NEUROBIOLOGICAL ASPECTS OF DEPRESSIVE DISORDER AND ANTIDEPRESSANT TREATMENT: ROLE OF GLIA

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Abbreviations:
cAMP- cyclic adenosine monophosphate, CNS- central nerve system, EAA- excitatory aminoacid, EGF- epidermal growth factor, GFAP- glial fibrillar acidic protein, IL- interleukin, NMDA -N-methyl D-aspartate, SSRI-selective serotonin reuptake inhibitor, TCA- tricyclic antidepressant, TNF- tumour necrosis factor

ABSTRACT

Depression is a complex disorder related to chronic inflammatory processes, chronic stress changes and hippocampal response. There is a widening knowledge of the role of glial cells in nutrient supply to neurons, maintenance of synaptic contacts and tissue homeostasis within CNS. Glial cells, viewed in the past as mostly passive elements with limited influence on the neuronal functioning, are becoming recognized as active partners of neurons and are starting to be discussed as a possible therapeutic target. Their role in the pathogenesis of depressive disorder is also reconsidered.

Attention is devoted to studies of the different types of antidepressants and their effects on transmembrane signalling, including levels of α subunits of G proteins in C6 glioma cells in vitro as a model of postsynaptic changes in vitro. These models indicate similarities in antidepressant effects on G proteins of brain cells and effector cells of natural immunity, natural
killers and granulocytes. Thus, antidepressant response can possess certain common characteristics in functionally different systems which participate also in the disease pathogenesis. There are, however, differences in the astrocyte G-protein responses to antidepressant treatment, indicating that antidepressants differ in their effect on the glial signalization. Today mainstream approach to neurobiological basis of depressive disorder and other mood illnesses is linked to abnormalities in transmembrane signal transduction via G-protein coupled receptors. Intracellular signalization cascade modulation results to the activation of transcription factors with subsequent increased production of a wide array of products including growth factors and to changes in cellular activity and reactivity.

DEPRESSION AS A SYSTEMIC DISORDER

Depression is one of the most prevalent mental disorders and one of leading causes of morbidity, mortality and economic burden worldwide. During four decades of research, biochemical research of depression has focused on the monoamine neurotransmitters and their receptors, in the past decade on the receptor transduction mechanisms and intracellular signalization cascades (Avissar and Schreiber, 2002, 2006). The family of G-proteins is a crucial convergence point in the signal transduction from many extracellular primary messengers to the intracellular second messengers and cell response (Kovářů and Kovářů, 2005). G-protein measurement is applied as one of state markers of the depressive patients or subjects under antidepressant, lithium or electroconvulsive treatment (Avissar and Schreiber, 2002, 2006). Besides biochemical research, many other approaches contributed significantly to the understanding of depression, one of the most fruitful contributions to this comprehension came from the field of psychoneuroimmunology and neuroimmunology (Haddad et al., 2002, Tafet and Bernardini, 2003, Shiepers et al., 2005, Havrdová, 2005).

Considerable evidence points to some similarities between depression and inflammatory response (Leonard, 2006). So-called "sickness behaviour" which is phenomenologically similar to depression with fatigue, anhedonia, loss of energy and anorexia as the prominent features is linked to increased levels of pro-inflammatory cytokines like IL-1, IL-6 or TNFα on the
periphery, or within CNS (Kelley et al., 2003, Shiepers et al., 2005). Main producers of cytokines on the periphery are activated macrophages and T- and B-cells, in the CNS the activated microglial cells (Hauwel et al., 2005, Shiepers et al., 2005). Chronic inflammatory diseases, e.g. rheumatoid arthritis, are often accompanied by depression (Covic et al., 2006). Some of the immune system cells, e.g. granulocytes or natural killers, show during the depression course and antidepressant treatment responses in many ways similar to the brain cells. Antidepressants affect cytokine production, promoting anti-inflammatory cytokine phenotype in human blood (Kovářů et al., 1997, Fišerová et al., 2002, Diamond et al., 2006).

