furthermore, the amygdala is strongly modulated by dopaminergic and cholinergic systems, which activate both glutamatergic and GABAergic neurons, and thus significantly affect the excitatory-inhibitory balance in this structure. This dissertation aimed to determine quantitative changes in the neuronal population and quantitative changes in GABAergic, dopaminergic, and cholinergic markers in the amygdala of ERβ knock-out female mice. These quantitative changes were estimated using immunohistochemical methods and automated line scan analysis. The results demonstrate severe neuronal deficits in all main amygdala regions of ERβ knock-out mice, accompanied by astroglia overexpression in particular in the medial, basomedial and cortical nucleus and a decrease in calbindin-expressing (CB) neurons in the amygdala of ERβ knock-out mice compared with controls. While other markers of the GABAergic system include parvalbumin (PV), calretinin (CR), somatostatin (SOM), γ-aminobutyric acid type A receptor with α1 subunit ($GABA_{A\alpha1}$) and vesicular GABA transporter (VGAT) remain unchanged. Furthermore, among dopaminergic markers, the expression of tyrosine hydroxylase (TH), dopamine transporter (DAT) and dopamine D2-like receptor (DA_2) was significantly elevated in the amygdala of mice with ERβ deprivation when compared to matched controls, whereas the content of dopamine D1-like receptor (DA_1) was not altered in the ERβ knock-out mice. Similar observations were also noted for some cholinergic markers, i.e., acetylcholinesterase (AChE) and vesicular acetylcholine transporter (VAChT). Further analysis of cholinergic markers showed a significant increase in the number of neurons, in the nuclei of the amygdala

of ERβ-deficient female mice, containing cholinergic receptors i.e., muscarinic acetylcholine type 1 receptor ($AChR_{M1}$) and alpha-7 nicotinic acetylcholine receptor ($AChR_{\alpha 7}$) compared to wild-type mice. Present studies have shown that the characteristic and distribution pattern of the main markers of the GABAergic, dopaminergic, and cholinergic systems in ERβ knock-out female mice were abnormal. It is assumed that the observed changes lead to, or are the result of, impaired signalling, which in turn may alter the synaptic plasticity of the amygdala in the female mice studied. As a further consequence, changes within these neuronal populations may lead to anxiety disorders. However, determining the exact role of ERβ in anxiety-related behaviour in this process requires further research.