Depression is becoming a serious social problem, especially in the so-called “developed countries” where technological progress, and thus placed before us ever new challenges and fierce competition on the job market is raising the stress of everyday life. Primary care physicians are faced with cases of various symptoms of unknown origin (i.e. somatic diseases) reported by their patients, which further diagnosis leads to qualification towards anxiety or depression. However, despite the fact that more than 50 years have passed since the first antidepressants were introduced into clinics, depression is still difficult to cure. Nowadays used antidepressants, regardless of the mechanism of their action, have one major drawback: it requires several weeks of treatment before the first signs of improvement will be noticed by patients. What is more, in this initial period preceding remission or even a complete cure, depressive episodes may increase, and undesired and unpleasant effects often associated with antidepressants intake discourage many patients to continue treatment. As if that was not enough, we must remember that approx. 30% of patients are completely resistant to pharmacotherapy of depression, regardless of various attempts of different pharmacotherapy.

Depression is a disease related to the central nervous system – to our brain. The brain consists of nerve cells – neurons – that communicate via a transmission of chemical signals called neurotransmitters. Neurotransmitters are synthesized in neurons, released from one neuron to a narrow interneuronal gap (synaptic cleft), and then acting on various receptors located on the neighboring neuron. After finishing their work, neurotransmitters are removed from the synaptic cleft. This is the principle of transmitting signals within the brain, also in the situation of stressful stimuli perceived from the environment through our senses. We do not know the exact cause of depression, although there are many plausible hypotheses regarding its etiology – e.g. the aforementioned stress (rather long-term, cumulative over a lifetime), childhood trauma, inflammatory processes, genetic predisposition. However, we know that the symptoms of depression are directly related to disturbances in neurotransmitter levels in the brain, mainly noradrenaline, dopamine or serotonin, which are usually considerably reduced. Thus, the mechanism of action of antidepressants relies on enhancing this neurotransmission by stimulating or blocking specific receptors, inhibiting neurotransmitter-degrading enzymes, or preventing the clearance (reuptake) from the synaptic cleft. All these strategies aim to restore neurotransmitter levels to normal values, and thus, ensuring the proper functioning of these parts of the brain that are responsible for controlling our emotions.

Effective treatment of depression remains still an open issue and studies conducted both in laboratories of research institutions and pharmaceutical companies tend to invent drugs with more effective and faster profile through searching of new or more selective substances. We have to realize that introduction of new drugs onto the market is a long, arduous way and requires many years of research at the experimental level, clinical trials, and finally the implementation of commercial production. Our knowledge of the mechanisms of intracellular signal transduction has been substantially expanded since many now commonly used antidepressants were introduced into the clinic. It has been recognized a number of proteins being the important targets for antidepressant treatment. New receptor subtypes putatively responsible for the modulatory effects of antidepressants were also identified by pharmacological and genetic methods. Namely, among alpha(1)- adrenergic receptors (alpha(1)-AR) being essential targets of the catecholamines, especially noradrenaline (norepinephrine), three subtypes (alpha(1A)-AR, alpha(1B)-AR, alpha(1D)-AR) were identified in late 90s of last century. They serve different functions and are fairly well described, but only in the peripheral organs. Their action in the central nervous system is still poorly understood and research impeded and often impossible due to the lack of so-called "pharmacological tools", that is highly specific ligands for particular subtypes, penetrating into the brain. An alternative approach is to apply the cell lines and genetically modified animals where it is possible to eliminate or overexpress (artificially enhance) particular receptor subtype and in this way to validate its role by assessing the disturbances caused by the mutation – the strategy utilized in our project. The project is based on the working hypothesis assuming that alpha(1)-AR subtypes possess different functions in the mechanisms of antidepressant treatment. The hypothesis has been already supported by our previous research conducted at the level of mRNA encoding alpha(1A)-AR and alpha(1B)-AR subtypes which revealed a different regulation of these subtypes after imipramine administration (a popular antidepressant acting on noradrenergic system).

The aim of the project is therefore a **functional dissection of three existing subtypes (A, B, and D) of alpha(1)-AR in the mechanisms of action of antidepressant drugs** using:

• ***in vitro* model** - cell overexpressing (i.e. showing excess) particular alpha(1)-AR subtypes, while not expressing other subtypes;
• ***in vivo* model** - mice with the deleted genes (*knock - out*, KO) encoding for individual alpha(1)-AR subtypes.

The initial phase of the project (research on cell lines) will uncover whether and how antidepressants affect the reactivity of alpha(1)-AR subtypes by their response to agonists (stimulants) measured with use of pharmacological methods (such as the so-called assessment of second messengers and levels of various proteins crucial for intracellular signaling). It will be carried out in parallel to gene expression profiling of mice lacking particular alpha(1)-AR subtypes (A-KO, B-KO, D-KO) by means of cDNA microarrays performed on the material isolated from the cerebral structures of particular relevance for modulation of the response following antidepressants. This method (expression profiling) involves the screening examination of virtually all genes of the mouse by determining the pattern of changes in their expression. This will be done both in terms of effect on the mutation itself (lack of specific alpha(1)-AR subtype) as well as of the effect of chronic antidepressants administration influence. In the case of gene expression profiling is not really important to identify specific, particular genes whose expression was modified, but to find out the whole groups of genes (clusters) responsible for changes in the molecular pathways transmitting signals inside the cell. Such analysis is predicted upon advanced bioinformatics tools and specialized software that help to determine whether the induced changes may lead to e.g. inflammation, apoptosis (programmed cell death), or opposite, their direction can result in increased defense mechanisms. Of course, such processes detected at the mRNA level does not necessarily need to be passed through to functional changes leading to the actual effects of cellular response. Likewise, *in vitro* studies carried out on an simple, one-cell model with the modulation of a particular alpha(1)-AR subtype will not give the whole spectrum of interactions in a complex multicellular organism. Nevertheless, these results obtained in the initial part of the project will be extremely helpful to determine further steps, implemented at the level of protein expression analysis focusing on changes in the molecular paths considered as important targets for antidepressant treatment (i.e. mitogen activated kinases, transcription factor CREB, glycogen synthase

kinases). Moreover, it is worth remembering that alpha(1)-ARs themselves are also a targets for many antidepressants which have a direct affinity for these receptors.

Experiments carried out in the framework of this project will provide new data and will define more precisely the functional role of alpha(1)-AR subtypes in response to antidepressant treatment. Further in-depth identification of molecular pathways involved in the intracellular mechanisms of action of antidepressants may help in better understanding of the factors involved in the etiology of depression and contribute to the development of new strategies in drug design resulting in more effective therapies.