Despite deepening understanding of systemic aspects of depressive disorder, majority of authors are still considering depression as a primarily “brain disorder”. There is a growing knowledge of the role of glial cells in neurogenesis, neuronal development and maturation, nutrient supply, involvement in the communication at the synaptic contacts and tissue homeostasis and even active participation in the signalization processes (Araque et al., 1999, Laming et al., 2000, Hertz and Zielke, 2004). Astrocytes are becoming recognized as active partners of neurons and there are several neuro-psychiatric disorders where the role of astrocytes is recognized, e.g. Alzheimer’s disease, multiple sclerosis, HIV dementia or dementia with Lewy bodies.

The objective of this paper is to review the literature concerning astrocyte neurobiology associated with depression and antidepressant treatment within the frame of the systemic concept of depression. We will try to show astrocytes as a cell population which can contribute to the depression development and is affecting neuronal functioning during the disease course. We will focus on the G-protein astrocytic signalling, in respect to the crucial role of G-proteins in the cell signal transduction. Antidepressant treatment is considered to exert its effect mainly on neuronal
population, we will try to demonstrate that astrocytes can be already regarded as one of the targets of antidepressant treatment which mediate a part of the beneficial effects.

DEPRESSION AS A STRESS INDUCED SELECTIVE NEURODEGENERATION

Major depression involves disturbances in emotional, cognitive, immune, autonomic and endocrine functions (Nestler et al., 2002). Nervous, endocrine and immune systems share neurotransmitters, peptide hormones and cytokines as well as their receptors as a common chemical language to communicate with each other (Haddad et al., 2002, Fišerová et al., 2002, Tafet and Bernardini, 2003, Kovářů and Kovářů, 2005). This interplay is especially important during stress response, and stressful life events are indeed often precipitating factors for the depression onset (Hayley et al., 2005, Sekot et al., 2005). Neurotransmitters alterations can affect functioning of these systems in many ways, for decades depression was linked particularly to disturbances in serotonergic and noradrenergic neurotransmission. Dysfunction in the neurotransmitter systems results, besides psychological and behavioral consequences, in the systemic effect with hyperactivation of stress hypothalamic-pituitary-adrenal axis (HPA) (Haddad et al., 2002, Tafet and Bernardini, 2003). Resulting prolonged hypercortisolemia causes a wide array of organ and immune changes (Tafet and Bernardini, 2003, Duman, 2004, Gubba et al., 2004).

One of the most affected structures is hippocampus which expresses high numbers of steroid receptors (Brown et al., 1999, Sheline et al., 2002). Hippocampus has a key role in the declarative memory tasks and many other cognitive functions. It is also interconnected in the limbic system, participating in the recognition and regulation of emotional states as well as in the vegetative and autonomic function control, including HPA and sympatho-adrenal system regulation (Sheline et al., 2002, Tafet and Bernardini, 2003). Adrenal steroids modulate
excitability of hippocampal neurons and interfere with the process of dendritic remodelling in CA3 hippocampal region, causing hippocampal dendritic atrophy (Brown et al., 1999, McEwen et al., 2002, Sheline et al., 2002, Hayley et al., 2005). Stress also impairs the process of adult neurogenesis, causing robust reduction of the number of newly generated cells in the hippocampal dentatus gyrus within various stress paradigms (Czech et al., 2002, Duman, 2004). Newly generated cells are demonstrated to be functionally connected in the neuronal circuitry, reduced adult neurogenesis is hypothesized to cause reduced ability of hippocampus to cope with novelty and complex tasks leading to the inadequate information processing at the interface systems involved in the learning and affect regulation (Jakobs et al., 2000, Nestler et al., 2002, Kempermann et al., 2004, Doetsch and Hen, 2005).

Today mainstream therapy of depression via G-protein receptors and intracellular signalization cascade modulation results in the activation of transcription factors, with subsequent increase of growth factor production, most notable are studies of brain-derived growth factor (Duman and Monteggia, 2006). This “neurotrophin hypothesis of depression” assumes that deficiency in the neurotrophin signalization systems with effects on the cellular plasticity, viability and neurogenesis together with an enhancement of apoptotic processes caused by increased cortisol and pro-inflammatory cytokines levels play an important role in the depression etiopathogenesis (Aberg et al., 2000, Jacobs et al., 2000, Gould and Manji, 2002, Nestler et al., 2002, Kempermann and Kronenberg, 2003, Duman, 2004, Duman and Monteggia, 2006, Leonard, 2006).

Data are supported by the study performed by Alfonso et al. (2004) relating psychosocial stress and hippocampus response using model tree shrews. The authors screened two subtractive hippocampal cDNA libraries generated from RNA of cortisol treated animals. Comparing transcript levels of stressed and control groups, four differences were demonstrated: nerve
growth factor (NGF), membrane glycoprotein protein 6a (M6a), CDC-like kinase 1 (CLK-1), and α subunit of Gq protein (GNAQ) transcript levels were reduced by chronic psychosocial stress. All genes are related to neuronal differentiation, in agreement with previous findings of dendrite retraction and impairment of neurogenesis. Treatment by antidepressant clomipramine prevents these processes (with the exception of unchanged NGF). This study also supports the concept that stress and/or depressive disorders are accompanied by the neuronal dedifferentiation at least in the hippocampal area and antidepressants can prevent these processes (Alfonso et al., 2004).

Brain imaging studies show reductions of a hippocampal volume correlated with lifetime duration of depression (Steffens et al., 2000, Sheline et al., 2002). Reduction of the hippocampal volume is more prominent in depressive subjects exposed to trauma during childhood (Vithingham et al., 2002). Cell-counting studies have established that major depressive disorder and bipolar illness are characterized by alterations in the density and size of neuronal and glial cells in fronto-limbic brain regions (Rajkowska, 2003). There is an observed decrease in the neuronal and glial cell sizes and densities in the orbito-frontal regions and dorsolateral prefrontal cortices of subjects with mood disorders and alcohol dependence, with more marked pathology of glial population (Miguel-Hidalgo and Rajkowska, 2003). Reduced subgenual glial numbers are more prominent in subjects with the family history of depression (Öngür et al., 1998). There are also reduced numbers of glial cells and glial/neuronal ratio in the amygdala (Bowley et al., 2002) and reduced astrocytic marker GFAP in the cerebellum of subjects with mood disorders (Fatemi et al., 2004). As a mechanism responsible for cell loss of both neurons and glia in the hippocampal region is considered apoptosis, requiring prolonged and severe stress exposure to occur (Tacuma et al., 2004, Lucassen et al., 2006)
These findings from histopathological and cell counting studies suggest that depression, as a complex disorder affecting many cell populations, is involving also glial cells. In this communication we will focus mainly on the astrocytes and model C6 glioma (astrocytoma) cells, keeping in mind that contribution of other glial elements, e.g. oligodendrocytes or microglia, is also very important.

ASTROCYTE AND DEPRESSION ETIOPATHOGENESIS

Astrocytes are prevailing glial cells population in the CNS, outnumbering neurons by 2-10 times, depending on brain area (O’Kusky and Collonier, 1982). They form a plexiform net of cells connected by gap junctions, providing thus a way for buffering extracellular ion dysbalances caused by neuronal activity (Syková, 2005). Astroglial cells are an essential component of blood brain barrier, and provide nutrient supply to neurons (Laming et al., 2000). Astrocytes express virtually all neuronal neurotransmitter receptors, ion channels and neurotransmitter uptake sites – see Scheme 1 (Hösli and Hösli, 1993, Porter and McCarthy, 1997, Deschepper, 1998, Verkhratsky and Steinhäuser, 2000, Nakagawa and Schwartz, 2004). There is an intensive bidirectional communication between neurons and glial cells at the synapses, a concept of ”gliotransmission“ and “tripartite synapse“ was postulated where astrocytes are seen as active partners of neurons (Araque et al., 1999). Astrocytes participate in the neurotransmitter uptake from synaptic cleft, their synthesis from precursors, supply of neurotransmitter precursors to neuron and disposal of excess neurotransmitter (Danbolt, 2001, Hertz and Zielke, 2004). Released neurotransmitter can evoke Ca\(^2+\) concentration increases in astrocytes ensheathing synaptic cleft which can signal back to the neuronal presynaptic terminal, and further increases or suppresses the release of neurotransmitter (Cotrina et al., 2000, Parpura and Haydon, 2000).

Astrocytes express both mineralocorticoid and glucocorticoid receptors, glucocorticoid levels regulate astrocytic reactivity and apoptosis, so downregulation of astrocytic growth factor
production by glucocorticoids is demonstrated (Gubba et al., 2004). Glucocorticoids also inhibit glucose uptake by both astrocytes and neurons impairing thus complex brain energy metabolism (Horner et al., 1990).

Hippocampal atrophy observed in depression illness might be related to the altered excitatory aminoacid system function. Excessive excitatory amino acid levels can be neurotoxic to neuronal terminals, neurons are in this respect greatly dependent on astrocyte clearance of elevated glutamate, because removal of glutamate is largely mediated by astrocytic transporters (Brown, 1999, Danbolt, 2001, Hertz and Zielke, 2004). Elevated cAMP levels are demonstrated to enhance expression of glutamate transporters (GLAST and GLT1) in rat astrocytes and regulate active calcium entry (Hughes et al., 2004, Pawlak et al., 2005). Growth factors, e.g. TGF-β and EGF, increase expression of glutamate transporters on astrocytes (Zelenaia et al., 2000). This can exert protective effect in the endangered hippocampal structures, e.g. apical dendrites of pyramidal neurons, enhance glutamate uptake and prevent excitatory amino acid induced impairments of long-term potentiation and dendritic remodelling.

Astrocytes are important regulators of synapse numbers during development and in the adulthood and are necessary for synapse maturation, proper receptor density and receptor subunit composition (Wilson et al., 1998, Donato, 2001, Slezak and Pfrieger, 2003). There is also an evidence for astrocyte influence in the process of adult neurogenesis taking place in the subventricular and subgranular hippocampal zones (Seri et al., 2001, Nakayama et al., 2003, Hagg, 2005). Interesting novel findings indicate glial gene Ndrg2, with putative roles in neuronal differentiation, synapse formation and axon-glial interactions regulated by glucocorticoids and antidepressants as a candidate vulnerability gene to depression development (Nichols et al., 2005).
Following noxious insults associated with neuronal damage, astrocytes proliferate, change cell morphology, accumulation of cytoplasmic fibrillary material and profile of expressed receptors and adhesion molecules (Ridet et al., 1997, Sofroniew, 2005). These reactive astrocytes produce neurotrophins, cytokines and chemokines which serve as mediators of the host defense system, inflammatory response and signals among astrocytes, neurons and microglia (Müller et al., 1995, Nakagawa and Schwartz, 2004, Hauwel et al., 2005). Astrocytes are main cell population, responsible for limiting inflammatory reactions within CNS, proinflammatory cytokines, prostaglandins and nitric oxide released during inflammatory response can loose negative feedback and rise to the levels which can be deleterious to the neurons and other cells. For example, astrocytes lacking β2 adrenoreceptors play a role in multiple sclerosis pathogenesis (De Keyser et al., 2004). There is a negative interference of inflammation with induction of long-term potentiation, neurite sprouting and neurogenesis in hippocampus (Vereker et al., 2001, Hayley et al., 2005). There is also an increase of apoptosis rate (Shiepers et al., 2005, Lucassen et al., 2006).

There is also an important involvement of astrocytes in the serotonin metabolism. Depletion of serotonin precursor tryptophane in diet is causing serotonin level reductions in the brain which is correlating with depressive symptomatology (Neumeister, 2003, Leonard, 2006). Tryptophane is metabolized by tryptophane hydroxylases to serotonin or alternatively by dioxygenases to kynurenine. Kynurenine is further metabolised to the neurotoxic metabolites or neuroprotective kynurenic acid. Activity of dioxygenases is increased by cortisol and pro-inflammatory cytokines, e.g. IL-6 or INF-γ. Activated microglia is producing mostly neurotoxic metabolites 3-hydroxyanthranil acid and quinolinic acid, main astrocyte metabolite is neuroprotective kynurenine Guillemin et al., 2005). Astrocytes are also metabolizing quinolinic acid produced by
microglia, reducing thus neurotoxicity associated with microglial activation (Guillemin et al., 2001)

Reductions in neuronal size and density in certain areas observed in depression can be indicative of diminished glial ability to support full cell size and proper dendrite arborisation. Given indispensable function of astrocytes in the neuronal energy metabolism, reductions of the number of glial cells in the frontal lobes can participate in the frontal hypometabolism observed in these regions during depressive episode. Astrocytes contain high levels of antioxidants and antioxidant enzymes. Reduced neuron supportive functions due to compromised astrocyte numbers or function can thus cause neurons to be more vulnerable to excessive corticosteroid mediated, excitotoxic glutamate, quinolinic acid or reactive oxygen species mediated damage (Brown et al., 1999, Brown, 1999, Drukarch et al., 1998, Tacuma et al., 2004). Astrocyte derived growth factors provide neuroprotection in various damage models (Junker et al., 2002, Nakagawa and Schwartz, 2004, Tacuma et al., 2004). Astrocytes can further support neuronal viability by secretion of other neuroprotective factors and by restricting inflammatory response and microglial activation (Müller et al., 1995, Villoslada and Genain, 2004, Hauwel et al., 2005).

EFFECT OF ANTIDEPRESSANTS ON ASTROCYTES

Several different classes of pharmacological agents are currently used for treatment of depression. These include monoamine oxidase inhibitors (MAO), nonselective inhibitors of monoamine uptake, tricyclic drugs - imipramine, amitriptyline or desipramine, selective inhibitors of serotonin reuptake (SSRIs) like fluoxetine, sertraline or citalopram, inhibitors of noradrenaline reuptake and drugs inhibiting uptake of both monoamines, e.g. venlafaxine or mirtazapine. Antidepressive effect proves also tianeptine which enhances uptake of monoamines
on the synapses and is supposed to reverse stress induced changes on the hippocampal formation and modulate glutamate receptors function (McEven et al., 2002). The main mechanism to terminate action of the synaptically released transmitters is uptake by transporters which are present both on the neurons and astrocytes (Fuller and Wong, 1990, Bal et al., 1997, Inazu et al., 2001, 2003).

Norepinephrine transporter (NET) is sensitive to tricyclic drugs like desipramine and imipramine as well as to the mixed serotonin/noradrenaline uptake inhibitor milnacipram (Inazu et al., 2003). Elevation of norepinephrine levels by blockade of uptake has a profound effect on astrocytic neurotransmitter receptors expression and cellular signalization cascade modulation. Astrocytes as a major cell population expressing \( \beta_1 \) and \( \beta_2 \) adrenoreceptors in the brain (Hösli and Hösli, 1993) are responding to antidepressant desipramine by decreasing density of receptors (Sapena et al., 1996). Norepinephrine present in the synapse or other extraneuronal spaces can also be taken up into adjacent glia by a mechanism known as uptake 2, the extraneuronal monoamine transporter. This uptake 2 transporter is inhibited by formation of normetanephrine in glia by catechol O-methyltransferase from epinephrine. Thus astrocytes actively participate in increase of synaptic norepinephrine and potentiate action of norepinephrine reuptake inhibitor antidepressants (Schildkraut and Mooney, 2004).

Astrocytic serotonin sodium-dependent transporter SERT is sensitive to tricyclic drugs as well as to SSRIs and its expression is downregulated by SSRIs (Bal et al., 1997, Inazu et al., 2001, Benmansour et al., 2002). Its expression and serotonin uptake are positively regulated by fibroblast growth factor (Kubota et al., 2001). By binding to astrocytic 5HT\(_1\) receptors, serotonin stimulates production of neurotrophic S100\(\beta\) which is also produced after exposition to SSRI fluoxetine (Wilson et al., 1998, Donato, 2001, Manev et al., 2001). Plasma level of S100\(\beta\) was
significantly higher in patients with major depression and positively correlated with treatment response after 4 weeks of treatment (Arolt et al., 2003). Stimulation of 5-HT$_{2A}$ receptors enhances the turnover of phosphoinositol and cAMP accumulation, there is a potentiation of $\beta$-adrenoreceptor stimulated accumulation of cAMP. Furthermore, in the presence of both noradrenaline and serotonin receptor agonists, the accumulation of cAMP is increased, and each of them alone can also cause accumulation (Hansson et al., 1990).

Influence of antidepressant treatment on glutamate system function is also studied, tricyclic antidepressants when administered chronically have modulatory effect on NMDA receptors, NMDA antagonist MK-801 produces similar effect (Nowak et al., 1993). There is also demonstrated a stress induced increase of glial glutamate transporter GLT-1a, administration of antidepressant tianeptine eliminates this effect, concurrent with downregulation of NMDA-receptor subunits (McEwen et al., 2002).

In respect to the suspicious neurotrophin shortage in depression, production of growth factors by astrocytes following antidepressant exposition is investigated. Chronic administration of antidepressants amitriptyline, clomipramine, mianserine, fluoxetine and paroxetine significantly increases glia derived neurotrophic factor (GDNF) release from C6 glioma cells (Hisaoka et al., 2001). This GDNF release is further potentiated by serotonin (Hisaoka et al., 2004). Activation of $\beta_2$ adrenoreceptors by clenbuterol induced the synthesis of nerve growth factor (NGF), fibroblast growth factor (FGF) and transforming growth factor $\beta$1 (TGF $\beta$1) and provided neuroprotection against glutamate induced and ischemic neuronal damage (Junker et al., 2002). Noradrenaline is demonstrated to stimulate C6 glioma and cortical astrocytic cells to produce NGF via $\beta$-adrenergic receptor stimulation and adenylylcyclase activation (Stone and Ariano, 1989).

Production of growth factors seems to be also regulated by dopamine D1 and D2 receptor
agonists which may be required for NGF and glia-derived growth factor (GDGF) production by astrocytes (Ohta et al., 2003). Production of brain-derived neurotrophic factor by mouse astrocytes is potentiated by dopaminergic stimulation as well (Inoue et al., 1997). MAO B inhibitor selegiline also stimulates growth factor (NGF, BDNF, and GDNF) production by mouse astrocytes (Mizuta et al., 2000).

All monoamines (noradrenaline, serotonin or dopamine) are able to increase significantly BDNF astrocyte synthesis and secretion, and suggest the existence of a positive reciprocal interaction between monoaminergic neuronal activity and astrocyte neurotrophic support in neuron–astrocyte crosstalk which has a dynamic role in mediating neuronal plasticity and trophic functions in the brain (Mojca-Juric et al., 2006).

MAJOR DEPRESSION AND CELL SIGNALLING

Biochemical approach to mood disorders and antidepressant action is based on monoamine theories describing lowered serotonin and norepinephrine amount in the synaptic cleft, and antidepressant influenced inhibition of the neurotransmitter reuptake into nerve terminals (Gould and Manji, 2002, Avissar and Schreiber, 2006). Attention is also focused on transmembrane signal transduction from neurotransmitter receptor coupled to heterotrimeric guanine-nucleotide binding proteins (G proteins), effector systems (enzymes, ion channels) and subsequent intracellular response.

Trimeric G proteins are composed of α, β and γ subunits. In activated state especially G α subunits play a key role in transmembrane signalling from receptor binding the ligand (neurotransmitter, hormone, chemokine, etc.) to effector enzymes - adenylylcyclase (G αs, G αi1,2) and phospholipase C (G αq/11), producing second messengers cAMP and 1,4,5...
inositoltriphosphate (Spiegel, 1996), respectively. Subsequent intracellular events predominantly regulate cascade of protein phosphorylation reactions by kinases. G proteins play a role in molecular switches in complex biological processes, such as synaptic plasticity, neuronal sprouting and cellular differentiation including early stages of neurogenesis, providing thus a mechanism for the epigenetic control of neuronal differentiation (Strubing et al., 1997, Avissar and Schreiber, 2006).

Various studies are concerned in the involvement of G proteins in pathophysiology, diagnosis and treatment of mood disorders, modulation of G protein coupled neurotransmitter receptors and regulation of G protein function by arrestins, etc. (Gould and Manji, 2002, Avissar and Schreiber, 2002, 2006). Furthermore, there was an association between G β subunit expression or G-protein β3 subunit gene polymorphism in major depressive disorder, and moreover an antidepressant effect was demonstrated (Kovářů et al., 2001, Lee et al., 2004). Altered signal transduction components, especially α subunit of G protein expression and/or function, as well as changed mRNA levels were found in the postmortem brain tissue of patients with major depression and bipolar disorder (Young et al., 1993). Another data demonstrated both G protein influenced cAMP synthesis and G protein induced activation of phosphoinositide signal transduction in postmortem brain cortex regions of suicide victims with major depression (Pacheco et al., 1996). In contrast, there is decreased phosphoinositide metabolism in postmortem brain samples of bipolar affective disorder subjects (Gonzales-Maeso et al., 2002).

Besides this, analyses of peripheral blood granulocytes or thrombocytes of depressive patients confirmed relationship between alteration in G proteins and decreased G protein function in depression, and increased levels and the function of G proteins in bipolar disorder (Gould and Manji, 2002, Avissar and Schreiber, 2006). We demonstrated dynamic changes in levels of both
G αq/11 and G αs subunits of peripheral blood granulocytes of patients with unipolar depression during the course of fluoxetine administration on days 3 – 28 (Kovářů et al., 2000, Kovářů and Kovářů, 2005). Granulocytes are effectors of natural immunity likewise natural killer (NK) cells. Blunted peripheral blood NK cytotoxicity was demonstrated in major depression (Reynaert et al., 1995).

ANTIDEPRESSANTS AND CELL SIGNALLING

Antidepressant effects during depressive disorder is based mainly on inhibition of reuptake of biogenic amines - serotonin (5-HT), norepinephrine and/or dopamine into presynaptic nerve terminals (Gould and Manji, 2002, Avissar and Schreiber, 2006). Furthermore, antidepressants cause downregulation of β-adrenergic, 5-HT1 and 5-HT2 receptors at the postsynaptic level, when administered chronically. Decreased monoamine receptor densities following antidepressant treatment can also be seen in cell culture systems, such as C6 glioma cells lacking presynaptic input. Thus, monoamine receptor downregulation is directly resulting from postsynaptic action of the antidepressants. Reports concerning antidepressant postreceptor effects on G proteins involve both proximal effects on receptor coupled to G protein and distal effects on G protein-effector enzymes, adenylylcyclase and phospholipase C which are producing second messengers cAMP and 1,4,5 inositoltriphosphate.

Receptor-G protein coupled interactions are tightly regulated by mechanisms of desensitization, internalization, downregulation, and resensitization which are protecting cells from over-stimulation. These mechanisms involve activities of two families of proteins: a) G–protein coupled receptor kinases, serine-threonine kinases, capable of phosphorylation of receptor and thus uncoupling receptor-G protein, and b) beta-arrestins with “scaffold“ function of G protein, transducing signal leading to activation of mitogen-associated protein (MAP)
kinase cascade. MAP kinase via specific kinases regulates a number of intracellular events, including apoptotic signals (Avissar and Schreiber, 2006).

In vitro studies contribute to the clarification of the molecular basis of antidepressant action involving G proteins as postreceptor components (Alt et al., 2001, Donati and Rasenick, 2005). Cell cultures represent alternative bioassay models to animal experiments. C6 glioma established cell line of astrocyte origin is used in the studies exploring antidepressant effects (Mareš et al., 1991, Kovářů et al., 2001, Donati and Rasenick, 2005). Acute antidepressant administration is lasting 24 hours, chronic 5-day administration to C6 glioma cell culture is comparable with 3-week antidepressant treatment of rats (Chen and Rasenick, 1995).

According to our results, acute fluoxetine effect evoked a decreased level of Gαq/11 subunit in C6 glioma cells, whereas after chronic exposition an increased Gαq/11 level was determined. Acute fluoxetine administration causing a reduced membrane Gαq/11 amount was linked to subunit translocation into cytoplasm where it was proved (Kovářů et al., 1997, 1998). Reduced membrane Gαq/11 subunit levels caused a lower degree of phospholipase C signalling with subsequent decreased substrate 1,4,5 inositoltrisphosphate formation. Antidepressants from different classes are able to induce Gα subunit translocation into the cytoplasm (Donati and Rasenick, 2005). Intracellular consequences of fluoxetine administration on C6 glioma cells were demonstrated by apoptotic events in contrast to ineffective imipramine or amitriptyline (Španová et al., 1997). MAP kinase activation participating in apoptotic signals was detected in fluoxetine treated cultures of rat astrocytes (Mercier et al., 2004).

We also studied in vitro chronic effects of sertraline, citalopram (SSRIs) and mirtazapine (noradrenergic and serotonergic antidepressant) in comparison with tricyclic imipramine, amitriptyline or desipramine. Results show that Gα subunit profiles are antidepressant dependent
and are not affected by TCA administration. Data demonstrate antidepressant induced cell signal transduction pathway modulation via both effector enzymes, adenylylcyclase (Gαs) and phospholipase C (Gαq/11) (Kovářů et al., 1998, 1999, 2001).

When citalopram effects on both rat C6 glioma cells after in vitro chronic treatment were compared with in vivo rat brain, similarities in Gα subunit profiles were observed (Fig.1). In vivo citalopram effects were comparable in the brain and the spleen, indicating similar cell signalling response in functionally different systems (Fišerová et al., 1997, Kovářů et al., 2000, 2001, Fišerová et al., 2002).

CONCLUSION

Despite several decades of research, the exact neurobiological substrate of depression and mechanisms of antidepressant drug action are still unknown. There is a complex disturbance of homeostatic functions in depression, comprising immune, endocrine and central nervous systems, which are regulated and/or corrected by antidepressant treatment. As suggested by many authors, disturbances in cellular plasticity processes, neurogenesis and suppression of reparation processes are the crucial changes at the cellular level in depression (Jacobs et al., 2000, Nestler et al., 2002, Kempermann and Kronenberg, 2003, Duman, 2004, Leonard, 2006). Impairment of neural connectivity results in the aberrant information processing in the neural circuits critical for mood regulation.

Depression is often accompanied by inflammatory changes and hypercortisolemia which are both pro-apoptotic, many findings demonstrate an increase of apoptotic processes and atrophic changes in hippocampus and frontal lobes as well as other structural neuronal alterations (Hayley et al., 2005, Shiepers et al., 2005, Lucassen et al., 2006).
Nevertheless, the today prevailing opinion considering only neuronal population as a substrate of a disorder and the only target of antidepressant medication is not further sustainable with a widening knowledge demonstrating close functional cooperation between neurons and supportive glia in health and disease. This close cooperation is possibly reflected also in the depression pathogenesis where degeneration of neuronal populations in certain regions is accompanied also by glial astheny and impairment of neuronal metabolism which is largely glia-dependent.

As we try to demonstrate, antidepressants affect glial cell signal transduction, as evidenced by changed G-protein levels and second messenger changes. Treatment also modulates complex cell responses and production of many substances, necessary for neuronal health and survival, including a wide array of growth factors. The antidepressant treatment effect which in some aspects opposes the effect of stress on neurons, is therefore not limited to neuronal population. Antidepressants affect also astrocytes which are the major source of the neurotrophic and neuroprotective substances, thus supporting neuroplasticity events. Therefore, modulation of astrocyte activity seem to be a logical step in complex pharmacological treatment of depression as well as of other neuro-psychiatric disorders with a neurodegenerative component. Considering significant differences in the antidepressant effects on the astrocyte cell signal transduction, it remains a matter of further research to identify drugs with specific modulatory effects on astrocytic function or production of neurotrophic molecules.

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Scheme 1.
Astrocyte receptors and membrane transporters.

Figure 1.
Effect of citalopram *in vitro* and *in vivo* on the rat. Each result is the mean +/- S.E.M of 7-9 measurements. For details see Kovářů *et al.*, 2001.
| Scheme 1 |
|-----------------|-------------------------------|
| **GABAₐ, GABAₐ₁a, GABAₐ₁b, GABAₐ₂** | Charles 2003, Porter *et al.*, 1997 |
| **Glutamate AMPA/kainate, NMDA, mGLURs₁, mGLURs₅** | Porter *et al.*, 1997, Hertz and Zielke 2004 |
| **Purinergic P₁, P₂X₁, P₂X₂, P₂X₃, P₂X₄, P₂X₅, P₂X₆, P₂Y₁, P₂Y₄** | Müller *et al.*, 1995, Washburn and Neary 2006 |
| **Acetylcholine nikotinic receptors** | Sharma and Vijayaraghavan 2001 |
| **Dopamine D₁, D₃, D₄, D₅** | Ohta *et al.*, 2003, Myiazaki *et al.*, 2004 |
| **Norepinephrine transporter NET, uptake 2 transporter** | Inazu *et al.*, 2003, Schildkraut and Money, 2004 |
| **Glutamate transporters GLAST and GLT1** | Danbolt 2001, Pawlak *et al.*, 2005 |
| **Receptors for cytokines (CNTF, IL 1β, IL1ra, INF-γ, IL4,IL 6, IL10, TGFβ, TNFα)** | Haddad *et al.*, 2002, Nakagawa and Schwartz 2004, |
| **Peptide receptors for VIP, somatostatin, oxytocin, vasopresin, ANP, bradykin, thrombin** | Deschepper 1998, Porter *et al.*, 1997 |
Figure 1